Environmental Health Criteria 188
Nitrogen Oxides
(Second Edition)
This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

Environmental Health Criteria 188

NITROGEN OXIDES
(SECOND EDITION)


Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

World Health Organization
Geneva, 1997
The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization and the Organization for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.
CONTENTS

ENVIRONMENTAL HEALTH CRITERIA FOR NITROGEN OXIDES

Preamble xiii

1. SUMMARY 1

1.1 Nitrogen oxides and related compounds 1
  1.1.1 Atmospheric transport 2
  1.1.2 Measurement 3
  1.1.3 Exposure 3

1.2 Effects of atmospheric nitrogen species, particularly nitrogen oxides, on vegetation 4

1.3 Health effects of exposures to nitrogen dioxide 7
  1.3.1 Studies of the effects of nitrogen compounds on experimental animals 7
    1.3.1.1 Biochemical and cellular mechanisms of action of nitrogen oxides 8
    1.3.1.2 Effects on host defence 10
    1.3.1.3 Effects of chronic exposure on the development of chronic lung disease 11
    1.3.1.4 Potential carcinogenic or co-carcinogenic effects 12
    1.3.1.5 Age susceptibility 12
    1.3.1.6 Influence of exposure patterns 12
  1.3.2 Controlled human exposure studies on nitrogen oxides 13
  1.3.3 Epidemiology studies on nitrogen dioxide 15
  1.3.4 Health-based guidance values for nitrogen dioxide 18

2. PHYSICAL AND CHEMICAL PROPERTIES, AIR SAMPLING AND ANALYSIS, TRANSFORMATIONS AND TRANSPORT IN THE ATMOSPHERE 19

2.1 Introduction 19
  2.1.1 The nomenclature and measurement of atmospheric nitrogen species 19

2.2 Nitrogen species and their physical and chemical properties 20
  2.2.1 Nitrogen oxides 21
    2.2.1.1 Nitric oxide 21
    2.2.1.2 Nitrogen dioxide 21
2.2.1.3 Nitrous oxide 21
2.2.1.4 Other nitrogen oxides 24
2.2.2 Nitrogen acids 24
2.2.2.1 Nitric acid 24
2.2.2.2 Nitrous acid 25
2.2.3 Ammonia 25
2.2.4 Ammonium nitrate 25
2.2.5 Peroxyacetyl nitrate 26
2.2.6 Organic nitrites and nitrates 26

2.3 Sampling and analysis methods 26
2.3.1 Nitric oxide 26
2.3.1.1 Nitric oxide continuous methods 26
2.3.1.2 Passive samplers for NO 32
2.3.1.3 Calibration of NO analysis methods 33
2.3.1.4 Sampling considerations for NO 33
2.3.2 Nitrogen dioxide 34
2.3.2.1 Chemiluminescence (NO + O₃) 34
2.3.2.2 Chemiluminescence (luminol) 35
2.3.2.3 Laser-induced fluorescence and tuneable diode laser absorption spectrometry 36
2.3.2.4 Wet chemical methods 36
2.3.2.5 Other methods 36
2.3.2.6 Passive samplers 37
2.3.2.7 Calibration 37
2.3.3 Total reactive odd nitrogen 38
2.3.4 Peroxyacetyl nitrate 38
2.3.5 Other organic nitrates 38
2.3.6 Nitric acid 39
2.3.7 Nitrous acid 39
2.3.8 Dinitrogen pentoxide and nitrate radicals 39
2.3.9 Particulate nitrate 39
2.3.10 Nitrous oxide 40
2.3.11 Summary 40

2.4 Transport and transformation of nitrogen oxides in the air 40
2.4.1 Introduction 40
2.4.2 Chemical transformations of oxides of nitrogen 40
2.4.2.1 Nitric oxide, nitrogen dioxide and ozone 40
2.4.2.2 Transformations in indoor air 43
2.4.2.3 Formation of other oxidized nitrogen species 43
2.4.3 Advection and dispersion of atmospheric nitrogen species 47
2.4.3.1 Transport of reactive nitrogen species in urban plumes 49
2.4.3.2 Air quality models 50
2.4.3.3 Regional transport 50
2.5 Conversion factor for nitrogen dioxide 50
2.6 Summary 50

3. SOURCES, EMISSIONS AND AIR CONCENTRATIONS 52

3.1 Introduction 52
3.2 Sources of nitrogen oxides 53
  3.2.1 Sources of NO\textsubscript{x} emission 53
    3.2.1.1 Fuel combustion 53
    3.2.1.2 Biomass burning 56
    3.2.1.3 Lightning 56
    3.2.1.4 Soils 57
    3.2.1.5 Oceans 59
  3.2.2 Removal from the ambient environment 60
  3.2.3 Summary of global budgets for nitrogen oxides 62
3.3 Ambient concentrations of nitrogen oxides 63
  3.3.1 International comparison studies of NO\textsubscript{x} concentrations 63
  3.3.2 Example case studies of NO\textsubscript{x} and NO\textsubscript{2} concentrations 75
3.4 Occurrence of nitrogen oxides indoors 79
  3.4.1 Indoor sources 84
    3.4.1.1 Gas-fuelled cooking stoves 84
    3.4.1.2 Unvented gas space heaters and water heaters 84
    3.4.1.3 Kerosene space heaters 85
    3.4.1.4 Wood stoves 85
    3.4.1.5 Tobacco products 88
  3.4.2 Removal of nitrogen oxides from indoor environments 89
3.5 Indoor concentrations of nitrogen oxides 92
  3.5.1 Homes without indoor combustion sources 92
  3.5.2 Homes with combustion appliances 95
  3.5.3 Homes with combustion space heaters 96
  3.5.4 Indoor nitrous acid concentrations 107
  3.5.5 Predictive models for indoor NO\textsubscript{2} concentration 107
3.6 Human exposure 109
3.7 Exposure of plants and ecosystems 114
4. EFFECTS OF ATMOSPHERIC NITROGEN COMPOUNDS (PARTICULARLY NITROGEN OXIDES) ON PLANTS

4.1 Properties of NO and NH₃

4.1.1 Adsorption and uptake
4.1.2 Toxicity, detoxification and assimilation
4.1.3 Physiology and growth aspects
4.1.4 Interactions with climatic conditions
4.1.5 Interactions with the habitat
4.1.6 Increasing pest incidence
4.1.7 Conclusions for various atmospheric nitrogen species and mixtures
  4.1.7.1 NO₂
  4.1.7.2 NO
  4.1.7.3 NH₃
  4.1.7.4 NH₄⁺ and NO₃⁻ in wet and occult deposition
  4.1.7.5 Mixtures
4.1.8 Appraisal
4.1.9 General conclusions

4.2 Effects on natural and semi-natural ecosystems

4.2.1 Effects on freshwater and intertidal ecosystems
  4.2.1.1 Effects of nitrogen deposition on shallow softwater lakes
  4.2.1.2 Effects of nitrogen deposition on lakes and streams
4.2.2 Effects on ombrotrophic bogs and wetlands
  4.2.2.1 Effects on ombrotrophic (raised) bogs
  4.2.2.2 Effects on mesotrophic fens
  4.2.2.3 Effects on fresh- and saltwater marshes
4.2.3 Effects on species-rich grasslands
  4.2.3.1 Effects of nitrogen on calcareous grasslands
  4.2.3.2 Critical loads for nitrogen in calcareous grasslands
  4.2.3.3 Comparison with other semi-natural grasslands
4.2.4 Effects on heathlands
  4.2.4.1 Effects on inland dry heathlands
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.4.2</td>
<td>Effects of nitrogen on inland wet heathlands</td>
<td>167</td>
</tr>
<tr>
<td>4.2.4.3</td>
<td>Effects of nitrogen on arctic and alpine heathlands</td>
<td>168</td>
</tr>
<tr>
<td>4.2.4.4</td>
<td>Effects on herbs of matgrass swards</td>
<td>170</td>
</tr>
<tr>
<td>4.2.5</td>
<td>Effects of nitrogen deposition on forests</td>
<td>172</td>
</tr>
<tr>
<td>4.2.5.1</td>
<td>Effects on forest tree species</td>
<td>172</td>
</tr>
<tr>
<td>4.2.5.2</td>
<td>Effects on tree epiphytes, ground vegetation and ground fauna of forests</td>
<td>180</td>
</tr>
<tr>
<td>4.2.6</td>
<td>Effects on estuarine and marine ecosystems</td>
<td>186</td>
</tr>
<tr>
<td>4.2.7</td>
<td>Appraisal and conclusions</td>
<td>190</td>
</tr>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>193</td>
</tr>
<tr>
<td>5.2</td>
<td>Nitrogen dioxide</td>
<td>193</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Dosimetry</td>
<td>193</td>
</tr>
<tr>
<td>5.2.1.1</td>
<td>Respiratory tract dosimetry</td>
<td>194</td>
</tr>
<tr>
<td>5.2.1.2</td>
<td>Systemic dosimetry</td>
<td>195</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Respiratory tract effects</td>
<td>195</td>
</tr>
<tr>
<td>5.2.2.1</td>
<td>Host defence mechanisms</td>
<td>195</td>
</tr>
<tr>
<td>5.2.2.2</td>
<td>Lung biochemistry</td>
<td>222</td>
</tr>
<tr>
<td>5.2.2.3</td>
<td>Pulmonary function</td>
<td>235</td>
</tr>
<tr>
<td>5.2.2.4</td>
<td>Morphological studies</td>
<td>238</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Genotoxicity, potential carcinogenic or co-carcinogenic effects</td>
<td>257</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Extrapulmonary effects</td>
<td>264</td>
</tr>
<tr>
<td>5.3</td>
<td>Effects of mixtures containing nitrogen dioxide</td>
<td>267</td>
</tr>
<tr>
<td>5.4</td>
<td>Effects of other nitrogen oxide compounds</td>
<td>270</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Nitric oxide</td>
<td>270</td>
</tr>
<tr>
<td>5.4.1.1</td>
<td>Endogenous formation of NO</td>
<td>270</td>
</tr>
<tr>
<td>5.4.1.2</td>
<td>Absorption of NO</td>
<td>271</td>
</tr>
<tr>
<td>5.4.1.3</td>
<td>Effects of NO on pulmonary function, morphology and host lung defence function</td>
<td>271</td>
</tr>
<tr>
<td>5.4.1.4</td>
<td>Metabolic effects</td>
<td>274</td>
</tr>
<tr>
<td>5.4.1.5</td>
<td>Haematological changes</td>
<td>275</td>
</tr>
<tr>
<td>5.4.1.6</td>
<td>Biochemical mechanisms for nitric oxide effects: reaction with iron and effects on enzymes and nucleic acids</td>
<td>275</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Nitric acid</td>
<td>276</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Nitrates</td>
<td>277</td>
</tr>
</tbody>
</table>
5.5 Summary of studies of the effects of nitrogen compounds on experimental animals 277

6. CONTROLLED HUMAN EXPOSURE STUDIES OF NITROGEN OXIDES 281

6.1 Introduction 281
6.2 Effects of nitrogen dioxide 282
   6.2.1 Nitrogen dioxide effects on pulmonary function and airway responsiveness to bronchoconstrictive agents 282
   6.2.1.1 Nitrogen dioxide effects in healthy subjects 283
   6.2.1.2 Nitrogen dioxide effects on asthmatics 292
   6.2.1.3 Nitrogen dioxide effects on patients with chronic obstructive pulmonary disease 306
   6.2.1.4 Age-related differential susceptibility 309
   6.2.2 Nitrogen dioxide effects on pulmonary host defences and bronchoalveolar lavage fluid biomarkers 309
   6.2.3 Other classes of nitrogen dioxide effects 316
6.3 Effects of other nitrogen oxide compounds 317
6.4 Effects of nitrogen dioxide/gas or gas/aerosol mixtures on lung function 322
6.5 Summary of controlled human exposure studies of oxides of nitrogen 329

7. EPIDEMIOLOGICAL STUDIES OF NITROGEN OXIDES 333

7.1 Introduction 333
7.2 Methodological considerations 333
   7.2.1 Measurement error 333
   7.2.2 Misclassification of the health outcome 334
   7.2.3 Adjustment for covariates 335
   7.2.4 Selection bias 336
   7.2.5 Internal consistency 336
   7.2.6 Plausibility of the effect 336
7.3 Studies of respiratory illness 337
   7.3.1 Indoor air studies 337
      7.3.1.1 St Thomas' Hospital Medical School Studies (United Kingdom) 337
7.3.1.2 Harvard University - Six Cities Studies (USA) 344
7.3.1.3 University of Iowa Study (USA) 349
7.3.1.4 Agricultural University of Wageningen (The Netherlands) 349
7.3.1.5 Ohio State University Study (USA) 351
7.3.1.6 University of Dundee (United Kingdom) 353
7.3.1.7 Harvard University - Chestnut Ridge Study (USA) 354
7.3.1.8 University of New Mexico Study (USA) 355
7.3.1.9 University of Basel Study (Switzerland) 356
7.3.1.10 Yale University Study (USA) 359
7.3.1.11 Freiburg University Study (Germany) 360
7.3.1.12 McGill University Study (Canada) 360
7.3.1.13 Health and Welfare Canada Study (Canada) 361
7.3.1.14 University of North Carolina Study (USA) 361
7.3.1.15 University of Tucson Study (USA) 362
7.3.1.16 Hong Kong Anti-Cancer Society Study (Hong Kong) 362
7.3.1.17 Recent studies 362

7.3.2 Outdoor studies 363
7.3.2.1 Harvard University - Six City Studies (USA) 363
7.3.2.2 University of Basel Study (Switzerland) 365
7.3.2.3 University of Wuppertal Studies (Germany) 367
7.3.2.4 University of Tubigen (Germany) 367
7.3.2.5 Harvard University - Chestnut Ridge Study (USA) 367
7.3.2.6 University of Helsinki Studies (Finland) 368
7.3.2.7 Helsinki City Health Department Study (Finland) 368
7.3.2.8 Oulu University Study (Finland) 369
7.3.2.9 Seth GS Medical College Study (India) 369

7.4 Pulmonary function studies 369
7.4.1 Harvard University - Six City Studies (USA) 369
7.4.2 National Health and Nutrition Examination Survey Study (USA) 370
7.4.3 Harvard University - Chestnut Ridge Study (USA) 371
7.4.4 Other pulmonary function studies 371
7.5 Other exposure settings 372
7.5.1 Skating rink exposures 372
7.6 Occupational exposures 373
7.7 Synthesis of the evidence for school-age children 375
7.7.1 Health outcome measures 375
7.7.2 Biologically plausible hypothesis 380
7.7.3 Publication bias 381
7.7.4 Selection of studies 381
7.7.4.1 Brief description of selected studies 382
7.7.4.2 Studies not selected for quantitative analysis 384
7.7.5 Quantitative analysis 385
7.8 Synthesis of the evidence for young children 391
7.9 Summary 393

8. EVALUATION OF HEALTH AND ENVIRONMENT RISKS ASSOCIATED WITH NITROGEN OXIDES 397
8.1 Sources and exposure 397
8.2 Evaluation of the effects of atmospheric nitrogen species on the environment 398
8.2.1 Guidance values - critical levels for air concentrations of nitrogen oxides 400
8.2.2 Environment-based guidance values - critical loads for total nitrogen deposition 400
8.3 Evaluation of health risks associated with nitrogen oxides 402
8.3.1 Concentration-response relationships 402
8.3.2 Subpopulations potentially at risk 407
8.3.3 Derivation of health-based guidance values 409

9. CONCLUSIONS AND RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH AND THE ENVIRONMENT 412

10. FURTHER RESEARCH 414
NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Chatelaine, Geneva, Switzerland (Telephone No. 9799111).
Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental
effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe every study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are only used when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and in vitro studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.
Content

The layout of EHC monographs for chemicals is outlined below.

- Summary – a review of the salient facts and the risk evaluation of the chemical
- Identity – physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and in vitro test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in: Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e., the substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.
Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. While observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson.
**EHCPREPARATION FLOW CHART**

- **Commitment to draft EHCP**

  - **Revision as necessary**

  - **Document preparation initiated**

  - **Draft sent to IPCS Responsible Officer (RO)**

  - **Responsible Officer, Editor, check for coherence of text and readability; preliminary reference cross-check**

  - **1st Draft**

  - **International circulation to Contact Points (150+)**

  - **Comments to IPCS (RO)**

  - **Review of comments, reference cross-check, preparation of Task Group (TG) draft**

  - **Editor**

  - **Task Group meeting**

  - **Insertion of TG changes**

  - **Post-TG draft, detailed reference cross-check**

  - **Publishing**

  - **Graphics**

  - **French/Spanish translations of Summary/Editorials**

  - **Library for CIP Data**

  - **Approval by Director, IPCS**

  - **WHO Publication Office**

  - **Printer**

  - **Proofs**

  - **Publication**

---

*Note: Diagram shows the flow of document preparation and approval, with various stages involving consultation, review, and final approval before publication.*
Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet in camera.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking, and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.
WHO TASK GROUP ON ENVIRONMENTAL HEALTH
CRITERIA FOR NITROGEN OXIDES

Members

Dr K. Bentley*, Health and Environment Policy Section,
Department of Community Services and Health, Canberra
ACT, Australia

Dr S. Dobson, Institute of Terrestrial Ecology, Monks Wood
Experimental Station, Abbots Ripton, Huntingdon,
Cambridgeshire, United Kingdom

Dr L. van der Eerden, Centre “De Bom” Wageningen, The
Netherlands

Dr L. Folinsbee, Health Effects Research Laboratory, US
Environmental Protection Agency, Research Triangle Park,
North Carolina, USA (Rapporteur)

Dr L. Grant*, National Center for Environmental Assessment,
US Environmental Protection Agency, Research Triangle
Park, North Carolina, USA

Mr L. Heiskanen, Health and Environment Policy Section,
Department of Community Services and Health, Canberra
ACT, Australia

Mr G.M. Johnson, CSIRO, Division of Coal and Energy
Technology, Centre for Pollution Assessment and Control,
North Ryde, NSW, Australia

Dr J. Kagawa, Professor of Hygiene and Public Health, Tokyo
Women’s Medical College, Shinjuku-ku, Tokyo, Japan

Dr R.R. Khan, Ministry of Environment and Forests,
Paryavaran Bhawan, New Delhi, India

Dr D.B. Menzel, University of California, Department of
Community & Environment and Medicine, California, USA

* Invited, but unable to attend
Dr L. Neas, Department of Environmental Health, Environmental Epidemiology Program, Harvard School of Public Health, Boston, Massachusetts, USA

Dr S.E. Paulson, Department of Atmospheric Sciences, University of California, Los Angeles, California, USA

Dr P.J.A. Rombout, Department for Inhalation Toxicology, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands (Chairman)

Dr W. Tyler, Veterinary Anatomy and Cell Biology, University of California, California, USA

Dr K. Victorin, Karolinska Institute, Institute of Environmental Medicine, Stockholm, Sweden

Dr A. Woodward, Department of Community Medicine, University of Adelaide, Adelaide, Australia

Dr R. Ye, Deputy Director, National Environmental Protection Agency, Xizhimennai Nanziaojie, Beijing, People's Republic of China

Observers

Professor M. Moore, National Research Centre for Environmental Toxicology, Nathan, Australia

Dr M. Pain, Department of Thoracic Medicine, Royal Melbourne Hospital, Melbourne VIC, Australia

Dr P. Psaila-Savona, WA Department of Health, Perth WA, Australia

Mr B. Taylor, Policy and Planning Group, Public and Planning Group, Public Health Commission, Wellington, New Zealand

Mr B. Saxby, AGL Gas Companies, North Sydney NSW, New Zealand
Secretariat

Dr B.H. Chen, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (Secretary)

Dr M. Younes, WHO European Centre for Environment & Health, Bilthoven, The Netherlands
ENVIRONMENTAL HEALTH CRITERIA FOR
NITROGEN OXIDES

A WHO Task Group on Environmental Health Criteria for Nitrogen Oxides met in Melbourne, Australia from 14 to 18 November 1994. The meeting was hosted by the Clean Air Society of Australia and New Zealand and the Victorian Departments of Health and Environment, Australia. Dr B.H. Chen, IPCS, opened the meeting and welcomed the participants on behalf of the Director, IPCS, and the three IPCS cooperating organizations (UNEP/ILO/WHO). The Task Group reviewed and revised the draft criteria monograph and made an evaluation of the risks for human health and the environment from exposure to nitrogen oxides.

The first draft of this monograph was prepared by Drs J.A. Graham, L.D. Grant, L.J. Folinebee, D.J. Kotchmar and J.H.B. Garner, US EPA. Drs W.G. Ewald, T.B. McMullen and B.E. Tilton, US EPA, contributed to the preparation of the first draft. The second draft was prepared by Dr L.D. Grant incorporating comments received following the circulation of the first draft to the IPCS Contact Points for Environmental Health Criteria. Drs R. Bobbink, L. Van der Eerden and S. Dobson prepared the final text of the environmental section. Mr G.M. Johnson contributed to the final text of the chemistry section.

Dr B.H. Chen and Dr P.G. Jenkins, both members of the IPCS Central Unit, were responsible for the overall scientific content and technical editing, respectively.

The efforts of all who helped in the preparation and finalization of the document are gratefully acknowledged.

Financial support for this Task Group meeting was provided by the Department of Community Services and Health, Australia, Victorian Departments of Health and Environment, Australia, and the Clean Air Society of Australia and New Zealand.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AM</td>
<td>alveolar macrophages</td>
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<tr>
<td>AQG</td>
<td>Air Quality Guidelines</td>
</tr>
<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
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<tr>
<td>BHPN</td>
<td>N-bis (2-hydroxypropyl) nitrosamine</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CLM</td>
<td>chemiluminescence method</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<tr>
<td>ECD</td>
<td>electron capture detection</td>
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<tr>
<td>FEF</td>
<td>forced expiratory flow</td>
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<tr>
<td>FEV</td>
<td>forced expiratory volume</td>
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<tr>
<td>FTIR</td>
<td>Fourier transformed infrared</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GDH</td>
<td>glutamate dehydrogenase</td>
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<tr>
<td>(c)GMP</td>
<td>(cyclic) guanosine monophosphate</td>
</tr>
<tr>
<td>GS</td>
<td>glutamine synthetase</td>
</tr>
<tr>
<td>HNO₂</td>
<td>nitrous acid</td>
</tr>
<tr>
<td>HNO₃</td>
<td>nitric acid</td>
</tr>
<tr>
<td>LIF</td>
<td>laser-induced fluorescence</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>N₂</td>
<td>nitrogen (elemental)</td>
</tr>
<tr>
<td>NH₃</td>
<td>ammonia</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>ammonium ion</td>
</tr>
<tr>
<td>NH₃</td>
<td>the sum of NH₃ and NH₄⁺</td>
</tr>
<tr>
<td>NiR</td>
<td>nitrate reductase</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NO₂</td>
<td>nitrogen dioxide</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>nitrite ion</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>nitrate ion</td>
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EHC 188: Nitrogen Oxides

N₂O  nitrous oxide
N₂O₅ nitrogen pentoxide
NO₂  nitric oxide plus nitrogen dioxide
NOₓ  gas-phase oxidized nitrogen species (except nitrous oxide)
NPSH  non-protein sulfhydryl
NR  nitrate reductase
O₃  ozone
PAN  peroxycetyl nitrate
PBzN peroxycbenzoxy nitrate
PEF  peak expiratory flow
PFC  plaque-forming cell
PMN  polymorphonuclear leukocyte
ppb  parts per billion (10⁻⁹)
ppm  parts per million (10⁻⁶)
ppt  parts per trillion (10⁻¹²)
ppbv  parts per trillion (by volume)
PSD  passive sampling device
Rₐw  airway resistance
ROC  reactive organic carbon
RUBISCO ribulose 1,5-biphosphate carboxylase
SD  standard deviation
SES  socioeconomic status
SGₐw  specific airway conductance
SO₂  sulfur dioxide
SOₓ  sulfur oxides
SPM  suspended particulate matter
SRₐw  specific airway resistance
TDLAS  tuneable diode laser absorption spectrometry
TSP  total suspended particulate
VOC  volatile organic carbon
1. SUMMARY

1.1 Nitrogen oxides and related compounds

Nitrogen oxides can be present at significant concentrations in ambient air and in indoor air. The types and concentrations of nitrogenous compounds present may vary greatly from location to location, with time of day, and with season. The main sources of nitrogen oxide emissions are combustion processes. Fossil fuel power stations, motor vehicles and domestic combustion appliances emit nitrogen oxides, mostly in the form of nitric oxide (NO) and some (usually less than about 10%) in the form of nitrogen dioxide (NO₂). In the air, chemical reactions occur that oxidize NO to NO₂ and other products. There are also biological processes that liberate nitrogen species from soils, including nitrous oxide (N₂O). Emissions of N₂O can cause perturbation of the stratospheric ozone layer.

Human health may be affected when significant concentrations of NO₂ or other nitrogenous species, such as peroxyacetyl nitrate (PAN), nitric acid (HNO₃), nitrous acid (HNO₂), and nitrated organic compounds, are present. In addition, nitrates and HNO₃ may cause health effects and significant effects on ecosystems when deposited on the ground.

The sum of NO and NO₂ is generally referred to as NOₓ. Once released into the air, NO is oxidized to NO₂ by available oxidants (particularly ozone, O₃). This happens rapidly under some conditions in outdoor air; in indoor air, it is generally a much slower process. Nitrogen oxides are a controlling precursor of photochemical oxidant air pollution resulting in ozone and smog formation; interactions of nitrogen oxides (except N₂O) with reactive organic compounds and sunlight form ozone in the troposphere and smog in urban areas.

NO and NO₂ may also undergo reactions to form a range of other oxides of nitrogen, both in indoor and outdoor air, including HNO₂, HNO₃, nitrogen trioxide (NO₃), dinitrogen pentoxide (N₂O₅), PAN and other organic nitrates. The complex range of gas-phase nitrogen oxides is referred to as NOₓ. The partitioning of oxides of nitrogen among these compounds is strongly dependent on the concentrations of other oxidants and on the meteorological history of the air.
HNO$_3$ is formed from the reaction of OH and NO$_2$. It is a major sink for active nitrogen and also a contributor to acidic deposition. Potential physical and chemical sinks for HNO$_3$ include wet and dry deposition, photolysis, reaction with OH radicals, and reaction with gaseous ammonia to form ammonium nitrate aerosol.

PANs are formed from the combination of organic peroxy radicals with NO$_2$. PAN is the most abundant organic nitrate in the troposphere and can serve as a temporary reservoir for reactive nitrogen, which may be regionally transported.

The NO$_3$ radical, a short-lived NO species that is formed in the troposphere primarily by the reaction of NO$_2$ with O$_3$, undergoes rapid photolysis in daylight or reaction with NO. Appreciable concentrations are observed during the night.

N$_2$O$_5$ is primarily a night-time constituent of ambient air as it is formed from the reaction of NO$_3$ and NO$_2$. In ambient air, N$_2$O$_5$ reacts heterogeneously with water to form HNO$_3$, which in turn is deposited.

N$_2$O is ubiquitous because it is a product of natural biological processes in soil. It is not known, however, to be involved in any reactions in the troposphere. N$_2$O participates in upper atmospheric reactions contributing to stratospheric ozone (O$_3$) depletion and is also a relatively potent greenhouse gas that contributes to global warming.

1.1.1 Atmospheric transport

The transport and dispersion of the various nitrogenous species in the lower troposphere is dependent on both meteorological and chemical parameters. Advection, diffusion and chemical transformations combine to dictate the atmospheric residence times. In turn, atmospheric residence times help determine the geographic extent of transport of given species. Surface emissions are dispersed vertically and horizontally through the atmosphere by turbulent mixing processes that are dependent to a large extent on the vertical temperature structure and wind speed.

As the result of meteorological processes, NO$_x$ emitted in the early morning hours in an urban area typically disperses vertically and moves downwind as the day progresses. On sunny summer days, most of the NO$_x$ will have been converted to HNO$_3$ and PAN.
by sunset, with concomitant formation of ozone. Much of the HNO₃ is removed by deposition as the air mass is transported, but HNO₃ and PAN carried in layers aloft (above the nighttime inversion layer but below a higher subsidence inversion) can potentially be transported long distances in oxidant-laden air masses.

1.1.2 Measurement

There are a number of methods available to measure airborne nitrogen-containing species. This document briefly covers methodologies currently available or in general use for in situ monitoring of airborne concentrations in both ambient and indoor environments. The species considered are NO, NO₂, NOₓ, total reactive odd nitrogen (NOₓ), PAN and other organic nitrates, HNO₃, HNO₂, N₂O, the nitrate radical, NO₃⁻, and N₂O.

Measuring concentrations of nitrogen oxides is not trivial. While a straightforward, widely available method exists for measuring NO (the chemiluminescent reaction with ozone), this is an exception for nitrogen oxides. Chemiluminescence is also the most common technique used for NOₓ. NO₂ is first reduced to NO. Unfortunately, the catalyst typically used for the reduction is not specific, and has various conversion efficiencies for other oxidized nitrogen compounds. For this reason, great care must be taken in interpreting the results of the common chemiluminescence analyser in terms of NO₂, as the signal may include many other compounds. Additional difficulties arise from nitrogen oxides that may partition between the gaseous and particulate phases both in the atmosphere and in the sampling procedure.

1.1.3 Exposure

Human and environmental exposure to nitrogen oxides varies greatly from indoors to outdoors, from cities to the countryside, and with time of day and season. The concentrations of NO and NO₂ typically present outdoors in a range of urban situations are relatively well established. The concentrations encountered indoors depend on the specific details of the nature of combustion appliances, chimneys and ventilation. When unvented combustion appliances are used for cooking or heating, indoor concentrations of nitrogen oxides typically greatly exceed those existing outside. Recent research has shown in these circumstances that HNO₂ can reach significant concentrations. One report showed that HNO₂ can represent over 10% of the concentrations usually reported as NO₂.
1.2 Effects of atmospheric nitrogen species, particularly nitrogen oxides, on vegetation

Most of earth's biodiversity is found in (semi-)natural ecosystems, both in aquatic and terrestrial habitats. Nitrogen is the limiting nutrient for plant growth in many (semi-)natural ecosystems. Most of the plant species from these habitats are adapted to nutrient-poor conditions, and can only compete successfully on soils with low nitrogen levels.

Human activities, both industrial and agricultural, have greatly increased the amount of biologically available nitrogen compounds, thereby disturbing the natural nitrogen cycle. Various forms of nitrogen pollute the air: mainly NO, NO$_2$ and ammonia (NH$_3$) as dry deposition; and nitrate (NO$_3$) and ammonium (NH$_4^+$) as wet deposition. NH refers to the sum of NH$_3$ and NH$_4^+$. Another contribution is from occult deposition (fog and clouds). There are many more nitrogen-containing air pollutants (e.g., N$_2$O, PAN, N$_2$O, amines), but these are neglected here, either because their contribution to the total nitrogen deposition is believed to be small, or because their concentrations are probably far below effect thresholds.

Nitrogen-containing air pollutants can affect vegetation indirectly, via photochemical reaction products, or directly after being deposited on vegetation, soil or water surface. The indirect pathway is largely neglected here although it includes very relevant processes, and should be taken into account when evaluating the entire impact of nitrogen-containing air pollutants: NO$_2$ is a precursor for tropospheric O$_3$, which acts both as a phytotoxin and a greenhouse gas.

The impacts of increased nitrogen deposition upon biological systems can be the result of direct uptake by foliage or uptake via the soil. At the level of individual plants, the most relevant effects are injury to the tissue, changes in biomass production and increased susceptibility to secondary stress factors. At the vegetation level, deposited nitrogen acts as a nutrient; this results in changes in competitive relationships between species and loss of biodiversity. The critical loads for nitrogen depend on (i) the type of ecosystem; (ii) the land use and management in the past and present; and (iii) the abiotic conditions (especially those that influence the nitrification potential and immobilization rate in the soil).
Adsorption on the outer surface of the leaves takes place and may damage wax layers of the cuticle, but the quantitative relevance for the field situation has not yet been proved. Uptake of NO and NH is driven by the concentration gradient between atmosphere and mesophyll. It generally, but not always, is directly determined by stomatal conductance and thus depends on factors influencing stomatal aperture. There is increasing evidence that foliar uptake of nitrogen reduces the uptake of nitrogen by the roots. Uptake and exchange of ions through the leaf surface is a relatively slow process, and thus is only relevant if the surface remains wet for longer periods.

NO is only slightly soluble in water, but the presence of other substances can alter the solubility. NO has a higher solubility, while that of NH is much higher. NO (the primary reaction product of NO), NH and NH are all highly phytotoxic, and could well be the cause of adverse effects of nitrogen-containing air pollutants. The free radical N=O may play a role in the phytotoxicity of NO.

More-than-additive effects (synergism) have been found in nearly all studies concerning SO plus NO. With other NO mixtures (NO, O, and CO), interactive effects are the exception rather than the rule.

When climatic conditions and supply of other nutrients allow biomass production, both NO and NH result in growth stimulation at low concentrations and growth reduction at higher concentrations. However, the exposure level at which growth stimulation turns into growth inhibition is much lower for NO than for NH.

Evidence exists that plants are more sensitive at low light intensity (e.g., at night and in winter) and at low temperatures (just above 0 °C). NO and NH can increase the sensitivity of plants to frost, drought, wind and insect damage.

An interaction exists between soil chemistry and sensitivity of vegetation to nitrogen deposition; this is related to pH and nitrogen availability.

The relative contribution of NO and NO to the NO effect on plants is unclear. The vast majority of information is on effects of NO, but available information on NO suggests that NO and NO have comparable phytotoxic effects.
Air quality guidelines refer to thresholds for adverse effects. Two different types of effect thresholds exist: critical levels (CLEs) and critical loads (CLOs). The critical level is defined as the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge. The critical load is defined as a quantitative estimate of an exposure (deposition) to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge.

According to current practice, critical levels have been derived from assessment of the lowest exposure concentrations causing adverse effects on physiology or growth of plants (biochemical effects were excluded), using a graphical method.

To include the impact of NO, a critical level for NO\(_2\) is proposed instead of one for NO\(_2\) for this purpose it has been assumed that NO and NO\(_2\) act in an additive manner. A strong case can be made for the provision of critical levels for short-term exposure. However, currently there are insufficient data to provide these with sufficient confidence. Current evidence suggests a critical level of about 75 pg/m\(^3\) for NO\(_2\) as a 24-h mean.

The critical level for NO\(_2\) (NO and NO\(_2\) added in ppb and expressed as NO\(_2\) in pg/m\(^3\)) is considered to be 30 pg/m\(^3\) as an annual mean.

Information on organisms in the environment is almost exclusively restricted to plants, with minimum data on soil fauna. This evaluation and guidance values are, therefore, expressed in terms of nitrogen species effects on vegetation. However, it is expected that plants will form the most sensitive component of natural systems and that the effect on biodiversity of plant communities is a sensitive indicator of effects on the whole ecosystem.

Critical loads are derived from empirical data and steady-state soil models. Estimated critical loads for total nitrogen deposition in a variety of natural aquatic and terrestrial ecosystems are given. Possible differential effects of deposited nitrogen species (NO\(_2\) and NH\(_3\)) are insufficiently known to differentiate between nitrogen species for critical load estimation.

The great majority of ecosystems for which there is sufficient information to estimate critical loads are from temperate climates.
The few arctic and montane ecosystems included, which might be expected to be representative of higher latitudes, have the least reliable basis. There is no information on tropical ecosystems and little on estuarine or marine ecosystems in any climatic zone. Nutrient-poor tropical ecosystems such as rain forests and mangrove swamps are likely to be adversely affected by nitrogen deposition. The lack of both deposition data and effect thresholds make it impossible to make risk assessments for these climatic regions.

The most sensitive ecosystems (ombrotrophic bogs, shallow soft-water lakes and arctic and alpine heaths) for which effects thresholds can be estimated show critical loads of 5-10 kg N ha\(^{-1}\) year\(^{-1}\) based on decreased biological diversity in plant communities. A more average value for the limited range of ecosystems studied is 15-20 kg N ha\(^{-1}\) year\(^{-1}\), which applies to forest trees.

The atmospheric chemistry of nitrogen oxides includes the capacity for ozone generation in the troposphere, ozone depletion in the stratosphere, and contribution to global warming as greenhouse gases. Nitrogen oxides and ammonia contribute to soil acidification (along with sulfur oxides) and thereby to increased bioavailability of aluminium.

The phytotoxic effects of nitrogen oxides on plants have little direct relevance to crop plants when concentrations marginally exceed the critical level. However, the role of NO\(_2\) in the generation of ozone and other phytotoxic substances, e.g., organic nitrates leads to crop loss. Nitrogen deposited on growing crops will represent a very small increase in total available nitrogen compared to that added as fertilizer.

1.3 Health effects of exposures to nitrogen dioxide

A large number of studies designed to evaluate the health effects of NO\(_2\) have been conducted. Of the NO\(_x\) compounds, NO\(_2\) has been most studied. The discussion in this section focuses on NO\(_2\), NO, HNO\(_2\) and HNO\(_3\), while nitrates are mentioned briefly.

1.3.1 Studies of the effects of nitrogen compounds on experimental animals

Extrapolating animal data to humans has both qualitative and quantitative components. As summarized below, NO\(_2\) causes a constellation of effects in several animal species; most notably,
effects on host defence against infectious pulmonary disease, lung metabolism/biochemistry, lung function and lung structure. Because of basic physiological, metabolic and structural similarities in all mammals (laboratory animals and humans), the commonality of the observations in several animal species leads to a reasonable conclusion that NO₂ could cause similar types of effects in humans. However, because of the differences between mammalian species, exactly what exposures would actually cause these effects in humans is not yet known. That is the topic of quantitative extrapolation. Limited modelling research on the dosimetric aspect (i.e., the dose to the target tissue/cell that actually causes toxicity) of quantitative extrapolation suggests that the distribution of the deposition of NO₂ within the respiratory tract of animals and humans is similar, without yet providing adequate values to use for animal-to-human extrapolation. Unfortunately, very little information is available on the other key aspect of extrapolation, species sensitivity (i.e., the response of the tissues of different species to a given dose). Thus, from currently available animal studies, we know which human health effects NO₂ may cause. We are unable to assert with great confidence the effects that are actually caused by a given inhaled dose of NO₂.

With the above issues in mind, the animal toxicology database for NO₂ is summarized below according to major classes of effects and topics of special interest. Although it is clear that the effects of NO₂ exposure extend beyond the confines of the lung, the interpretation of these systemic effects relative to potential human risk is not clear. Therefore they are not summarized further here, but are discussed in later chapters. Although interactions of NO₂ and other co-occurring pollutants, such as O₃ and sulfuric acid (H₂SO₄), can be quite important, especially if synergism occurs, the database does not yet allow conclusions that enable assessment of real-world potential interactions.

1.3.1.1 Biochemical and cellular mechanisms of action of nitrogen oxides

NO₂ acts as a strong oxidant. Unsaturated lipids are readily oxidized with peroxides as the dominant product. Both ascorbic acid (vitamin C) and α-tocopherol (vitamin E) inhibit the peroxidation of unsaturated lipids. When ascorbic acid is sealed within bilayer liposomes, NO₂ rapidly oxidizes the sealed ascorbic acid. The protective effects of α-tocopherol and ascorbic acid in animals and humans are due to the inhibition of NO₂ oxidation. NO₂ also oxidizes membrane proteins. The oxidation of either membrane lipids or proteins results in the loss of cell
permeability control. The lungs of NO₂-exposed humans and experimental animals have larger amounts of protein within the lumen. The recruitment of inflammatory cells and the changes in the lung are due to these events.

The oxidant properties of NO₂ also induce the peroxide detoxification pathway of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase. Following NO₂ exposure the increase in the peroxide detoxification pathway in animals follows an exposure-response relationship.

The mechanism of action of NO is less clear. NO is readily oxidized to NO₂ and peroxidation then occurs. Because of the concurrent exposure to some NO₂ in NO exposures, it is difficult to discriminate NO effects from NO₂. NO functions as an intracellular second messenger modulating a wide variety of essential enzymes, and it inhibits its own production (e.g., negative feedback). NO activates guanylate cyclase which in turn increases intracellular cGMP levels. A possible mechanism of action of nitrates may be through the release of histamine from mast cell granules. Acidic nitrogenous air pollutants, particularly HNO₃, may act by alteration of intracellular pH.

PAN decomposes in water, generating hydrogen peroxide. Little is known of the mechanism of action, but oxidative stress is likely for PAN and its congeners.

Inorganic nitrates may act through alterations in intracellular pH. Nitrate ion is transported into alveolar type 2 cells acidifying the cell. Nitrate also mobilizes histamine from mast cells. HNO₂ could also act to alter intracellular pH, but this mechanism is unclear.

The mechanisms of action of the other nitrogen oxides are unknown.

Acute exposure to NO₂ at a concentration of 750 µg/m³ (0.4 ppm) can result in lipid peroxidation. NO₂ can oxidize polyunsaturated fatty acids in cell membranes as well as functional groups of proteins (either soluble proteins in the cell, such as enzymes, or structural proteins, such as components of cell membranes). Such oxidation reactions (mediated by free radicals) are a mechanism by which NO₂ exerts direct toxicity on lung cells. This mechanism of action is supported by animal studies showing the importance of lung antioxidant defences, both endogenous
(e.g., maintenance of lung glutathione levels) and exogenous (e.g.,
dietary vitamins C and E), in protecting against the effects of
NO₂. Many studies have suggested that various enzymes in the
lung, including glutathione peroxidase, superoxide dismutase and
catalase, may also serve to defend the lung against oxidant attack.

1.3.1.2 Effects on host defence

Although the primary function of the respiratory tract is to
ensure an efficient exchange of gases, this organ system also
provides the body with a first line of defence against inhaled
viable and non-viable airborne agents. An extensive database
clearly shows that exposure to NO₂ can result in the dysfunction
of these host defences, increasing susceptibility to infectious
respiratory disease. The host-defence parameters affected by NO₂
include the functional and biochemical activity of cells in lungs,
alveolar macrophages (AMs), immunological competence, suscep-
tibility to experimentally induced respiratory infections, and the
rate of mucociliary clearance.

Alveolar macrophages are affected by NO₂. These cells are
responsible for maintaining the sterility of the pulmonary region,
clearing particles from this region, and participating in
immunological functions. Functional changes that have been
reported include the following: the suppression of phagocytic
ability and stimulation of lung clearance at 560 μg/m³ (0.3 ppm)
2 h/day for 13 days; a decrease in bactericidal activity at
4320 μg/m³ (2.3 ppm) for 17 h; and a decreased response to
migration inhibition factor at 3760 μg/m³ (2.0 ppm) 8 h/day,
5 days/week for 6 months. The morphological appearance of these
defence cells changes after chronic exposure to NO₂.

The importance of host defences becomes evident when
animals have to cope with laboratory-induced pulmonary
infections. Animals exposed to NO₂ succumb to bacterial or viral
infection in a concentration-dependent manner. Mortality also
increases with increased NO₂ concentration or duration of
exposure. After acute exposure, effects are observed at
concentrations as low as 3760 μg/m³ (2 ppm). Exposure to
concentrations as low as 940 μg/m³ (0.5 ppm) will cause effects in
the infectivity model after 6 months.

Both humoral and cell-mediated defence systems are changed
by NO₂ exposure. In the cases in which the immune system has
been investigated, effects have been observed after short-term
exposure to concentrations $\geq 9400$ pg/m$^3$ (5 ppm). The effects are complex since the direction of the change (i.e., increase or decrease) is dependent upon NO$_2$ concentration and the length of exposure.

1.3.1.3 Effects of chronic exposure on the development of chronic lung disease

Humans are chronically exposed to NO$_2$. Therefore, such exposures in animals have been studied rather extensively, typically using morphological and/or morphometric methods. This research has generally shown that a variety of pulmonary structural and correlated functional alterations occur. Some of these changes may be reversible when exposure ceases.

Pulmonary function may be altered following chronic NO$_2$ exposure of experimental animals. Impaired gas exchange occurred following exposure to 7520 pg/m$^3$ (4.0 ppm) NO$_2$ for four months and this was reflected in decreased arterial O$_2$ tension, impaired physical performance and increased anaerobic metabolism.

Although NO$_2$ produces morphological changes in the respiratory tract, the database is sometimes confusing due to quantitative and qualitative variability in responsiveness between, and even within, species. The rat, the most commonly used experimental animal in morphological assessments of exposure, appears to be relatively resistant to NO$_2$. Short-term exposures to concentrations of 9400 pg/m$^3$ (5.0 ppm) or less generally have little effect in the rat, where similar exposures in the guinea-pig may result in some centriacinar epithelial damage.

Longer-term exposures result in lesions in some species with concentrations as low as 560 to 940 pg/m$^3$ (0.3 to 0.5 ppm). These are characterized by epithelial remodelling similar to that described above, but with the involvement of more proximal airways and thickening of the interstitium. Many of these changes, however, will resolve even with continued exposure, and long-term exposures to levels above about 3760 pg/m$^3$ (2.0 ppm) are required for more extensive and permanent changes in the lungs. Some effects are relatively persistent (e.g., bronchiolitis), whereas others tend to be reversible and limited even with continued exposure. In any case, it seems that for either short- or long-term exposure, the response is more dependent upon concentration than duration of exposure.
There is substantial evidence that long-term exposure of several species of laboratory animals to high concentrations of NO_2 results in morphological lung lesions. Destruction of alveolar walls, an essential additional criterion for human emphysema, has been reliably reported in lungs from animals in a limited number of studies. The lowest NO_2 concentration for the shortest exposure duration that will result in emphysematous lung lesions cannot be determined from these published studies.

1.3.1.4 Potential carcinogenic or co-carcinogenic effects

NO_2 has been shown to be mutagenic in *Salmonella* bacteria, but was not mutagenic in one study with a mammalian cell culture. Other studies using cell cultures have demonstrated sister chromatid exchanges (SCE) and DNA single strand breaks. No genotoxic effects have been demonstrated *in vivo* concerning lymphocytes, spermatocytes or bone marrow cells, but two inhalation studies with high concentrations (50 760 and 56 400 µg/m³, 27 and 30 ppm) for 3 h and 16 h, respectively, have demonstrated such effects in lung cells.

Literature searches revealed no published reports of NO_2 studies using classical whole-animal chronic bioassays for carcinogenesis. Research with mice having spontaneously high tumour rates was equivocal. In one study, NO_2 at 18 800 µg/m³ (10 ppm) slightly enhanced the incidence of lung adenomas in a sensitive strain of mice (A/J). Although several co-carcinogenesis investigations have been undertaken, conclusions are precluded because of problems with methodology and interpretation. Reports on whether NO_2 facilitates the metastasis of tumours to the lung are also inadequate to form conclusions. Other investigations have centred on whether NO_2 could produce nitrates and nitrites that, by reacting with amines in the body, could produce nitrosamines. A few studies suggest that nitrosamines are formed in animals treated with high doses of amines and exposed to NO_2, but other studies have indicated that nitrosamine formation is unlikely.

1.3.1.5 Age susceptibility

Investigations into age dependency are inadequate and results so far are equivocal.

1.3.1.6 Influence of exposure patterns

Several animal toxicological studies have elucidated the relationships between concentration (C) and duration (T) of
exposure, indicating that the relationship is complex. Most of this research has used the infectivity model. Early C x T studies demonstrated that concentration had more impact on mortality than did duration of exposure. An evaluation of the toxicity of NO₂ exposures cannot be delineated by C x T relationships.

1.3.2 Controlled human exposure studies on nitrogen oxides

Human responses to a variety of oxidized nitrogen compounds have been evaluated. By far, the largest database and the one most suitable for risk assessment is that available for controlled exposures to NO₂. The database on human responses to NO, HNO₂ vapour, HNO₃ vapour and inorganic nitrate aerosols is not as extensive. A number of sensitive or potentially sensitive subgroups have been examined, including adolescent and adult asthmatics, older adults, and patients with chronic obstructive pulmonary disease (COPD) and pulmonary hypertension. Exercise during exposure increases the total uptake and alters the distribution of the deposited inhaled material within the lung. The relative proportion of NO₂ deposited in the lower respiratory tract is also increased by exercise. This may increase the effects of the above compounds in people who exercise during exposure.

As is typical with human biological response to inhaled particles and gases, there is variability in the biological response to NO₂. Healthy individuals tend to be less responsive to the effects of NO₂ than individuals with lung disease. Asthmatics are clearly the most responsive group to NO₂ that has been studied to date. Individuals with COPD may be more responsive than healthy individuals, but they have limited capacity to respond to NO₂ and thus quantitative differences between COPD patients and others are difficult to assess. Sufficient information is not available at present to evaluate whether age and sex play a role in the response to NO₂.

Healthy subjects can detect the odour of NO₂, in some cases at concentrations below 188 pg/m³ (0.1 ppm). Generally, NO₂ exposure did not increase respiratory symptoms in any of the subject groups tested.

NO₂ causes decrements in lung function, particularly increased airway resistance in resting healthy subjects at 2-h concentrations as low as 4700 μg/m³ (~2.5 ppm). Available data are insufficient to determine the nature of the concentration-response relationship.
Exposure to \( \text{NO}_2 \) results in increased airway responsiveness to bronchoconstrictive agents in exercising healthy, non-smoking subjects exposed to concentrations as low as 2800 \( \mu \text{g/m}^3 \) (~1.5 ppm) for 1 h or longer.

Exposure of asthmatics to \( \text{NO}_2 \) causes, in some subjects, increased airway responsiveness to a variety of provocative mediators, including cholinergic and histaminergic chemicals, \( \text{SO}_2 \), and cold air. The presence of these responses appears to be influenced by the exposure protocol, particularly whether or not the exposure includes exercise. These responses may begin at concentrations as low as 380 \( \mu \text{g/m}^3 \) (0.2 ppm). A meta-analysis suggests that effects may occur at even lower concentrations. However, an unambiguous concentration-response relationship is observed between 350 to 1150 \( \mu \text{g/m}^3 \) (~0.2 to 0.6 ppm).

The implications of this overall trend are unclear, but increased airway responsiveness could potentially lead to increased response to aeroallergens or temporary exacerbation of asthma, possibly leading to increased medication usage or even increased hospital admissions.

Modest increases in airway resistance may occur in COPD patients from brief exposure (15-60 min) to concentrations of \( \text{NO}_2 \) as low as 2800 \( \mu \text{g/m}^3 \) (~1.5 ppm), and decrements in spirometric measures of lung function (3 to 8% change in FEV1, (forced expiratory volume in 1 second)) may also be observed with longer exposures (3 h) to concentrations as low as 600 \( \mu \text{g/m}^3 \) (~0.3 ppm).

Exposure to \( \text{NO}_2 \) at levels above 2800 \( \mu \text{g/m}^3 \) (~1.5 ppm) may alter the numbers and types of inflammatory cells in the distal airways or alveoli. \( \text{NO}_2 \) may alter the functioning of cells within the lungs and production of mediators that may be important in lung host defences. The constellation of changes in host defences, alterations in lung cells and their activities, and changes in biochemical mediators is consistent with the epidemiological findings of increased host susceptibility associated with \( \text{NO}_2 \) exposure.

In studies on mixtures of \( \text{NO}_2 \) with other pollutants, \( \text{NO}_2 \) has not been observed to increase responses to other co-occurring pollutant(s) beyond that which would be observed for the other pollutant(s) alone. A notable exception is the observation that pre-exposure to \( \text{NO}_2 \) enhanced the ozone-induced change in airway responsiveness in healthy exercising subjects during a
subsequent ozone exposure. This observation suggests the possibility of delayed or persistent responses to NO₂.

Within an NO₂ concentration range that may be of interest with regard to risk evaluation (i.e., 100–600 μg/m³), the characteristics of the concentration–response relationship for acute changes in lung function, airway responsiveness to bronchoconstricting agents or symptoms cannot be determined from the available data.

On the basis of an effect at 400 μg/m³ and the possibility of effects at lower levels, based on a meta analysis, a one-hour average daily maximum NO₂ concentration of 200 μg/m³ (~0.11 ppm) is recommended as a short-term guideline.

NO is acknowledged as an important endogenous second messenger within several organ systems. Inhaled NO concentrations above 6000 μg/m³ (~5 ppm) can cause vasodilation in the pulmonary circulation without affecting the systemic circulation. The lowest effective concentration has not been established. Information on pulmonary function and lung host defences consequent to NO exposure are too limited for any conclusions to be drawn at this time. Relatively high concentrations (> 40 000 μg/m³) have been used in clinical applications for brief periods (~1 h) without reported adverse reactions.

Nitric acid levels in the range of 250–500 μg/m³ (~97–194 ppb) may cause some pulmonary function responses in adolescent asthmatics, but not in healthy adults.

Limited information on HNO₃ suggests that it may cause eye inflammation at 760 μg/m³ (0.40 ppm). There are currently no published data on human pulmonary responses to HNO₃.

Limited data on inorganic nitrates suggest that there are no lung function effects of nitrate aerosols at concentrations of 7000 μg/m³ or less.

13.3 Epidemiology studies on nitrogen dioxide

Epidemiological studies on the health effects of nitrogen oxides have mainly focused on NO₂. Many indoor and outdoor epidemiological studies designed to evaluate the health effects of NO₂ have been conducted. Two health outcome measurements of NO₂ exposure are generally considered: lung function measurements and respiratory symptoms and diseases.
The evidence from individual studies of the effect of NO$_2$ on lower respiratory symptoms and disease in school-aged children is somewhat mixed. The consistency of these studies was examined and the evidence synthesized in a combined quantitative analysis (meta-analysis) of the subject studies. Most of the indoor studies showed increased lower respiratory morbidity in children associated with long-term exposure to NO$_2$. Mean weekly NO$_2$ concentrations in bedrooms in studies reporting NO$_2$ levels were predominantly between 15 and 122 µg/m$^3$ (0.008 and 0.065 ppm). Combining the indoor studies as if the end-points were similar gives an estimated odds ratio of 1.2 (95% confidence limits of 1.1 and 1.3) for the effect per 28.3 µg/m$^3$ (0.015 ppm) increase of NO$_2$ on lower respiratory morbidity. This suggests that, subject to assumptions made for the combined analysis, an increase of about 20% in the odds of lower respiratory symptoms and disease corresponds to each increase of 28.3 µg/m$^3$ (0.015 ppm) in estimated 2-week average NO$_2$ exposure. Thus, the combined evidence is supportive for the effects of estimated exposure to NO$_2$ on lower respiratory symptoms and disease in children aged 5 to 12 years.

In individual indoor studies of infants 2 years of age or younger, no consistent relationship was found between estimates of NO$_2$ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analysis of these indoor infant studies, subject to the assumptions made for the meta-analysis, the combined odds ratio for the increase in respiratory disease per increase of 28.2 µg/m$^3$ (0.015 ppm) NO$_2$ was 1.09 with a 95% confidence interval of 0.95 to 1.26, where mean weekly NO$_2$ concentrations in bedrooms were predominantly between 9.4 and 94 µg/m$^3$ (0.005 and 0.050 ppm) in studies reporting levels. The increase in risk was very small and was not reported consistently by all studies. We cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children. The reasons for these age-related differences are not clear.

The measured NO$_2$ studies gave a higher estimated odds ratio than the surrogate estimates, which is consistent with a measurement error effect. The effect of having adjusted for covariates such as socioeconomic status, smoking and sex was that those studies that adjusted for a particular covariate found larger odds ratios than those that did not.

Although many of the epidemiological studies that involved measured NO$_2$ levels used measurements over only 1 or 2 weeks,
these levels were used to characterize children's exposures over a much longer period. The standard respiratory symptom questionnaire used by most of these studies summarizes information on health status over an entire year. The 28.2 µg/m³ (0.015 ppm) difference in NO₂ levels used in the meta-analyses relates to a difference in the household annual average exposure between gas and electric cooking stoves. Some studies measured NO₂ levels only in the winter and may have overestimated annual average exposures. This would tend to have underestimated the health effect of a 28.2 µg/m³ (0.015 ppm) difference in the annual NO₂ exposure. A study based on a household annual average exposure measured in both the winter and summer found a stronger health effect than many of the other studies. The true biologically relevant exposure period is unknown, but these exposures extended over a lengthy period up to the entire lifetime of the child.

The association between outdoor NO₂ and respiratory health is not clear from current research. There is some evidence that the duration of respiratory illness may be increased at higher ambient NO₂ levels. A major difficulty in the analysis of outdoor studies is distinguishing possible effects of NO₂ from those of other associated pollutants.

Several uncertainties need to be considered in interpreting the above studies and meta-analysis. Error in measuring exposure is potentially one of the most important methodological problems in epidemiological studies of NO₂. Although there is evidence that symptoms are associated with indicators of NO₂ exposure, the quality of these exposure estimates may be inadequate to determine a quantitative relationship between exposure and symptoms. Most of the studies that measured NO₂ exposure did so only for periods of 1 to 2 weeks and reported the values as averages. Few of the studies attempted to relate the observed effects to the pattern of exposure (e.g., transient NO₂ peaks). Furthermore, measured NO₂ concentration may not be the biologically relevant dose; estimating actual exposure requires knowledge of pollutant species, levels and related human activity patterns. However, only very limited activity and aerometric data are available that examine such factors. The extrapolation to possible patterns of ambient exposure is difficult. In addition, although the level of similarity and common elements between the outcome measures in the NO₂ studies provide some confidence in their use in the quantitative analysis, the symptoms and illnesses combined are to some extent different and could indeed reflect different
underlying processes. Thus, caution is necessary in interpreting the meta-analysis results.

Other epidemiological studies have attempted to relate some measure of indoor and/or outdoor NO$_2$ exposure to changes in pulmonary function. These changes were marginally significant. Most studies did not find any effects, which is consistent with controlled human exposure study data. However, there is insufficient epidemiological evidence to draw any conclusions about the long- or short-term effects of NO$_2$ on pulmonary function.

On the basis of a background level of 15 μg/m$^2$ (0.008 ppm) and the fact that significant adverse health effects occur with an additional level of 28.2 μg/m$^2$ (0.015 ppm) or more, an annual guideline value of 40 μg/m$^2$ (0.023 ppm) is proposed. This value will avoid the most severe exposures. The fact that a no-effect level for subchronic or chronic NO$_2$ exposure concentrations has not yet been determined should be emphasized.

1.3.4 Health-based guidance values for nitrogen dioxide

On the basis of human controlled exposure studies, the recommended short-term guidance value is for a one-hour average NO$_2$ daily maximum concentration of 200 μg/m$^2$ (0.11 ppm). The recommended long-term guidance value, based on epidemiological studies of increased risk of respiratory illness in children, is 40 μg/m$^2$ (0.023 ppm) annual average.
2.1 Introduction

Nitrogen oxides are produced by combustion processes and are emitted to the air mainly as NO together with some NO\textsubscript{a}. Natural biological processes and lightning also emit NO and N\textsubscript{2}O. In the atmosphere nitrogen oxides undergo complex chemical and photochemical reactions; NO is oxidized to NO\textsubscript{2} and other products and eventually to HNO\textsubscript{3} and nitrates. Nitrogenous species are removed from the air to the ground by wet and dry deposition processes. Oxidized nitrogen compounds can have impacts on human health and the environment, and are important to the formation of photochemical smog and tropospheric ozone.

In this chapter the properties of nitrogen compounds are briefly described and techniques for their sampling and analysis outlined. Atmospheric chemical reactions that cause the oxidation of NO to NO\textsubscript{2} and the production of ozone, organic nitrates and HNO\textsubscript{3} are described. The differences between night-time and day-time chemistry and the composition of the atmosphere are discussed. The nature of the nitrogen species and their chemical reactions in urban regions, in chimney plumes such as those from power stations, in air advected away from urban regions and in rural and remote areas are described. The role of nitrogen oxides in photochemical smog production and the effects of nitrous oxide on stratospheric ozone are briefly discussed.

2.1.1 The nomenclature and measurement of atmospheric nitrogen species

There are several methods available for determining nitrogen species, but many of these techniques are nonspecific.

To denote various mixtures of nitrogen species, the terms NO\textsubscript{x}, NO\textsubscript{a} and NO\textsubscript{2} are often employed. It is customary to refer to the sum of NO and NO\textsubscript{2} emitted from a source as NO\textsubscript{x}, the unit of measure for NO\textsubscript{x} being the NO\textsubscript{2} mass equivalent of the NO plus NO\textsubscript{2}.

The term NO\textsubscript{a} is frequently used to denote the sum of the gas phase oxidized nitrogen species (except N\textsubscript{2}O) and NO\textsubscript{a} to denote the sum of NO\textsubscript{a} plus the oxidized nitrogen present as particulate
matter. Measurement of NO requires a combination of particulate and gas phase sampling and analysis.

A confusion arises because one of the most commonly used methods for determining NO in ambient air (thermal conversion of NO to NO and measurement of the resultant NO by chemiluminescent reaction with O) is nonspecific and responds to several gaseous species in addition to NO. These include organic nitrogen compounds and, depending on the converter, HNO, although HNO can be readily lost to the sampling system. Therefore, depending on the composition of the air being sampled, the results from this type of instrument can be representative of NO rather than NO (or NO) concentrations. This technique is used in most routine determinations of ambient NO and NO concentrations but the discrepancy between these values and true NO and NO can be considerable for air in which the pollutant emissions have undergone substantial exposure to sunlight.

Nitrous oxide is ubiquitous in the atmosphere because it is a product of biological processes in soil as well as anthropogenic activities. It is not involved to any appreciable extent in chemical reactions in the lower atmosphere, but it is an active “greenhouse” gas. In the stratosphere NO forms NO by reaction with excited oxygen atoms, and this NO then acts to deplete the stratospheric O concentration.

Although NO, dinitrogen trioxide (N,O), dinitrogen tetroxide (N,O), and N,O may play a role in atmospheric chemical reactions leading to the transformation, transport, and ultimate removal of nitrogen compounds from ambient air, they are present in very low concentrations, even in polluted environments.

NH is generated during decomposition of nitrogenous matter in natural ecosystems and may be locally produced in high concentrations by human activities such as intensive animal husbandry and feedlots. Under suitable conditions NH can react with oxidized nitrogen species to form ammonium nitrate aerosol.

### 2.2 Nitrogen species and their physical and chemical properties

There are seven oxides of nitrogen that may be present in ambient air, namely: NO, NO, N, NO, N,O, N,O, and N,O. In addition these can be present as HNO, HNO and various organic nitrogen species, such as PAN, other organic nitrates and
particles containing oxidized nitrogen compounds (particularly adsorbed nitric acid). Of these species, NO and NO₂ are the ones most often measured and are present in the greatest concentrations in urban and industrial air.

The chemical and physical properties of individual nitrogen species are given below and are summarized in Table I.

2.2.1 Nitrogen oxides

2.2.1.1 Nitric oxide

NO is a colourless, odourless gas that is only slightly soluble in water. It is a by-product of combustion processes, arising from (i) high temperature oxidation of molecular nitrogen from the combustion air, and (ii) from oxidation of nitrogen present in certain fuels such as coal and heavy oil.

2.2.1.2 Nitrogen dioxide

NO₂ is a reddish-orange-brown gas with a characteristic pungent odour. The boiling point is 21.1 °C, but the low partial pressure of NO₂ in the atmosphere prevents condensation. NO₂ is corrosive and highly oxidizing. About 5 to 10% by volume of the total emissions of NO from combustion sources is usually in the form of NO₂, although substantial variations from one source type to another have been observed.

In the atmosphere, photochemical reactions involving ozone and organic compounds convert NO to NO₂. NO₂ is an efficient absorber of light over a broad range of ultraviolet (UV) and visible wavelengths. Because of its brown colour, NO₂ can contribute to discoloration and reduced visibility of polluted air. Photolysis of NO₂ by sunlight produces NO and an oxygen atom, which usually adds to an oxygen molecule to produce ozone.

2.2.1.3 Nitrous oxide

N₂O is a colourless gas with a slight odour at high concentrations. It is emitted to the atmosphere as a trace component from some combustion sources and from the consumption of nitrate by an ubiquitous group of denitrification bacteria that use nitrate as their terminal electron acceptor in the absence of oxygen (Delwiche, 1970; Brezonik, 1972; Keeney, 1973; Focht & Verstraete, 1977). At atmospheric concentrations N₂O has no
<table>
<thead>
<tr>
<th>Oxide</th>
<th>Relative molecular mass (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Solubility in water at 0 °C (cm³ per 100 g)</th>
<th>Thermodynamic functions (ideal gas, 1 atm, 25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enthalpy of formation (kcal/mol)  Entropy (cal/mol-deg)</td>
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<tr>
<td>NO</td>
<td>30.01</td>
<td>-163.6</td>
<td>-151.8</td>
<td>7.34</td>
<td>21.56</td>
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<tr>
<td>NO₂</td>
<td>46.01</td>
<td>-11.2</td>
<td>21.2</td>
<td>Reacts with H₂O forming HNO₂ and HNO₃</td>
<td>7.91</td>
</tr>
<tr>
<td>N₂O</td>
<td>44.01</td>
<td>-90.6</td>
<td>-88.5</td>
<td>130.52</td>
<td>19.61</td>
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<tr>
<td>N₂O₃</td>
<td>78.01</td>
<td>-102</td>
<td>47 (decomposes)</td>
<td>Reacts with H₂O forming HNO₂</td>
<td>19.80</td>
</tr>
<tr>
<td>N₂O₄</td>
<td>92.02</td>
<td>-11.3</td>
<td>21.2</td>
<td>Reacts with H₂O forming HNO₂ and HNO₃</td>
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</tr>
<tr>
<td>Compound</td>
<td>Molecular Weight</td>
<td>Boiling Point</td>
<td>Condensation Temperature</td>
<td>Standard Enthalpy of Condensation (kJ/mol)</td>
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<td>-------------------</td>
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<td>--------------------------</td>
<td>------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>N₂O₅</td>
<td>108.01</td>
<td>30</td>
<td>-</td>
<td>3.24 - H₂O forming HNO₂</td>
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<tr>
<td>HNO₂</td>
<td>47.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>HNO₃</td>
<td>63.01</td>
<td>-42</td>
<td>83</td>
<td>-32.1 - H₂O at 118.3 g/100 cm³</td>
<td></td>
</tr>
<tr>
<td>(CH₃COONO₂) PAN</td>
<td>121.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>80.04</td>
<td>109.6</td>
<td>210 at 11 torr</td>
<td>118.3 g/100 cm³ H₂O at 0 °C -87.37</td>
<td></td>
</tr>
</tbody>
</table>

* Matheson Gas Data Book (Matheson Company, 1966)
* Handbook of Chemistry and Physics (Weast et al., 1986)
* At 0 °C and 1 atm pressure
significant physiological effects in humans, although at higher concentrations it is employed as an anaesthetic.

$N_2O$ does not play a significant role in atmospheric reactions in the lower troposphere. In the stratosphere it reacts with singlet oxygen to produce NO, which participates in $O_3$ decomposition in the stratosphere. These reactions are of concern because of the possibility that increasing $N_2O$ concentrations resulting from fossil fuel use, and also from denitrification of excess fertilizer, may contribute to a decrease in stratospheric $O_3$ (Council for Agricultural Science and Technology, 1976; Crutzen, 1976) with consequent potential for adverse impacts on ecosystems and human health. Also of concern is the fact that $N_2O$ absorbs long-wave radiation, and therefore serves as a radiatively important greenhouse gas that may contribute to global warming.

2.2.1.4 Other nitrogen oxides

Other nitrogen oxides can be present in trace quantities in the air. $NO_3$ has been identified in laboratory systems containing $NO_2/O_3$, $NO_2/O$ and $N_2O_5$ as an important reactive transient (Johnston, 1966). It is likely to be present in photochemical smog. In the presence of sunlight, $NO_3$ is rapidly converted to either NO or $NO_2$ (Wayne et al., 1991). Nitrogen trioxide is highly reactive towards both NO and $NO_2$. Its expected concentration in polluted air is very low (about $10^4 \mu g/m^3$). However, traces of $NO_3$ may play an important role in atmospheric chemistry, especially at night when it may serve as a reservoir for $NO_3$ (Wayne et al., 1991). In the atmosphere $N_2O_3$ is in equilibrium with NO and $NO_2$. It reacts with water to form $HNO_2$. $N_2O_3$ is the dimer of $NO_2$, formed in equilibrium with $NO_2$ molecules, and it readily dissociates to $NO_2$. $N_2O_5$ can be a trace night-time component of the air because it is formed by a reaction between $NO_2$ and $NO_3$. Since $NO_3$ can exist in appreciable quantities only in the absence of sunlight, $N_2O_5$ is only important at night, when its reaction with water can be a significant source of nitric acid.

2.2.2 Nitrogen acids

2.2.2.1 Nitric acid

$HNO_3$ is the most oxidized form of nitrogen. In the gaseous state it is colourless. It is photochemically stable in the troposphere. $HNO_3$ is volatile, so that at typical concentrations and temperatures in the atmosphere the vapour does not coalesce.
into aerosol and is not retained on particles unless the aerosol contains reactants such as sodium chloride or ammonium salts to react with the acid, when it produces particulate nitrates (Wolff, 1984).

In the aqueous phase (e.g., rain drops), HNO₃ dissociates to form the nitrate ion (NO₃⁻). Because nitrate is chemically unreactive in dilute aqueous solution, nearly all of the transformations involving nitrate in natural waters result from biochemical pathways. The nitrate salts of all common metals are quite soluble.

2.2.2 Nitrous acid

HNO₂ is formed when NO and NO₂ are present in the atmosphere, as a result of their reaction with water. In sunlight, the dominant pathway for HNO₂ formation is the reaction of NO with hydroxyl radicals. During the daytime, atmospheric concentrations of HNO₂ are limited by the photolysis of HNO₂ to produce NO and hydroxyl radical.

Nitrous acid is a weak reducing agent and is oxidized to nitrate only by strong chemical oxidants and by nitrifying bacteria.

2.2.3 Ammonia

NH₃ is the completely reduced form of nitrogen. It is a colourless gas with a pungent odour. It is extremely soluble in water, forming ammonium (NH₄⁺) and hydroxyl (OH⁻) ions. In the atmosphere, NH₃ has been reported to be converted into NO by reaction with hydroxyl radicals (Søderlund & Svensson, 1976). In the stratosphere, NH₃ can be dissociated by irradiation with sunlight at wavelengths below 230 nm (McConnell, 1973).

2.2.4 Ammonium nitrate

Gas-phase ammonia reacts with nitric acid to form ammonium nitrate (NH₄NO₃). Ammonium nitrate is a solid at room temperature. Like ammonia, it is very soluble in water and hence will be absorbed by any water droplets present. Thus it readily forms an aerosol in the atmosphere. Pathways to aerosol formation include nucleation and condensation on existing particles. The presence of NH₄NO₃ particles can result in a visible haze.
2.2.5 Peroxyacetyl nitrate

Of the various peroxy nitrates found in ambient air, peroxyacetyl nitrate (CH\textsubscript{3}COOONO\textsubscript{2}), or PAN, is found at the highest concentrations. PAN undergoes a temperature-dependent decomposition to its precursors, NO\textsubscript{2} and acetyl peroxy radicals. At low ambient temperatures PAN can have a substantial lifetime in the atmosphere (Cox & Roffey, 1977). In polluted air PAN concentrations can reach several parts per billion.

2.2.6 Organic nitrites and nitrates

A wide variety of organic nitrites (RNO\textsubscript{2}) and nitrates (RNO\textsubscript{3}), where R denotes CH\textsubscript{3}, CH\textsubscript{2}CH\textsubscript{3}, benzyl, etc., may be found in ambient air. Some of these are emitted directly while others are formed by photochemical reactions in the atmosphere.

2.3 Sampling and analysis methods

This section outlines methods for measuring nitrogen-containing species in the atmosphere. The main focus is on methodologies currently available and in general use for monitoring concentrations in both ambient and indoor air.

Table 2 summarizes sampling and analytical methods for selected species and addresses relevant characteristics, including the type of method (i.e., \textit{in situ}, remote, active, passive, continuous or integrative), the stage of development of the method, sampling duration, precision, accuracy and detection limits.

2.3.1 Nitric oxide

2.3.1.1 Nitric oxide continuous methods

Nitric oxide reacts rapidly with O\textsubscript{3} to give NO\textsubscript{2} in an excited electronic stage. The transition of excited NO to the grand state can be accompanied by the emission of light in the red-infrared spectral range. When this chemiluminescent reaction occurs under controlled conditions, the intensity of the emitted light is proportional to the concentration of the NO reactant. This provides the basis of the chemiluminescence method (CLM) for analysis of NO. This method is a continuous technique and is the most commonly used method for measuring NO in ambient air. Commercial instruments for measuring NO and NO\textsubscript{2} are available.
<table>
<thead>
<tr>
<th>Species</th>
<th>Methods²</th>
<th>Type²</th>
<th>Development stage²</th>
<th>Sample duration</th>
<th>Performance</th>
<th>Comments</th>
<th>References</th>
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<tr>
<td>NO</td>
<td>CLM (NO + O₃)</td>
<td>I, A, C</td>
<td>C</td>
<td>5 min</td>
<td>≤ 10%</td>
<td>≤ 20%</td>
<td>≤ 9 ppb</td>
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<tr>
<td>TP-LIF</td>
<td>I, A, C</td>
<td>R</td>
<td>30 sec</td>
<td>-</td>
<td>16%</td>
<td>10 ppt</td>
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<tr>
<td>TDLAS</td>
<td>I, A, C</td>
<td>R, C</td>
<td>60 sec</td>
<td>-</td>
<td>-</td>
<td>0.5 ppb</td>
<td>40-m path length</td>
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<tr>
<td>PSD</td>
<td>I, P, IN</td>
<td>C</td>
<td>24 h</td>
<td>-</td>
<td>-</td>
<td>70 ppb/h*</td>
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<tr>
<td>NO₂</td>
<td>CLM (NO + O₃)</td>
<td>I, A, C</td>
<td>C</td>
<td>5 min</td>
<td>10%</td>
<td>20%</td>
<td>9 ppb</td>
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<td></td>
<td>CLM (NO + O₃)</td>
<td>I, A, C</td>
<td>R</td>
<td>&lt; 100 sec</td>
<td>20 ppt</td>
<td>30%</td>
<td>10-25 ppt</td>
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<td>I, A, C</td>
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<td>R</td>
<td>2 min</td>
<td>20 ppt</td>
<td>15%</td>
<td>12 ppt</td>
</tr>
<tr>
<td></td>
<td>TD-LIF</td>
<td>I, A, C</td>
<td>R</td>
<td>60 sec</td>
<td>-</td>
<td>-</td>
<td>100 ppt</td>
</tr>
<tr>
<td></td>
<td>DOAS</td>
<td>R, A, C</td>
<td>R, C</td>
<td>12 min</td>
<td>-</td>
<td>15%</td>
<td>4 ppb</td>
</tr>
<tr>
<td></td>
<td>Bubbler</td>
<td>I, A, IN</td>
<td>R</td>
<td>24 h</td>
<td>6 ppb</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>TEA filter</td>
<td>I, A, L</td>
<td>R</td>
<td>24 h</td>
<td>6 ppb</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>TES filter</td>
<td>I, A, IN</td>
<td>L</td>
<td>1 h</td>
<td>0.1 ppb*</td>
<td>-</td>
<td>0.1 ppb*</td>
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Table 2 (cont'd).
Table 2 (contd).

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<tr>
<th>Method</th>
<th>Detection</th>
<th>Sample</th>
<th>Time</th>
<th>Recovery</th>
<th>LOD (ppb)</th>
<th>Notes</th>
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<tr>
<td>DPA Cartridge</td>
<td>I, A, IN</td>
<td>L</td>
<td>8 h</td>
<td>8%</td>
<td>0.1 ppb</td>
<td>DPA may volatilize; Lipari (1984)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>interferences: HNO$_2$ and PAN</td>
</tr>
<tr>
<td>TEA PSD</td>
<td>I, P, IN</td>
<td>L</td>
<td>24 h</td>
<td>30%</td>
<td>30 ppb-h</td>
<td>Similar to Palms Tube; interferences as above</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_x$</td>
<td>CLM (NO + O$_3$)</td>
<td>R</td>
<td>10 sec</td>
<td>-</td>
<td>15%</td>
<td>10 ppt CO with Au reducing catalyst</td>
</tr>
<tr>
<td></td>
<td>I, A, C</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAN</td>
<td>GC-ECD</td>
<td>R, RM</td>
<td>15 min</td>
<td>-</td>
<td>30%</td>
<td>10 ppt$^*$ Sensitivity can be enhanced by using cryogenic sampling</td>
</tr>
<tr>
<td></td>
<td>I, A, IN</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td>and capillary columns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-CLM</td>
<td>I, A, IN</td>
<td>L</td>
<td></td>
<td></td>
<td>-</td>
<td>CLM (NO + O$_3$) and (luminol) reported</td>
</tr>
<tr>
<td>Other organic</td>
<td>GC-ECD/MS</td>
<td>R</td>
<td>24 h</td>
<td>-</td>
<td>1 ppt$^*$</td>
<td>Sample collected on charcoal</td>
</tr>
<tr>
<td>Nitrates</td>
<td>I, A, C</td>
<td>R</td>
<td></td>
<td></td>
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Table 2 (contd).

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<tr>
<th>Species</th>
<th>Methods</th>
<th>Type</th>
<th>Development stage</th>
<th>Sample duration</th>
<th>Performance</th>
<th>Comments</th>
<th>References</th>
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<tr>
<td>NHO₃</td>
<td>Filter</td>
<td>I, A, IN</td>
<td>R, RM</td>
<td>24 h</td>
<td>10%</td>
<td>20%</td>
<td>8 ppt²</td>
</tr>
<tr>
<td></td>
<td>Denuder</td>
<td>I, A, IN</td>
<td>R, RM</td>
<td>24 h</td>
<td>8%</td>
<td>-</td>
<td>8 ppt²</td>
</tr>
<tr>
<td></td>
<td>TDLAS</td>
<td>I, A, C</td>
<td>R, C</td>
<td>5 min</td>
<td>-</td>
<td>20%</td>
<td>100 ppt</td>
</tr>
<tr>
<td>HNO₂</td>
<td>Denuder</td>
<td>I, A, IN</td>
<td>R, RM</td>
<td>24 h</td>
<td>15%</td>
<td>-</td>
<td>10 ppt⁷</td>
</tr>
<tr>
<td></td>
<td>LIF</td>
<td>I, A, C</td>
<td>R</td>
<td>15 min</td>
<td>-</td>
<td>-</td>
<td>20 ppt</td>
</tr>
<tr>
<td></td>
<td>DOAS</td>
<td>R, A, C</td>
<td>R, C</td>
<td>12 min</td>
<td>-</td>
<td>30%</td>
<td>600 ppt</td>
</tr>
<tr>
<td>NO₃</td>
<td>DOAS</td>
<td>R, A, C</td>
<td>R, C</td>
<td>12 min</td>
<td>-</td>
<td>15%</td>
<td>20 ppt</td>
</tr>
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Table 2 (contd.).

<table>
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<tr>
<th>Particulate/NO&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Denuder/Filter(s)</th>
<th>I, A, IN</th>
<th>R, RM</th>
<th>24 h</th>
<th>10%</th>
<th>-</th>
<th>40 ng/m&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Vossler et al. (1988) Use of denuders avoids artifacts; denuders collect HNO&lt;sub&gt;3&lt;/sub&gt; and NH&lt;sub&gt;3&lt;/sub&gt;; teflon and nylon filters used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen Oxide</td>
<td>GC-ECD</td>
<td>1, A, IN</td>
<td>R, RM</td>
<td>15 min</td>
<td>3%</td>
<td>-</td>
<td>20 ppb&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> CLM (NO + O<sub>3</sub>) = Chemiluminescent using NO + O<sub>3</sub> reaction
TP-LIF = Two-photon laser-induced fluorescence
TDLAS = Tuneable diode laser absorption spectroscopy
TTFMS = Two-tone frequency modulated spectroscopy
PSD = Passive sampling device
CLM (Luminol) = Chemiluminescent using reaction with Luminol
DOAS = Differential optical absorption spectroscopy
DIAS = Differential absorption lidar
TEA = Triethanolamine
DPA = Diphenylamine
GC-ECD = Gas chromatography with electron capture detector
GC-CLM = Gas chromatography with CLM detector
LIF = Laser-induced fluorescence
GC-MS = gas chromatography with mass spectrometer

<sup>b</sup> I = In situ
A = Active
C = Continuous
P = Passive
IN = Integrative
R = Remote

<sup>c</sup> C = Commercially available
R = Research tool
L = Laboratory prototype
RM = Routine method

<sup>d</sup> MDL = Minimum detection limit

<sup>e</sup> Depends on the sampled air volume (i.e., flow rate and sampling duration)

<sup>f</sup> Uses ion chromatographic or colorimetric analytical finish
with detection limits of approximately 5 ppb and response times of the order of minutes. CLM measurement of NO\textsubscript{2} can also be accomplished by firstly converting the NO\textsubscript{2} of the sample to NO. This is discussed in section 2.3.2.1.

Other NO analytical methods include laser-induced fluorescence (LIF) (Bradshaw et al., 1985), absorption spectroscopy (e.g., tuneable diode laser absorption spectroscopy, TDLAS) and passive samplers.

### 2.3.1.2 Passive samplers for NO

Passive samplers are used for air with higher-than-typical ambient concentrations, which may be found indoors or in the workplace. They are often used to obtain data at a large number of sites. Sampling typically lasts a few hours.

The Palmes tube is a passive sampler that relies on diffusion of an analyte molecule through a quiescent diffusion path of known length and cross-sectional area to a reactive surface where the molecule is captured by chemical reaction (Palmes et al., 1976). The Palmes tube does not measure NO directly. Two tubes are required; the first one has reactive grids coated with triethanolamine (TEA) to collect NO\textsubscript{2}, the second tube is similar but has an additional reactive surface coated with chromic acid to convert NO to NO\textsubscript{2}, which is in turn collected by the TEA-coated grids. The NO concentration of the air is determined from the difference in the results from the two tubes. The data is corrected for the effects of the different diffusivities of NO and NO\textsubscript{2} molecules. To ensure reliable results, contact between the chromic-acid-coated surface and the TEA-coated grids for longer than 24 h must be avoided. Analysis of the material contained in the TEA is accomplished by extracting the grids into solution and analysing the extract for NO\textsubscript{2} by the use of the spectrophotometric or ion chromatographic method (Miller, 1984). The colorimetric analysis is calibrated by dilution of gravimetrically prepared nitrite solutions. The Palmes Tube method was proposed for sampling occupational exposures where the dosage does not exceed 25 ppm for 8 h (i.e., 200 ppm-h). The reliability of this method for measuring NO in the field at the parts-per-billion or parts-per-million level remains to be demonstrated.

A badge-type sampler similar to the Palmes tube has been devised by Yanagisawa & Nishimura (1982). This device uses a series of 12 layers of chromium-trioxide-impregnated glass fibre
to oxidize NO to NO\textsubscript{2}. This technique is claimed to be more sensitive by approximately a factor of 10 than the Palmes tube and to have a lower limit dosage of 0.07 ppm-h.

### 2.3.1.3 Calibration of NO analysis methods

Calibration of CLM, TP-LIF and TDLAS measurement systems for NO all rely on compressed gas mixtures of known concentration being available. Typically compressed gas mixtures are supplied in passivated aluminium/stainless steel gas bottles certified by the manufacturer and with NO diluted with \text{N\textsubscript{2}} concentration in the rage of 1 to 50 ppm (Schiff et al., 1983; Carroll et al., 1985; Bradshaw et al., 1985). Calibrations are performed by dynamic dilution of the reference NO/\text{N\textsubscript{2}} mixture with air to give NO concentrations within the range of 0.1 to 5 ppm.

For passive NO samplers, only the analysis portion of the procedure is routinely calibrated (using gravimetrically prepared nitrite solution).

### 2.3.1.4 Sampling considerations for NO

Oxides of nitrogen are reactive species and exhibit various solubilities (Table I). The most inert materials (i.e. glass and Teflon\textsuperscript{TM}) are recommended for use in sampling trains. Since ambient air contains water vapour that may be sorbed on sampling lines, surface effects may influence the integrity of air samples containing the more reactive and more soluble NO\textsubscript{2} species. In hot, humid conditions condensation in the sample lines of liquid water from the air can cause difficulties when analysis equipment is installed in an air-conditioned environment. To minimize contamination of the system by dust and foreign matter, it is common practice to sample through an inert (teflon) sample inlet filter. Of the NO\textsubscript{2} species, NO is probably the least susceptible to surface effects, whereas surface effects are very important in the sampling of HNO\textsubscript{3}.

Nitric oxide reacts rapidly with \text{O\textsubscript{3}} to form NO\textsubscript{2}. In the presence of sunlight NO\textsubscript{2} in air photolyses to yield NO and \text{O\textsubscript{3}}. Thus in daylight NO, \text{O\textsubscript{3}} and NO\textsubscript{2} can exist simultaneously in ambient air in a condition known as a "photostationary state". The relative amounts of the three species at any time are influenced by the intensity of the sunlight present at that moment. Photolysis ceases when a sample is drawn into a dark sampling
line, but NO and O\textsubscript{3} can continue to react to form NO\textsubscript{2}. Therefore residence times in sampling lines must be minimized to maintain the intensity of the NO/NO\textsubscript{2} ratio of the sample.

2.3.2 Nitrogen dioxide

Airborne concentrations of NO\textsubscript{2} can be determined by several methods including CLM, LIF, absorption spectroscopy, including differential optical absorption spectroscopy (DOAS) and TDLAS, bubbler and passive collection with subsequent wet chemical analysis. The most common techniques are chemiluminescence and passive sampling.

2.3.2.1 Chemiluminescence (NO + O\textsubscript{3})

Instruments discussed in this section do not detect NO\textsubscript{2} directly. They sample continuously and rely on the conversion of some or all of the NO\textsubscript{2} in the air sample to NO, followed by the CLM reaction of NO and O\textsubscript{3}. The NO\textsubscript{2} concentration is calculated from the difference in the signal given by the sample after passing through the converter compared to that when the converter is bypassed.

Several methods have been employed to reduce NO\textsubscript{2} to NO (Kelly, 1986). They include catalytic reduction using heated molybdenum or stainless steel, reaction with carbon monoxide over a gold catalyst surface, reaction with iron sulfate at room temperature, reaction with carbon at 200 °C, and photolysis of NO\textsubscript{2} to NO by light in the wavelength range of 320 to 400 nm.

CLM instruments for the determination of NO\textsubscript{2} are readily available commercially. Field evaluation of nine instruments showed that the minimum detection limits (MDLs) ranged from 5 to 13 ppb (Michie et al., 1983; Holland & McElroy, 1986).

Converters may be non-specific for NO\textsubscript{2} and may convert several other nitrogen-containing compounds to NO, giving rise to overestimates for NO\textsubscript{2} concentrations. Using commercial instruments, Winer et al. (1974) found over 90% conversion of PAN, ethyl nitrate and ethyl nitrite to NO with a molybdenum converter, and similar responses to PAN and n-propyl nitrate with a carbon converter. With a stainless steel converter at 650 °C, Matthews et al. (1977) reported 100% conversion for NO\textsubscript{2}, 86% for NH\textsubscript{3}, 82% for CH\textsubscript{3}NH\textsubscript{2}, 68% for HCN, 1% for N\textsubscript{2}O and 0% for N\textsubscript{2}. Using a commercial instrument, Joseph & Spicer (1978) found...
quantitative conversion of HNO₃ to NO with a molybdenum converter at 350 °C. Similar responses to PAN, methyl nitrate, n-propyl nitrate, n-butyl nitrate and HNO₃, substantial response to nitrocresol, and no response to peroxycetyl nitrate (PBzN) were reported with a commercial instrument using a molybdenum converter at 450 °C (Grosjean & Harrison, 1985). These results were confirmed for PAN and HNO₃ by Rickman & Wright (1986) using commercial instruments with a molybdenum converter at 375 °C and a carbon converter at 285 °C.

Interference from species that do not contain nitrogen have also been reported. Joshi & Bufalini (1978), using a commercial instrument with a carbon converter, found significant apparent NO₂ responses to phosgene, trichloroacetyl chloride, chloroform, chlorine (Cl₂), hydrogen chloride, and photochemical reaction products of a perchloroethylene-NO₂ mixture. Grosjean & Harrison (1985) reported substantial responses to photochemical reaction products of Cl₂—NO₂ and Cl₂—methanethiol mixtures and small negative responses to methanethiol, methyl sulfide, and ethyl sulfide. Sickles & Wright (1979), using a commercial instrument with a molybdenum converter at 450 °C, found small negative responses to 3-methylthiophene, methanethiol, ethanethiol, ethyl sulfide, ethyl disulfide, methyl disulfide, hydrogen sulfide, 2,5-dimethylthiophene, methyl sulfide and methyl ethyl sulfide, and negligible responses to thiophene, 2-methylthiophene, carbonyl sulfide and carbon disulfide.

Methods of sample trapping followed by batch measurement of NO and NO₂ in the desorbed sample using a chemiluminescence instrument have been reported. Gallagher et al. (1985) used cryosampling of stratospheric whole-air samples, and Braman et al. (1986) used copper(I) iodide coated denuder tubes to sample NO₂ in ambient air.

### 2.3.2.2 Chemiluminescence (luminol)

A method for the direct chemiluminescence determination of NO₂ was reported by Maeda et al. (1980) and is based on the CLM reaction of gaseous NO₂ with a surface wetted with an alkaline solution of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The light emission is strong at wavelengths between 380 and 520 nm. The intensity of the light can be proportional to the NO₂ concentration in the sampled air, and the NO₂ concentration can be determined by calibration of the instrument with air of known NO₂ concentration.
Since the introduction of the luminol method by Maeda et al. (1980), improvements have been made to develop an instrument suitable for use in the field (Wendel et al., 1983), and additional modifications have been made recently to produce a continuous commercial instrument (Schiff et al., 1986). Detection limits of 5 to 30 ppt and a response time of seconds have been claimed, based on laboratory tests (Wendel et al., 1983; Schiff et al., 1986). Recent laboratory evaluation of two instruments has revealed a detection limit (i.e., twice the standard deviation of the clean air response) of 5 ppt, and 95% rise and fall times of 110 and 15 seconds (Rickman et al., 1988). Field tests of the same instruments have shown an operating precision of ± 0.6 ppb.

2.3.2.3 Laser-induced fluorescence and tuneable diode laser absorption spectrometry

Two newer techniques that show considerable promise for measuring NO₂ specifically are photofragmentation/2-photon LIF and TDLAS. The LIF and TDLAS techniques provide specific spectroscopic methods to measure NO₂ directly and compare favourably to the sample photolysis-chemiluminescence technique (Fehsenfeld et al., 1990; Gregory et al., 1990b). For NO₂ concentrations above 0.2 ppb, no interferences were found for TDLAS (Fehsenfeld et al., 1990).

2.3.2.4 Wet chemical methods

Most wet chemical methods for measuring NO₂ involve the collection of NO₂ in solution, followed by a colorimetric finish using an azo dye. Many variations of this method exist, including both manual and automated versions. These include the Griess-Saltzman method, the continuous Saltzman method, the alkaline guiacol method, the sodium arsenite method (manual or continuous), the triethanolamine-guaiacol-sulfite (TGS) method and the TEA method. These methods have been reviewed by Purdue & Hauser (1980).

2.3.2.5 Other methods

Several other methods for the determination of NO₂ have been reported. Atmospheric pressure ionization mass spectrometry has been investigated for the continuous measurement of NO₂ and SO₂ in ambient air (Benoit, 1983). Methods employing photothermal detection of NO₂ have been reported (Poizat & Atkinson, 1982; Higashi et al., 1983; Adams et al., 1986).
Physical and Chemical Properties

A portable, battery-powered analyser specific to NO\textsubscript{2}, which uses an electrochemical cell as the detector, is commercially available. By careful selection and design of the cell, levels down to approximately 0.1 ppm (v/v) can be detected, although with uncertainties of approximately 20-50%. The detection cell has a finite life, dependent on the time integral of the NO\textsubscript{2} concentrations measured. When the cell deteriorates, the instrument typically develops a gradual drift.

2.3.2.6 Passive samplers

Passive samplers are frequently used in industrial hygiene, indoor air and personal exposure studies and are less frequently used for ambient air analysis. Namiesnik et al. (1984) have provided an overview of passive samplers.

One type of passive NO\textsubscript{2} sampler for ambient application is the nitration plate. It is essentially an open petri dish containing TEA-impregnated filter paper. Mulik & Williams (1986) have adapted the nitration plate concept by adding diffusion barriers in their design of a passive sampling device (PSD) for NO\textsubscript{2} in ambient and personal exposure applications. The device employs a TEA-coated cellulose filter paper, two 200-mesh stainless steel diffusion screens and two stainless steel perforated plates on each side of the coated filter to act as diffusion barriers and permit NO\textsubscript{2} collection on both faces of the filter paper. After sampling, the paper is removed from the PSD, extracted in water, and analysed for NO\textsubscript{2} by ion chromatography. A sensitivity of 0.03 ppm-h and a rate of 2.6 cm\textsuperscript{3}/second were claimed. Comparison of PSD results with chemiluminescence determinations of NO\textsubscript{2} in laboratory tests at concentrations between 10 and 250 ppb showed a linear relation and high correlation (i.e., r = 0.996) (Mulik & Williams, 1987). Interference from PAN and l-INO\textsubscript{2} would be expected (Sickles, 1987). Results of TDLAS and triplicate daily PSD NO\textsubscript{2} measurements in a 13-day field study showed good agreement between the study average values but a correlation coefficient for daily results of only 0.47 (Mulik & Williams, 1987; Sickles et al., 1990). The Palmes tube described in section 2.3.1.2 has been used to sample air in the workplace and indoor environments to assess personal exposure to NO\textsubscript{2} (Palmes et al., 1976; Wallace & Ott, 1982).

2.3.2.7 Calibration

Calibration methods for NO\textsubscript{2} use permeation tubes or gas-phase titration (GPT) to generate known concentrations of NO\textsubscript{2}.
Calibrations are performed dynamically using dilution with purified air.

GPT employs the rapid, quantitative gas-phase reaction between NO, usually supplied as a known concentration from a gas cylinder, and O\textsubscript{3} supplied from a stable O\textsubscript{3} generator, to produce one NO\textsubscript{2} molecule for each NO molecule consumed by reaction. When O\textsubscript{3} is added to excess NO in a titration system, the decrease in NO concentration (and O\textsubscript{3}) is equivalent to the increase in NO\textsubscript{2} produced (US EPA, 1987b).

Use of cylinders of compressed gas containing NO\textsubscript{2} for calibration purposes (Fehsenfeld et al., 1987; Davis, 1988) is unwise because of the uncertain stability of the NO\textsubscript{2} concentrations delivered; this is a consequence of its relatively high boiling point.

2.3.3 Total reactive odd nitrogen

In this monograph, gas-phase total reactive odd nitrogen is represented by N\textsubscript{0}. Individual components comprising N\textsubscript{0} are gas phase NO, NO\textsubscript{2}, NO\textsubscript{3}, N\textsubscript{2}O\textsubscript{5}, HNO\textsubscript{2}, peroxynitric acid (\textit{HO}\textsubscript{2}NO\textsubscript{2}), PAN, and other organic nitrates. NH\textsubscript{3} and N\textsubscript{2}O are not components of N\textsubscript{0}.

Researchers have successfully combined highly sensitive research-grade CLM NO detectors with catalytic converters that are sufficiently active to reduce most of the important gas phase NO\textsubscript{y} species to NO for subsequent detection (Helas et al., 1981; Dickerson, 1984; Fahey et al., 1986; Fehsenfeld et al., 1987).

2.3.4 Peroxacetyl nitrate

Several methods have been used to measure the concentration of PAN in ambient air. Roberts (1990) has provided an overview of many of these methods. A well-developed method is gas chromatography using electron capture detection (GC-ECD) (Darley et al., 1963; Smith et al., 1972; Stephens & Price, 1973; Singh & Salas, 1983).

2.3.5 Other organic nitrates

Other organic nitrates (e.g., alkyl nitrates, peroxypivaloyl nitrate and PBzN) can also be present in the atmosphere, but usually at lower concentrations than PAN (Fahey et al., 1986).
general, similar methods for sampling, analysis and calibration may be used for other organic nitrates as are used for PAN (Stephens, 1969). FTIR, GC-ECD and GC-MS may be used to measure these compounds.

2.3.6 Nitric acid

Several methods are available for the determination of HNO₃ concentrations in the atmosphere. These include filtration (Okita et al., 1976; Spicer et al., 1978a), denuder tubes (Forrest et al., 1982; De Santis et al., 1985; Ferm, 1986), CLM (Joseph and Spicer, 1978) and absorption spectroscopy (Tuazon et al., 1978; Schiff et al., 1983; Biermann et al., 1988). Many of these techniques carry significant uncertainties, which have been compared by Hering et al. (1988).

2.3.7 Nitrous acid

Available techniques for the measurement of HNO₂ in ambient atmospheres employ denuders (Ferm & Sjodin, 1985), annular denuders (De Santis et al., 1985), CLM (Braman et al., 1986), PF/LIF (Rodgers & Davis, 1989), absorption spectroscopy (Tuazon et al., 1978; Biermann et al., 1988) and FTIR (Finlayson-Pitts & Pitts, 1986).

2.3.8 Dinitrogen pentoxide and nitrate radicals

N₂O₅ is readily reduced to NO at temperatures above 200 °C and may be measured nonspecifically as NO₂ with CLM NO₂ analysers (Bollinger et al., 1983; Fahey et al., 1986).

Ambient concentrations of the NO₂ radical have been measured using DOAS; concentrations between 1 and 430 ppt have been observed (Atkinson et al., 1986).

2.3.9 Particulate nitrate

Many methods are available for sampling ambient aerosols, including impactors, filtration, and filtration coupled with devices to remove particles larger than a specified size (e.g., elutriators, impactors and cyclones).

Particulate nitrate samples are generally collected by filtration, extracted, and analysed directly or indirectly for nitrate by ion chromatography or colorimetry.
2.3.10 Nitrous oxide

The most commonly used analytical method for N$_2$O employs GC-ECD. It has a detection limit of 20 ppb (Thijisse, 1978) and a precision of ± 3% at the background level of 330 ppb (Cicerone et al., 1978).

2.3.11 Summary

Gas-phase CLM instruments have replaced manual (wet) methods to a large extent in air quality monitoring network applications. Gas-phase CLM measurement technology permits the determination of NO, NO$_2$ and NO$_X$ in the low ppt range. Although CLM NO detectors coupled with catalytic NO$_2$ to NO converters are still not specific for NO$_2$, they have proved to be useful for measuring NO$_X$. CLM NO detectors coupled with photolytic NO$_2$ to NO converters have shown improved specificity for NO$_2$. Most ambient NO$_2$ monitoring data reported are from the nonspecific thermal converting technique.

Passive samplers for NO$_2$ have been used primarily for workplace and indoor applications, but hold promise for averaged ambient measurements as well. GC-ECD is useful in the determination of PAN, other organic nitrates and N$_2$O.

2.4 Transport and transformation of nitrogen oxides in the air

2.4.1 Introduction

Oxides of nitrogen are transformed by and removed from the atmosphere by a complex web of reactions that are fundamental to the formation and destruction of ozone and other oxidants. The predominant form of oxidized nitrogen (NO, NO$_2$, HNO$_3$, etc.) in the lower atmosphere varies, depending upon sunlight intensity, temperature, pollutant emissions, period of time since these emissions occurred and the meteorological history of an airmass.

2.4.2 Chemical transformations of oxides of nitrogen

2.4.2.1 Nitric oxide, nitrogen dioxide and ozone

The dominant source of nitrogen oxides in the air is combustion processes (see chapter 3); 90-95% of these nitrogen
Oxides are usually emitted as NO and 5–10% as NO₂. NO may be oxidized to NO₂ by atmospheric oxygen according to reaction 2-1:

\[ \text{NO} + \text{NO} + \text{O}_2 \rightarrow 2 \text{NO}_2 \]  

(2-1)

However at low NO concentrations this reaction is slow and is important only when NO > 1 ppm (Bostrom C, 1993). NO concentrations greater than 1 ppm are not frequently found in ambient air, but they may possibly occur in indoor air and in plumes from industrial sources (see Chapter 3). When concentrations are below 1 ppm, NO is oxidized to NO₂ by two types of reaction. The first type of reaction is given in equations 2-2 to 2-4. NO can react with O₃:

\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2 \]  

(2-2)

Also O₃ is formed when NO₂ is photolysed, forming NO plus an O atom

\[ \text{NO}_2 + \text{hv} \rightarrow \text{O} + \text{NO} \]  

(2-3)

and O atoms react rapidly with O₂ to form ozone:

\[ \text{M} \]

\[ \text{O} + \text{O}_2 \rightarrow \text{O}_3 \]  

(2-4)

Thus reactions 2-2, 2-3 and 2-4 recycle O₃ rather than producing a net increase in O₃ concentrations, where the "M" represents a third molecule such as N₂, O₂, etc., that absorbs excess vibrational energy from the newly formed O₃ molecules. However, a second oxidation path involving the reaction of organic species can lead to increases in O₃ concentrations and in the conversion rate of NO to NO₂ (2-9 and 2-10). Organic compounds in the air are commonly referred to as VOC (volatile organic carbon), ROC (reactive organic carbon) and non-methane hydrocarbons (NmHC). Urban areas are usually characterized by significant sources of both nitrogen oxides and ROC emissions. With suitable atmospheric conditions this can lead to the formation of photochemical smog. The smog-forming reactions are initiated by photolytic reactions which produce free radicals, for example:

(i) the photolysis of O₃
\[ \text{O}_3 + h\nu \rightarrow \text{O}_2 + \text{O}^* \]  

(2-5)

\( \text{O}^* \) is an excited form of atomic oxygen, which can react with water to produce the hydroxyl radical (OH):

\[ \text{O}^* + \text{H}_2\text{O} \rightarrow 2\text{OH} \]  

(2-6)

(ii) the photolysis of aldehydes, which also results in the production of OH. Aldehydes are emitted in motor vehicle exhaust and are produced in the air by reaction of ROC species with OH. OH is the most important oxidizing agent in the lower atmosphere; it can react with all organic compounds, usually forming water and producing an organic radical.

For a generalized organic compound, R-H (R = CH\(_3\), CHO, CH\(_2\)CH\(_3\), etc.), the principal elements of the reaction sequence are:

\[ \text{R-H} + \text{OH} \rightarrow \text{H}_2\text{O} + \text{R} \]  

(2-7)

\[ \text{M} \]

\[ \text{R + O}_3 \rightarrow \text{RO}_2 \] (fast)  

(2-8)

\( \text{RO}_2 \) provides a pathway to oxidize NO to NO\(_2\) without destroying \( \text{O}_3 \) (unlike reaction 2-2):

\[ \text{RO}_2 + \text{NO} \rightarrow \text{NO}_2 + \text{RO} \]  

(2-9)

\( \text{RO} \) can undergo reactions that form additional \( \text{HO}_2 \) or \( \text{RO}_2 \). \( \text{HO}_2 \) reacts with NO to form NO\(_2\) and regenerate OH:

\[ \text{HO}_2 + \text{NO} \rightarrow \text{NO}_2 + \text{OH} \]  

(2-10)

In the case of photochemical smog episodes, the quantity of NO\(_x\) emitted into the air determines the ultimate quantity of \( \text{O}_3 \) that may be produced. The ROC concentration and sunlight intensity are the major determinates of the rates at which NO will be oxidized to produce net increases in NO\(_2\) and \( \text{O}_3 \) concentrations. Ozone production is terminated when NO and NO\(_2\) are consumed by reaction to form products such as HNO\(_3\) (see below), resulting in insufficient NO concentration for reactions 2-9 and 2-10 to proceed at significant rates.

In large cities with sunny climates and poor dispersion of emissions (e.g., Los Angeles and Mexico City), \( \text{O}_3 \) concentrations in excess of 200 ppb are not uncommon.
2.4.2.2 Transformations in indoor air

Oxides of nitrogen in indoor air arise from two sources: a) outdoor air; and b) indoor sources, such as combustion appliances and heaters. Photochemical reactions do not take place under artificial lighting, so chemical transformations are limited by the amounts of oxidizing species (HO, O, etc.) that arrive in outdoor air, or are generated by combustion sources.

2.4.2.3 Formation of other oxidized nitrogen species

Oxidation products of NO, arising from tropospheric photochemical reactions include HNO₃, HO₂NO₂, HNO₂, peroxyacylnitrates (RC(O)O₂NO₂), N₂O₅, nitrate radical (NO₃) and organic nitrates (RNO₃).

Fig. 1 shows a summary for the interconversion pathways for oxides of nitrogen. These pathways govern urban and indoor air, as well as “clean” air, but the partitioning between the nitrogen oxide species varies according to the specific conditions characteristic of each type of airmass.

a) Nitric acid

Nitric acid is a strong mineral acid that contributes to acidic deposition from the air. In terms of atmospheric chemistry, HNO₃ is a major sink for active nitrogen. In daylight, HNO₃ is formed by the reaction of NO₂ with the OH radical:

\[
\text{M} \quad \text{NO}_2 + \text{OH} \rightarrow \text{HNO}_3
\]  (2-11)

This reaction is a chain-terminating step in the free radical chemistry that produces urban photochemical smog and it removes reactive nitrogen as well as the hydroxyl radical. Reaction 2-11 is a relatively fast reaction that can produce significant amounts of HNO₃ over a period of a few hours. At night, in polluted air containing significant ozone concentrations, the heterogeneous reaction between gaseous N₂O₅ and liquid water is thought to be a source of HNO₃ (N₂O₅ is produced from NO₃ (see section 2.4.3.5) and NO₂). This pathway to HNO₃ production is negligible during daytime, because the NO₃ radical photolyses rapidly and is not present in sufficient quantities to react with NO₂. The NO₃ radical can also abstract a hydrogen atom from certain organic compounds (such as aldehydes, dicarbonyls and cresols) to provide another night-time source of HNO₃.
Logan (1983) has estimated a lifetime of 1 to 10 days for HNO₃ in the lower troposphere. The primary removal mechanism is deposition. The loss of HNO₃ by rain-out is subject to precipitation frequency while the loss rate by dry deposition varies with the nature of the ground and vegetation and atmospheric mixing characteristics of the boundary layer. Chemical destruction mechanisms for HNO₃ also exist, but their importance is not well understood and is suspected to be minor for the lower troposphere.
Plrysical and Chemical Properties

In the presence of NH₃, HNO₃ may form the salt, ammonium nitrate:

\[
\text{HNO}_3(g) + \text{NH}_3(g) \rightarrow \text{NH}_4\text{NO}_3 \quad (2-12)
\]

Ammonium nitrate gas readily condenses to the particulate phase. Ammonium nitrate aerosol can be responsible for significant visibility reduction and particulate pollution, e.g., where nitric acid is produced in air from urban areas and this interacts with NH₃ emitted from agricultural operations.

b) Nitrous acid

\[
\text{HNO}_2 \text{ is produced from the reaction of NO and OH:}
\]

\[
\text{NO} + \text{OH} \rightarrow \text{HNO}_2 \quad (2-13)
\]

In indoor air other reactions (particularly surface reactions) may be important sources of nitrous acid.

There have been a few measurements of nitrous acid in urban environments (Harris et al., 1982; Winer et al., 1987). Daytime levels of nitrous acid are expected to be low because it photolyses rapidly, yielding NO and \( \cdot \text{OH} \). This reaction probably serves as a source of \( \cdot \text{OH} \) radicals during the morning in urban regions, where nitrous acid may form (from NO, NO₂ and H₂O) and accumulate during the night-time hours. Reaction 2-13 may lead to a build up of nitrous acid in urban air only during the late afternoon and evening hours when sunlight intensities are low but some \( \cdot \text{OH} \) radicals are still present.

c) Peroxynitric acid

While peroxynitric acid (\( \text{HO}_2\text{NO}_2 \)) has never been measured in the atmosphere, it is expected to be present in the upper troposphere. Models suggest concentrations in the 10 to 100 ppt range at altitudes above 6 kilometres (Logan, 1983; Singh, 1987). \( \text{HO}_2\text{NO}_2 \) is thermally unstable, so that boundary layer concentrations are expected to be extremely low (< 1 ppt). Peroxynitric acid is formed through the combination of a hydroperoxy (\( \text{HO}_2 \)) radical with NO₂. In the upper troposphere, \( \text{HO}_2\text{NO}_2 \) is destroyed by photolysis or by reaction with \( \cdot \text{OH} \) radicals.
Peroxyacetyl nitrates

Peroxyacetyl nitrate (PAN) is the most abundant of this family of organic nitrates. The second most abundant homologue, peroxypropionyl nitrate (PPN), is generally less than 10% of the PAN concentration, and species with higher relative molecular mass, such as PBzN, are expected to have even lower concentrations. PAN is a strong oxidant and is known to be phytotoxic; it is formed from the reaction of acetylperoxy radical with NO:

\[ \text{CH}_3\text{C}()\text{O}+\text{NO}_2+\text{M} \rightarrow \text{CH}_3\text{C}()\text{O}_2\text{NO}_2+\text{M} \quad (2-14) \]

PAN is thermally unstable and so its lifetime is very dependent on ambient temperature. For example, PAN lifetimes of about 5 and 20 h have been calculated for 20 °C and 10 °C, respectively.

In cold conditions PAN can serve as a reservoir for reactive nitrogen, which is liberated when the temperature of the air is increased. PAN can be lost from the atmosphere by dry deposition over land, but it is very likely that a significant fraction of PAN produced in urban plumes can be transported into the regional environment.

e) Nitrate radical

The nitrate (NO₃) radical is a short-lived species formed mainly by the reaction of NO₂ with O₃ although other sources of NO₃ radicals exist (Wayne et al., 1991).

\[ \text{NO}_2 + \text{O}_3 \rightarrow \text{NO}_3 + \text{O}_2 \quad (2-15) \]

NO₃ also reacts with NO₃ to form N₂O₅

\[ \text{M} \text{NO}_2 + \text{NO}_3 \rightarrow \text{N}_2\text{O}_5 \quad (2-16) \]

Nitrate radicals rapidly photolyse, resulting in a lifetime of about 5 seconds at midday. They also react rapidly with NO, which limits their lifetime both during the day- and night-time hours. At night if atmospheric NO concentrations are approximately 320 pptv, then the lifetime of NO₃ radicals is similar to that at midday (about 5 seconds).

At night, NO₃ concentrations range from about 0.3 ppt in clean tropospheric air to 70 ppt in urban areas (Biermann et al., 1988).
In clean background environments, it has been reported that measured NO₃ radical levels are significantly less than those predicted by the above reactions. Several loss mechanisms have been suggested (Noxon et al., 1980; Platt et al., 1981): (i) NO₃ radical reaction with organic compounds; (ii) heterogeneous losses of NO₃ radicals and/or N₂O₅ on particle surfaces; (iii) reactions of NO₃ radicals with H₂O vapour; and (iv) reaction of NO₃ radicals with NO.

N₂O₅ is formed from NO₃ and NO₂ (reaction 2-15). Since NO₃ is present only at night, N₂O₅ is also primarily a night-time species. N₂O₅ is thermally unstable, decomposing to NO₃ and NO₂ (reaction 2-15). At high altitudes in the troposphere, where temperatures are low, N₂O₅ can act as a temporary reservoir for NO₃. Dinitrogen pentoxide photolyses at wavelengths less than 330 nm to give NO₃ and NO₂.

Dinitrogen pentoxide reacts heterogeneously with water to form HNO₃. This serves as the main night-time production mechanism for HNO₃ and it provides an important route for removal of oxidized nitrogen from the atmosphere, since HNO₃ is readily removed by dry and wet deposition. Other atmospheric reactions of N₂O₅ include its reaction with gas-phase water to form HNO₃ and possible reactions with aromatic VOCs such as naphthalene and pyrene (Pitts et al., 1985; Atkinson et al., 1986). Nitroarenes appear to be the product of N₂O₅-aromatic reactions.

2.4.3 Advection and dispersion of atmospheric nitrogen species

The transport and dispersion of the various nitrogen species is dependent on both meteorological and chemical parameters. Advection, diffusion and chemical transformations dictate the atmospheric residence time of a particular trace gas. Nitrogenous species that undergo slow chemical changes in the troposphere and are not readily removed by depositional processes can have atmospheric lifetimes of several months. Gases with lifetimes of the order of months can be dispersed over continental scales and possibly even over an entire hemisphere. At the other extreme are gases that undergo rapid chemical transformation and/or depositional losses limiting their atmospheric residence times to a few hours or less. Dispersion of these short-lived species may be limited to only a few kilometres from their point of emission.
Surface emissions are dispersed vertically and horizontally through the atmosphere by turbulent mixing processes. These processes are dependent to a large extent on the vertical temperature structure of the boundary layers and on wind speed. In the vertical dimension, transport occurs as follows (see also Fig. 2.):

a) the daytime and/or night-time mixed layer; this layer can extend from the surface up to a few hundred metres at night or to several thousand metres during the daytime;

b) a layer that can exist during the night-time above a low level surface inversion and below the daytime mixing height; this layer generally is situated between 200 and 2000 m altitude;

c) the free troposphere; this transport zone is above the boundary layer mixing region.

Fig. 2. Schematic diagram of the combined reactions of nitrogen, oxygen and hydrogen (From: Finlayson-Pitts & Pitts, 1986)
During the warm, summertime period, vertical mixing follows a fairly predictable diurnal cycle. A surface inversion normally develops during the evening hours and persists throughout the night-time and morning period until broken by sunlight heating the surface of the earth. While the inversion is in place, surface NO\textsubscript{2} emissions can lead to relatively high local concentrations because of restricted vertical dispersion. Following the break-up of the night-time surface inversion, vertical mixing will increase and surface-based emissions will disperse to higher altitudes. The depth of the vertical mixing during the daytime is often controlled by synoptic weather features. Temperature inversions aloft, associated with high pressure systems, are common in many parts of the world.

The dispersion processes described above, coupled with the chemical transformations of reactive nitrogen compounds, determine the distances oxidized nitrogen will be transported in the troposphere. A reasonable understanding exists concerning the short-term (daylight hours) fate of NO\textsubscript{2} emitted in urban areas during the morning hours. As described above, NO\textsubscript{2} emitted in the early morning hours in an urban area will disperse vertically and move downwind as the day progresses. On sunny summer days, most of the NO\textsubscript{2} will have been converted to HNO\textsubscript{3} and PAN by sunset. Much of the HNO\textsubscript{3} will be removed by depositional processes as the air mass moves along. After dusk, an upper portion of the daytime mixed layer will be decoupled from the surface because of formation of a low-level radiation inversion. Transport will continue in this upper level during the night-time hours and, although photochemical processes will cease, dark-phase chemical reactions can proceed. Peroxyacetyl nitrate and HNO\textsubscript{3}, if carried along in this layer, can be transported long distances.

2.4.3.1 Transport of reactive nitrogen species in urban plumes

Overall removal rates for reactive nitrogen species during daytime at mid-latitudes have been measured or calculated for a few areas. For example, in the plume from Boston, USA, after correction for dilution, removal rates ranged from 0.14 to 0.24 h\textsuperscript{-1} on 4 days (Spicer, 1982, Altshuller, 1986). In Los Angeles and Detroit, the removal rate has been estimated to be 0.04-0.1 h\textsuperscript{-1} (Calvert, 1976; Chang et al., 1979; Kelly, 1987). Formation and removal of HNO\textsubscript{3} is thought to be the rate-controlling step for removal of reactive nitrogen.
2.4.3.2 Air quality models

Air quality models are mathematical descriptions of pollutant emissions, atmospheric transport, diffusion and chemical reactions of pollutants. However, air quality models are very complex and difficult to test for validity. Inputs include emissions, topography and meteorology of a region. Air quality models represent an integration of knowledge for the chemistry and physics of the atmospheric system; they offer some predictive capability for the effectiveness of pollution control strategies. Models have also been developed for indoor air.

2.4.3.3 Regional transport

Transport of reactive nitrogen species in regional air masses can involve several mechanisms. Mesoscale phenomena, such as land-sea breeze circulations or mountain-valley wind flows, will transport pollutants over distances of ten to hundreds of kilometres. On a larger scale, synoptic weather systems such as the migratory highs that cross the eastern USA and other areas of the world in the summertime influence air quality over many hundreds of kilometres. The accumulation and fate of nitrogen compounds will differ somewhat between the mesoscale and synoptic systems. Mountain-valley and land-water transport mechanisms have dual temporal scales because of their dependence on solar heating. However, in the larger-scale synoptic systems, reactive nitrogen species can build up over multiday periods. The residence time of air parcels within a slow-moving high pressure system can be as long as 6 days (Vukovich et al., 1977).

In many cases, the transport mechanisms mentioned above are interrelated. Mountain-valley or land-water breezes can dictate pollutant transport in the immediate vicinity of sources, but the eventual fate of reactive nitrogen species will be distribution into the synoptic system.

2.5 Conversion factor for nitrogen dioxide

1 ppm = 1.88 mg/m³
1 mg/m³ = 0.53 ppm

2.6 Summary

Combustion provides the major source of oxides of nitrogen in both indoor and outdoor air, producing mostly NO with some NO₂.
The sum of NO and NO$_2$ is generally referred to as NO$_x$. Once released into the air, NO is oxidized to NO$_2$ by available oxidants, particularly O$_3$, and by photochemical reactions involving reactive organic compounds. This happens rapidly under some conditions in outdoor air; for indoor air, it is generally a much slower process. Nitrogen oxides are a controlling precursor of ozone and smog formation; interactions of nitrogen oxides (except N$_2$O) with reactive organic compounds and sunlight form ozone in the troposphere and smog in urban areas.

In both indoor and outdoor air, NO and NO$_2$ may undergo reactions to form a suite of other nitrogenous species including HNO$_2$, HNO$_3$, NO$_3$, N$_2$O$_5$, PAN and other organic nitrates. The complete suite of gas-phase nitrogen oxides is referred to as NO$_x$. The partitioning of nitrogen among these compounds is strongly dependent on the concentrations of other oxidants, sunlight exposure, the presence of reactive organic compounds and the meteorological history of the air.

A sensitive, specific and reliable analytical method exists for measuring NO (by the chemiluminescent reaction with ozone), but this is an exception for NO$_x$ species. Chemiluminescence is also the most common technique used for NO$_2$, which is first reduced to NO. Unfortunately, the method of reduction usually used is not specific for NO$_2$, and it has various conversion efficiencies for other oxidized nitrogen compounds that may also be present in the air sample. For this reason, care must be taken in interpreting the NO$_2$ values given by the common chemiluminescence analyser, as the signal may include responses from interfering compounds. Additional difficulties arise from nitrogen species such as HNO$_3$ that may partition between the gas and particulate phases both in the atmosphere and in the sampling procedure.
3. SOURCES, EMISSIONS AND AIR CONCENTRATIONS

3.1 Introduction

Oxides of nitrogen can have significant concentrations in ambient air and in indoor air. The types and concentrations of nitrogenous compounds present can vary greatly from location to location, with time of day, and with the season. The main sources of nitrogen oxides emissions are combustion processes. Fossil fuel power stations, motor vehicles and domestic combustion appliances emit nitrogen oxides, mostly in the form of NO but with some (usually less than about 10%) in the form of NO₂. In the air chemical reactions occur which oxidize NO to NO₂ and other products (chapter 2). Also, there are biological processes in soils which liberate nitrogen species, including N₂O. Emissions of N₂O can cause perturbation of the stratospheric ozone layer.

Human health maybe affected when significant concentrations of NO₂ or other nitrogenous species, such as PAN, HNO₃, HNO₂ and nitrated organic compounds, are present. In addition, nitrates and nitric acid can cause significant effects on ecosystems when deposited on the ground.

Indoors, the use of combustion appliances for cooking and heating can give rise to greater NO and NO₂ concentrations than are present outdoors, especially when the appliance is not vented to the outside. Recent research has shown that in these circumstances nitrous acid can reach significant concentrations (Brauer et al., 1993).

This chapter discusses both ambient and indoor sources of nitrogenous compounds, their emissions, and the resulting concentrations that may directly affect human health or participate in atmospheric chemical pathways leading to effects on human health and welfare. Nitrogen-containing compounds are also of particular interest because of their secondary impacts. For example, production of photochemical smog and ozone pollution depends on emissions to the air of nitrogen oxides together with volatile organic compounds. Nitric acid, which is produced in the air by the reaction of hydroxyl radicals (OH) with NO₂, is one of the major components of acidic precipitation. As well as being present in the gas phase, oxidized nitrogen can, by reaction and adsorption, become incorporated into aerosol particles. Graedel et al. (1986) identified 20 inorganic nitrogen-containing species.
detectable in the atmosphere. Near cities and urban regions the species usually present in greatest concentrations are NO and NO₂, and these are the most reliably measured and frequently monitored nitrogen oxide species.

Knowledge of emission patterns and concentrations of nitrogenous compounds is critically important for air quality planning and human health and environment risk assessments. Because nitrogen oxides and their reaction products have lifetimes of several days in the atmosphere, they can be transported long distances by the wind and give rise to environmental impacts far from their source of emission.

3.2 Sources of nitrogen oxides

Combustion systems emit NO and NO₂, and together these species are usually denoted as NOₓ.

When NOₓ emissions are expressed in mass units, the mass is expressed as if all the NO had been converted to NO₂. Another convention adopted in some of the following sections is to report the emissions on a mass basis in terms of the nitrogen content.

3.2.1 Sources of NOₓ emission

3.2.1.1 Fuel combustion

Annual production of NOₓ from combustion of fossil fuels is typically estimated from emission factors for various combustion processes, combined with worldwide consumption data for coal, oil and natural gas. Logan (1983) provided a tabular summary of emission factors, which has been updated by the US National Acid Precipitation Assessment Program (Placet et al., 1991). Owing to variations in process operating conditions, the emission factors must be considered to be uncertain by about ±30%. Table 3 provides a summary of global emission estimates for NOₓ according to fuel type. The estimates of Logan (1983) are slightly higher than those of Ehhalt & Drummond (1982), the largest discrepancies being in emission estimates for the transportation sector. The differences arise because Logan (1983) based estimates of emissions on fuel usage, while Ehhalt & Drummond (1982) scaled the totals somewhat indirectly by using world automobile population numbers.
Table 3. Estimates of global emissions of nitrogen oxides (NO\textsubscript{x}) from combustion of fossil fuels and biomass (from: US EPA, 1993)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Source type</th>
<th>Annual consumption\textsuperscript{b} (10\textsuperscript{6} tonnes, unless indicated otherwise)</th>
<th>Emission factors\textsuperscript{c}</th>
<th>Global source strength\textsuperscript{d} (10\textsuperscript{6} tonnes nitrogen/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(E &amp; D)</td>
<td>(L)</td>
<td>(C et al.)</td>
</tr>
<tr>
<td>Fossil fuels\textsuperscript{e}</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hard coal</td>
<td>2150</td>
<td>2696</td>
<td>-</td>
</tr>
<tr>
<td>Lignite</td>
<td>810</td>
<td>-</td>
<td>-</td>
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<td>Light fuel oil</td>
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<tr>
<td>Heavy fuel oil</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Natural gas</td>
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<td>1.2 x 10\textsuperscript{6} m\textsuperscript{3}</td>
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<tr>
<td>Industrial sources</td>
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<td>-</td>
</tr>
<tr>
<td>Automobiles</td>
<td>(4.1-5.4) x 10\textsuperscript{12} km</td>
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</tr>
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<td>Total</td>
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<table>
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<tr>
<th>Source type</th>
<th>Annual consumption (10^6 tonnes, unless indicated otherwise)</th>
<th>Emission factors^b</th>
<th>Global source strength (10^5 tonnes nitrogen/year)</th>
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<td>(L)</td>
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<td><strong>Biomass burning</strong></td>
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<td>Savanna</td>
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<td>Forest clearings</td>
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<td>1900</td>
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<td></td>
<td>11.2 (5.6-16.4)</td>
<td>12.0</td>
<td>10.6</td>
</tr>
</tbody>
</table>

^a Estimates according to Ehhalt & Drummond (1982) (E & D) and Logan (1983) (L). Ranges are given in parentheses.

^b Emission factors refer to grams of nitrogen per kg of fuel consumed, unless indicated otherwise.

^c Combustion of petroleum refining and manufacture of nitric acid and cement; global emissions were obtained by scaling USA emissions for each industrial process.

^d Grams of nitrogen per m^3 of fuel consumed.

^e Grams of nitrogen per km.

^f For biomass-burning, Czutzen et al. (1979) (C et al.) have given annual consumption rates differing somewhat from those of the other authors. The data of Czutzen et al. (1979) and the resulting nitrogen oxides production rates are included for comparison.
Dignon (1992) has assembled a database for mapping (with a resolution of one degree in latitude and longitude) and estimated global NO$_x$ and sulfur oxides emissions from their common principal anthropogenic source, i.e. fossil fuel combustion. For 1980, the global total was estimated to be 22 million tonnes, as nitrogen. Countries heading the list (in millions of tonnes of nitrogen per year) were: USA, 6.4; USSR, 4.4; China, 1.7; Japan, 0.80; and Federal Republic of Germany, 0.66. An estimated 95% of NO$_x$ emissions from fossil fuel combustion originates in the northern hemisphere.

For oceanic regions, shipping is a source of NO$_x$ emissions. Aircraft also emit nitrogen oxides and this may be significant for the upper troposphere and stratosphere.

3.2.1.2 Biomass burning

Table 3 includes a breakdown of estimates for release of NO$_x$ from burning of biomass. In natural fires and the burning of wood, temperatures are rarely high enough to cause oxidation of nitrogen molecules of the air. The emissions are thereby more closely related to the fixed nitrogen content of the fuel. Logan (1983) reviewed a number of experimental determinations of nitrogen emission factors that indicate yields are highest for grass and agricultural refuse fires (1.3 g nitrogen/kg fuel), less for prescribed forest fires (0.6 g nitrogen/kg fuel), and still lower for burning of fuel wood in stoves and fireplaces (0.4 g nitrogen/kg fuel). The values roughly reflect differences in nitrogen content of the materials burned. Biomass burning is mainly associated with agricultural practices in the tropics, which include plant, slash, and shift practices as well as natural or intentional burning of savanna vegetation at the end of the dry season. Forest wildfires and use of wood as fuel make a lesser contribution.

3.2.1.3 Lightning

Thunderstorm activity has been viewed as a major NO$_x$ source since 1827, when Von Liebig proposed it as a natural mechanism for fixation of atmospheric nitrogen. Electrical discharges in air generate NO$_x$ by thermal dissociation of nitrogen molecules due to ohmic heating inside the discharge channel and shockwave heating of the surroundings. Laboratory studies by Chameides et al. (1977) and Levine et al. (1981) indicate an NO$_x$ yield of $6 \times 10^{16}$ molecules per joule of spent energy. Great uncertainties exist, however, about the total energy generated by lightning in the
Sources, Emissions and Air Concentrations

atmosphere. Noxon (1976, 1978) first studied the increase of NO\textsubscript{x} in the air during a thunderstorm. His results provide the basis for many of the estimates shown in Table 4. Reviews by Kowalczyk & Bauer (1981) Borucki & Chameides (1984) and Albritton et al. (1984) provide a best estimate of annual generation by lightning: 1 million tonnes of NO\textsubscript{x} in North America and 13 million tonnes globally (Placet et al., 1991).

3.2.1.4 Soils

The biochemical release of NO\textsubscript{x} from soils is poorly understood, and the flux estimates must be viewed with caution. Both rely on the observations by Galbally & Roy (1978), who used the flux box method in conjunction with chemiluminescence detection of NO\textsubscript{x}. They found average fluxes of 5.7 and 12.6 \( \mu \)g nitrogen/m\textsuperscript{2}\cdot h on ungrazed and grazed pastures, respectively, where NO was the main product. More recent measurements of Slemr & Seiler (1984) indicate that the release of NO\textsubscript{x} from soils depends critically on the temperature and moisture content of the soil, which in turn complicates the estimate of the global emissions. Slemr & Seiler (1984) also found an average release rate of 20 \( \mu \)g nitrogen/m\textsuperscript{2} per h for uncovered natural soils, evenly divided between NO and NO\textsubscript{2}. Grass coverage reduced the escape flux, whereas fertilization enhanced it. Ammonium fertilizers were about five times more effective than nitrate fertilizers. This suggests that nitrification as a source of NO\textsubscript{x} is more important than denitrification. According to Slemr & Seiler (1984), an annual global flux of 10 million tonnes of nitrogen represents an upper limit to the release of NO\textsubscript{x} from soils. Galbally et al. (1985) presented more detailed estimates for arid lands, and Table 4 provides a compilation of current literature used to develop the global budgets. Soil is also a source of N\textsubscript{2}O and NH\textsubscript{3} emissions.

In the presence of low concentrations, plants can emit NH\textsubscript{3}, rather than absorb it. This is especially true with senescing and with highly fertilized plants (Grünhage et al., 1992; Holtan-Hartwig & Bockman 1994; Fangmeijer et al., 1994). Release to the atmosphere of N\textsubscript{2} and NO by plants has also been reported. In some cases this was part of the response following exposure to nitrogen-containing pollutants, but other mechanisms are involved (Wellburn, 1990). NO and N\textsubscript{2}O are emitted in significant quantities by the soil. The reason why the deposition velocity of NO is relatively low see (see Table 5) is partly due to the fact that the downward flux (and uptake by the canopy) is "mathematically" compensated by soil emissions. In other words: a low deposition
Table 4. Global and North America natural emissions (average and range) of nitrogen oxides (NO\textsubscript{x}) from lightning, soils and oceans

<table>
<thead>
<tr>
<th></th>
<th>Global (10\textsuperscript{8} tonnes/year)</th>
<th>North America (10\textsuperscript{8} tonnes/year)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lightning</strong></td>
<td>8.6 (2.6-26)</td>
<td>18</td>
<td>Borucki &amp; Chameides (1984)</td>
</tr>
<tr>
<td></td>
<td>13 (7-26)</td>
<td>1.7</td>
<td>Abirzon et al. (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3-2</td>
<td>Kowalczyk &amp; Bauer (1981); Placet et al. (1991)</td>
</tr>
<tr>
<td><strong>Soils</strong></td>
<td>50 (as NO\textsubscript{2})</td>
<td>30 (as NO)</td>
<td>Lipschultz et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>2</td>
<td>Levine et al. (1984); Galbally &amp; Roy (1978)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slemr &amp; Seiler (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Placet et al. (1991)</td>
</tr>
<tr>
<td><strong>Oceans</strong></td>
<td>0.35</td>
<td></td>
<td>Zafiriou &amp; McFarland (1981); Logan (1983)</td>
</tr>
</tbody>
</table>
Table 5. Deposition velocity of nitrogen-containing gases and aerosols

<table>
<thead>
<tr>
<th>Deposition velocity (mm/second)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂</td>
<td>0.1-10</td>
</tr>
<tr>
<td>NO</td>
<td>0.2-1</td>
</tr>
<tr>
<td>NH₃</td>
<td>12 (-5 - 40)</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>1.4 (0.03-15)</td>
</tr>
</tbody>
</table>

Velocity does not always mean that the uptake by the vegetation is low. In the case of NO₂, soil emissions are mostly larger than deposition; this emission is the result of denitrification and is positively related to the nitrogen and water content and the temperature of the soil. This is why the release of nitrogen from the ecosystem in the form of NO₂ is dependent on the ecosystem type, climate and land use (fertilization and water table height). Skiba et al. (1992) estimated for the United Kingdom the NO and NO₂ emissions from agricultural land to be 2-6% of the nationwide NO emissions and 16-64% of the NO₂ emissions, respectively.

Estimates of global emissions of NO₂ and ammonia are summarized in Table 6.

3.2.1.5 Oceans

There have been few measurements of NO₃, NO₂ or NH₄ fluxes over the ocean, and current literature suggests that the sea is a negligible source of NO. Zafiriou & McFarland (1981) observed a supersaturation of seawater with regard to NO in regions of relatively high concentrations of nitrite, owing to upwelling conditions. The excess NO must, in this case, arise from photochemical decomposition of nitrite by sunlight. Logan (1983) estimated a local source strength of $1.3 \times 10^{12}$ molecules/m² per second under these conditions. Linear extrapolation results in an annual global flux estimate of 350,000 tonnes of nitrogen.
3.2.2 Removal from the ambient environment

Wet precipitation and dry deposition provide two of the major mechanisms for removal of NO₃ from the atmosphere. The addition to the plant soil ecosystem of nitrate (and ammonium) by rainwater constitutes an important source of fixed nitrogen to the terrestrial biosphere, and until 1930 practically all studies of nitrate in rainwater were concerned with the input of fixed nitrogen into agricultural soils. Eriksson (1952) and Boettger et al. (1978) have compiled many of the available data. Despite the wealth of information, it remains difficult to derive a global average for the deposition of nitrate, because of an uneven global coverage of the data, unfavourably short measurement periods at many locations, and inadequate collection and handling techniques for rainwater samples. In addition, the concentration of nitrate in rainwater has increased in those parts of the world where the utilization of fossil fuels has led to a rise in the emissions of NOₓ, i.e. primarily western Europe and the USA.
Dry deposition is important as a sink for those gases that are readily absorbed by materials covering the earth surface. In the budget of NO\(_x\), the gases affected most by dry deposition are NO\(_2\) and HNO\(_3\). The deposition velocity of NO is too small and the concentration of peroxyacetyl nitrates is not high enough for a significant contribution.

According to Grennfelt et al. (1983) and Wellburn (1990), NO and HNO\(_3\) have a higher deposition velocity than NH\(_3\), but this was not quantified. HNO\(_3\) is assumed to have a deposition velocity equal to SO\(_2\): 1-30 mm/second (Table 5).

There are several other nitrogen-containing air pollutants with relatively high deposition velocities. These generally add only small amounts to the total nitrogen deposition, because most of the time their ambient concentrations are relatively low.

Atmospheric nitrogen deposition can significantly change the chemical composition of the soil. In the rooting zone these changes have an impact on vegetation. The changes in deeper soil layers are particularly relevant if groundwater is used as a source of drinking-water. Groundwater under fertilized agricultural land can be heavily polluted with nitrate (and aluminium), but this is beyond the scope of this chapter. Due to atmospheric nitrogen deposition, the groundwater under forests and other non-fertilized vegetation can become polluted with nitrate. For instance, in 20% of the forested area of the Netherlands, the nitrate concentration in phreatic groundwater is higher than 50 mg/litre (the EC drinking-water standard); in 37% it is higher than 25 mg/litre (Boumans & Beltman, 1991). The average annual nitrogen deposition in the Netherlands is 45 kg/ha; approximately 10 kg/ha is from dry deposition of NO\(_x\). The nitrate concentration in groundwater is strongly related to the soil type. With the same atmospheric deposition, the nitrate concentration increases as follows: peaty soils < moderately drained sandy soils < well-drained rich sandy soils (Boumans, 1994). A distinct relation also exists concerning the age of the trees: tree stands in Wales showed nitrate leaching (measured in the stream water draining the catchments), but only with stands older than 30 years. Younger trees used the nitrogen as nutrient, but the nitrogen demand of the older trees was lower. The annual nitrogen deposition in that region was estimated to be 20 kg/ha (Emmett et al., 1993).
3.2.3 Summary of global budgets for nitrogen oxides

The principal routes to the production of NO, are combustion processes, nitrification and denitrification in soils, and lightning discharges. The major removal mechanism is oxidation to HNO₃, followed by wet and dry deposition. In developing Table 7, the dry deposition velocities for NO₂ over bare soil, grass and agricultural crops were assumed to fall in the range of 3 to 8 mm/second. However, over water the velocities are significantly smaller, so that losses of NO₂ by deposition onto the ocean surface can be ignored. The absorption of nitric acid by soil, grass and water is rapid, and dry deposition correspondingly important, but the global flux is difficult to estimate because information on HNO₃ mixing ratios is still sparse. Logan (1983) adopted NO mixing ratios of 50 pptv over the oceans and 100 pptv over the continents. The mixing ratios assumed for NO₂ were 100 and 400 pptv, respectively. Allowance was made for higher mixing ratios in industrialized areas affected by pollution. Logan (1983) included the deposition of particulate nitrate over the oceans, using a settling velocity of 3 mm/second. This process contributes 2 million tonnes nitrogen/year to a total dry deposition rate of 12 to 22 million tonnes nitrogen/year.

Efforts by Boettger et al. (1978), Ehhalt & Drummond (1982), Galbally et al. (1985) and Warneck (1988) to quantify the sources and sinks have led to an improved understanding of the global budget of NOₓ, in which the flux of NOₓ into the troposphere and the rate of nitrate deposition are approximately balanced. Ehhalt & Drummond (1982) relied on the detailed evaluation of data by Boettger et al. (1978). Their analysis emphasized measurements from the period 1950 to 1977, and they prepared a world map for nitrate deposition rates, which were then integrated along 5° latitude belts. Logan (1983) considered recent network data from North America and Europe; Galloway et al. (1982) reported measurements of nitrate in precipitation at remote locations in Alaska, South America, Australia and the Indian Ocean. Both estimates gave wet nitrate deposition rates in the range of 2 to 14 million tonnes nitrogen/year for the marine environment and 8 to 30 million tonnes nitrogen/year on the continents. An earlier appraisal by Soederlund & Svensson (1976) led to rather similar values, i.e. 5 to 16 and 13 to 30 million tonnes nitrogen/year, respectively, although it was primarily based on Eriksson's (1952) compilation of data from the period 1880 to 1930.
On continents, one should also consider the interception of aerosol particulates by high growing vegetation. The interception of nitrate is expected to be particularly effective. Hoefken & Gravenhorst (1982) studied the enrichment of nitrate in rainwater collected underneath forest canopies compared to that collected in open areas outside forests. The effect is caused by the wash-off of dry-deposited material from foliage. Hoefken & Gravenhorst (1982) found that, in a beech forest, nitrate was enhanced by a factor of 1.4, whereas in a spruce forest enhancement by a factor of 4.1 occurred. Unfortunately, they were unable to differentiate between contributions of particulate nitrate versus gaseous nitrate to the total dry deposition.

If losses of NO\textsubscript{2} and HNO\textsubscript{3} by dry deposition are included in the total budget of NO\textsubscript{2}, one obtains a reasonable balance between the sources and sinks, as Table 7 shows. Ehhalt & Drummond (1982) noted that an appreciable part of their dry deposition is already included in their wet deposition rates, because rain gauges frequently are left open continuously, so that the collection of nitrate occurs during both wet and dry periods. For NO\textsubscript{2}, they estimated a dry deposition rate of 7 million tonnes nitrogen/year. Because of the uncertainty, they chose to include it in the error bounds and not in the mean value of total NO\textsubscript{2}-derived nitrogen deposition. Clearly, the total budget of NO\textsubscript{2} is far from being well defined. Moreover, in view of the relatively short residence times of chemical species involved in the NO\textsubscript{2} cycle, it is questionable whether a global budget gives an adequate description of the tropospheric behaviour of NO\textsubscript{2} and its reaction products. Supplemental regional budgets could be more appropriate.

### 3.3 Ambient concentrations of nitrogen oxides

Because cities usually have an aggregation of emissions sources, ambient concentrations of NO and NO\textsubscript{2} tend to be greatest in cities. High concentrations of NO are common in street canyons, owing to motor vehicle emissions. In rural areas the emissions may have spent considerable time in the atmosphere and have undergone reactions to produce significant concentrations of other species, such as HNO\textsubscript{3} and PAN.

#### 3.3.1 International comparison studies of NO\textsubscript{2} concentrations

Data for monthly average concentrations of NO\textsubscript{2} collected by the World Meteorological Organization at five background locations in Europe for the period 1983 to 1985 are summarized in
Table 7. Global budget (average and range) of nitrogen oxides in the troposphere (from US EPA, 1993)\(^a\)

<table>
<thead>
<tr>
<th>Type of source or sink</th>
<th>Global flux (10^8 tonnes nitrogen/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td></td>
</tr>
<tr>
<td>Fossil-fuel combustion</td>
<td>13.5 (8.2-18.5)</td>
</tr>
<tr>
<td>Biomass burning</td>
<td>11.2 (5.6-16.4)</td>
</tr>
<tr>
<td>Release from soils</td>
<td>5.5 (1-10)</td>
</tr>
<tr>
<td>Lightning discharges</td>
<td>5.0 (2-8)</td>
</tr>
<tr>
<td>NH(_3) oxidation</td>
<td>3.1 (1.2-4.9)</td>
</tr>
<tr>
<td>Ocean surface (biologic)</td>
<td>-</td>
</tr>
<tr>
<td>High-flying aircraft</td>
<td>0.3 (0.2-0.4)</td>
</tr>
<tr>
<td>Stratosphere</td>
<td>0.6 (0.3-0.9)</td>
</tr>
<tr>
<td>Total production</td>
<td>39 (19-59)</td>
</tr>
<tr>
<td>Losses</td>
<td></td>
</tr>
<tr>
<td>Wet deposition of NO(_2), land</td>
<td>17 (10-24)</td>
</tr>
<tr>
<td>Wet deposition of NO(_2), oceans</td>
<td>8 (2-14)</td>
</tr>
<tr>
<td>Wet deposition, combined</td>
<td>24 (15-33)</td>
</tr>
<tr>
<td>Dry deposition of NO(_x)</td>
<td>-</td>
</tr>
<tr>
<td>Total loss</td>
<td>24 (15-40)</td>
</tr>
</tbody>
</table>

\(^a\) Derived from estimates according to Ehhalt & Drummond (1982) and Logan (1983)

Fig. 3 (WMO, 1988, 1989). Fig. 4 presents published monthly averages of NO\(_2\) in 1987 for 12 stations in a cooperative network under the Organisation for Economic Co-operation and Development (OECD) (Grennfelt et al., 1989). These two figures show that concentrations of both NO\(_x\) and NO\(_2\) tend to be higher during winter months.

Measurements of NO\(_2\) in several countries during the late 1970s and early 1980s are summarized in "Assessment of Urban Air Quality" (WHO, 1988). The trends in composite annual averages...
Fig. 3. Monthly average NO\textsubscript{x} concentrations at five WMO background stations, 1983-1985 (data derived from: WMO, 1988, 1989)

C = Czechoslovakia, Svratouch
G = Germany, Wank Peak
H = Hungary, Kacskermet
P\textsubscript{J} = Poland, Jarczew
P\textsubscript{S} = Poland, Suwalki
Fig. 4. Monthly average NO₂ concentrations at 12 OECD stations, 1987
(from: Grennfelt et al., 1989)
Sources, Emissions and Air Concentrations

for urban NO\(_2\) monitoring stations in five countries are portrayed in Fig. 5 for the period 1975 to 1983. The trend in the Canadian data appears to have been downward, but essentially stable trends were evident for data from the other countries. Annual averages in the 1980-1984 period for 42 cities around the world are summarized in the same report (WHO, 1988). During that period, only one city, Sao Paulo, reported an annual average greater than 0.053 ppm (100 \(\mu g/m^3\)).

Short-term peak values (1-h or 30-min maxima, or 98th or 95th percentile values) have been reported for 18 cities during the 1980-1984 period (WHO, 1988). Ten of these cities (Amsterdam, Brussels, Hamilton, Hong Kong, Jerusalem, Montreal, Munich, Rotterdam, Tel Aviv and Toronto) reported values above the WHO 1-h guideline level of 400 \(\mu g/m^3\) (0.21 ppm) for at least one year during that 5-year period. For eleven cities in the WHO report, both the annual average and a “1-hour” peak statistic were reported for the 1980-1984 period. Fig. 6 compares these two statistics. It shows that three cities, Amsterdam, Jerusalem and Tel Aviv, reported an average peak value above the WHO 1-hour guideline value of 400 \(\mu g/m^3\) (0.21 ppm). It should be kept in mind that the peak-value statistic is more susceptible to undetected spurious measurements than is the annual average. Data from the remaining eight cities place them in the quadrant below the target levels for both the annual average and the 1-hour peak. A similar situation is seen in the majority of cities in the USA and is discussed in the next section.

More recent data on NO\(_2\) trends in the world’s largest cities have been reported by WHO/UNEP (1992) in the monograph “Urban Air Pollution in Megacities of the World”. Such trends for six selected cities from various regions of the world are illustrated in Fig. 7, a composite of figures extracted directly from the WHO/UNEP (1992) report. In general, the overall trends appeared to be relatively stable for most of the cities (and/or specific neighbourhoods). However, there were a few exceptions, e.g., an apparent decrease in the late 1980s for Bombay and an apparent increase during the same period for some areas of Moscow. There are substantial differences in the concentrations reported for different cities.

Table 8 summarizes emissions of nitrogen oxides and ambient monitoring data from the WHO/UNEP (1992) report for the years indicated. Included are estimates for total emissions and percentages attributed to mobile sources, primarily private motor
Fig. 5. Year-to-year composite annual NO₂ averages for urban stations in five countries (adapted from: WHO, 1988)
Fig. 6. Annual averages and peak values of NO₂ concentrations reported for 11 cities, 1980-1984 (adapted from: WHO, 1988)
Fig. 7. Graphs illustrating trends in NO\textsubscript{2} ambient air concentrations for representative major cities (from: WHO/UNEP, 1992)
Annual mean nitrogen dioxide concentrations at selected sites

Fig. 7 (contd).
Annual mean nitrogen dioxide concentrations at selected sites.

Moscow

Annual average nitrogen dioxide concentrations

Fig. 7 (cont'd).
<table>
<thead>
<tr>
<th>City</th>
<th>Total emissions of nitrogen oxides (tonnes/year)</th>
<th>Mobile source contribution (%)</th>
<th>Ambient concentration (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangkok</td>
<td>60 000 (1990)</td>
<td>30</td>
<td>max 1 h NOₓ (as NO₂) 270 at one site; &lt; 320 at three stations (1987)</td>
</tr>
<tr>
<td>Beijing</td>
<td>na</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bombay</td>
<td>56 000 (1990)</td>
<td>52</td>
<td>NOₓ 70-85 (annual 98th percentile, 1990)</td>
</tr>
<tr>
<td>Buenos Aires</td>
<td>27 000 (1989)</td>
<td>48</td>
<td>na</td>
</tr>
<tr>
<td>Cairo</td>
<td>24 700 (1989)</td>
<td>23</td>
<td>NOₓ 380-1400 (1979, monthly means; single study)</td>
</tr>
<tr>
<td>Calcutta</td>
<td>36 550 (1990)</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Delhi</td>
<td>73 000 (1990)</td>
<td>20 (mostly diesel)</td>
<td>NO₂ 500 (1990, 8 h)</td>
</tr>
<tr>
<td>Jakarta</td>
<td>20 500 (1989)</td>
<td>75</td>
<td>NOₓ 28 (1990, annual mean)</td>
</tr>
<tr>
<td>Karachi</td>
<td>50 000 (1989)</td>
<td>38</td>
<td>38-544 (12-13 June 1986; single study)</td>
</tr>
<tr>
<td>London</td>
<td>79 000 (1983)</td>
<td>75 (1984)</td>
<td>NOₓ max 1 h 867; &gt; 600 for 8 h; &gt; 205 for 72 h (episode 12-15 Dec. 1991); 98th percentile &gt; 135; 50th percentile &gt; 50 (1989); NO recorded but not reported</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>440 000 (1987)</td>
<td>75</td>
<td>NOₓ max 1 h 526; &gt; 400 at 8 out of 24 stations (1990)</td>
</tr>
<tr>
<td>Manilla</td>
<td>119 000 (1990 - dubious accuracy)</td>
<td>90</td>
<td>na</td>
</tr>
<tr>
<td>Mexico City</td>
<td>177 300 (1991)</td>
<td>75</td>
<td>NOₓ hourly maxima 301-714 (1986-91)</td>
</tr>
<tr>
<td>Moscow</td>
<td>210 000 (1990)</td>
<td>19</td>
<td>NOₓ max daily means 100-150</td>
</tr>
</tbody>
</table>
Table 8 (contd).

<table>
<thead>
<tr>
<th>City</th>
<th>Total emissions of nitrogen oxides (tonnes/year)</th>
<th>Mobile source contribution (%)</th>
<th>Ambient concentration (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>120 000 New York City; 513 000 New York metropolitan area (1985)</td>
<td>na</td>
<td>NO₂ 1 h max 402; daily max 180; annual mean 87 (1980)</td>
</tr>
<tr>
<td>Rio de Janeiro</td>
<td>63 000 (1978)</td>
<td>92</td>
<td>na</td>
</tr>
<tr>
<td>Sao Paulo</td>
<td>245 000 (1988)</td>
<td>82</td>
<td>NO₂ max 1 h 600-1500 (1988)</td>
</tr>
<tr>
<td>Seoul</td>
<td>270 000 (1990)</td>
<td>78</td>
<td>NO₂ annual means only</td>
</tr>
<tr>
<td>Shanghai</td>
<td>127 000 (1983); 1991 emissions assumed 50% higher; i.e. = 190 000</td>
<td>na</td>
<td>NO₂ annual mean 50; indoor level 90</td>
</tr>
<tr>
<td>Tokyo</td>
<td>52 700 (1965)</td>
<td>67% from motor vehicles; 5% from ship and aircraft</td>
<td>daily mean 98th percentile &gt; 115 tolerable level at 25% of stations</td>
</tr>
</tbody>
</table>

* na = not available

vehicles and public land transport systems. However, the quality and type of information contained in the report is mixed, reflecting a variety of monitoring methods and reporting policies in different countries. Ambient data in some cities was reported as NOₓ, and in others as NO₂; reporting periods varied from one hour to one year.

As shown in Table 8, of importance for air quality management is the large contribution of NOₓ from motor vehicles reported for some cities and the continuing growth in this contribution. For example, emissions from vehicles in Bombay (about 29 000 tonnes per year in 1990) are expected to increase by an additional 14 600 tonnes/year by the year 2000 (WHO/UNEP, 1992).

Estimates for Jakarta attribute some three-quarters of NOₓ emissions to motor vehicles, which is comparable with London,
Los Angeles and Mexico City. Data from Manila indicate that some 90% of NO\textsubscript{2} originates from motor vehicles.

3.3.2 Example case studies of NO\textsubscript{2} and NO\textsubscript{2} concentrations

Data from a range of countries and locations are given in Table 9 (Agra, India) and Tables 10 and 11 (various cities in China).

Table 9. Concentrations of NO\textsubscript{2} measured in the vicinity of the Taj Mahal, Agra, India\textsuperscript{a}

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean monthly concentration range (µg/m\textsuperscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>5.5 to 41.9</td>
</tr>
<tr>
<td>1988</td>
<td>6.3 to 33.1</td>
</tr>
<tr>
<td>1989</td>
<td>4.2 to 15.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Highest concentrations tend to occur in winter.

Personal communication from R.R. Khan, Ministry of Environment and Forests, New Delhi, India (1994)

In urban areas in the USA, hourly patterns at fixed-site ambient air monitors often follow a bimodal pattern of morning and evening peaks, related to motor vehicular traffic patterns. Sites affected by large stationary sources of NO\textsubscript{2} (or NO that reacts to produce NO\textsubscript{2}) are often characterized by short episodes at relatively high concentrations, as the plume moves to downwind areas.

Since 1980, the annual average level among NO\textsubscript{2}-reporting stations in the USA has been below 0.03 ppm, with no significant trend evident. This is exemplified in Fig. 8 (US EPA, 1991) by annual averages for the period 1980 to 1989 for 60 metropolitan areas subdivided into three population categories: 16 areas with a population of 250 000 to 500 000, 14 with 500 000 to one million, and 30 with over one million. No group exhibited a time trend, but the areas with more than one million people clearly reported levels higher than the smaller metropolitan areas. For 103
Table 10. Annual average NO\textsubscript{x} concentration (μg/m\textsuperscript{3}) in China from 1981 to 1990

<table>
<thead>
<tr>
<th>Year</th>
<th>Cities all over China</th>
<th>Southern cities</th>
<th>Northern cities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration range</td>
<td>Annual average</td>
<td>Concentration range</td>
</tr>
<tr>
<td>1981</td>
<td>10-90</td>
<td>50</td>
<td>10-80</td>
</tr>
<tr>
<td>1982</td>
<td>10-110</td>
<td>45</td>
<td>10-90</td>
</tr>
<tr>
<td>1983</td>
<td>9-94</td>
<td>46</td>
<td>9-79</td>
</tr>
<tr>
<td>1984</td>
<td>10-95</td>
<td>42</td>
<td>13-75</td>
</tr>
<tr>
<td>1985</td>
<td>13-49</td>
<td>50</td>
<td>13-84</td>
</tr>
<tr>
<td>1986</td>
<td>14-108</td>
<td>48</td>
<td>14-98</td>
</tr>
<tr>
<td>1987</td>
<td>17-199</td>
<td>56</td>
<td>17-60</td>
</tr>
<tr>
<td>1988</td>
<td>9-110</td>
<td>45</td>
<td>9-110</td>
</tr>
<tr>
<td>1989</td>
<td>10-140</td>
<td>47</td>
<td>10-133</td>
</tr>
<tr>
<td>1990</td>
<td>7-130</td>
<td>43</td>
<td>12-71</td>
</tr>
</tbody>
</table>

* General Environmental Monitoring Station of China (1991)
<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cities</th>
<th>Minimum value</th>
<th>Percentile</th>
<th>Maximum value</th>
<th>Arithmetic</th>
<th>Geometric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>1986</td>
<td>71</td>
<td>14</td>
<td>17</td>
<td>20</td>
<td>30</td>
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<tr>
<td>1987</td>
<td>71</td>
<td>13</td>
<td>16</td>
<td>21</td>
<td>33</td>
<td>46</td>
</tr>
<tr>
<td>1988</td>
<td>73</td>
<td>8</td>
<td>11</td>
<td>18</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>1989</td>
<td>63</td>
<td>10</td>
<td>14</td>
<td>19</td>
<td>30</td>
<td>44</td>
</tr>
<tr>
<td>1990</td>
<td>59</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>27</td>
<td>38</td>
</tr>
</tbody>
</table>

* General Environmental Monitoring Station of China (1991)
Fig. 8. Metropolitan area trends in the composite annual average NO$_2$ concentration for three population classes, 1980-1989 (US EPA, 1991) (MSA = Metropolitan Statistical Area)
Metropolitan Statistical Areas (MSA) reporting a valid year's data for at least one station in 1988 and/or 1989, peak annual averages ranged from 0.007 to 0.061 ppm (Fig. 9). The only recently measured concentrations exceeding the USA annual average standard (0.053 ppm) have occurred at stations in southern California.

The seasonal patterns at stations in California are usually quite marked and reach their highest levels through the autumn and winter months. Stations elsewhere in the USA usually have less prominent seasonal patterns and may peak in the winter or summer, or may contain little discernable variation (Fig. 10) (US EPA, 1991).

One-hour NO₂ values at stations in the USA can exceed 0.2 ppm, but in 1988 only 16 stations (12 of which are in California) reported an apparently credible second high 1-h value above 0.2 ppm (Fig. 11). Because at least 98% of 1-h values at most stations are below 0.1 ppm, these values above 0.2 ppm are quite rare excursions whose validity should be verified (US EPA, 1991).

3.4 Occurrence of nitrogen oxides indoors

This section summarizes emissions of NOₓ from sources that affect indoor air quality and are commonly found in residential environments. There are several reasons for considering these emissions. Firstly, examining emissions from several types of sources and source categories can help identify the relative impact of each source on indoor air quality and thus its influence on human exposure. Secondly, such information is needed to understand the fundamental physical and chemical processes influencing emissions. This understanding can be used to help develop strategies for reducing emissions. Finally, studying emissions from indoor sources can provide source strength input data needed for indoor air quality modelling. Knowledge of indoor concentrations is an important component in estimating the total exposure of individuals to nitrogen oxides.

An important factor for indoor air quality is how (or if) the combustion products from appliances are vented outside the building. It should be noted that several common types of vented appliances usually emit NOₓ to the outdoors; examples include gas-fired furnaces, water heaters and clothes dryers, as well as stoves and furnaces using wood, coal and other fuels. Under some circumstances even these vented emissions may filter back inside
Fig 9. Distribution of peak annual NO$_2$ averages in 103 Metropolitan Statistical Areas (MSA) in the USA, 1988-1989.
Fig. 10. Monthly 50th, 90th, and 95th percentiles of 1-h \( \text{NO}_2 \) concentrations at selected stations in the USA, 1986-1989. (Annual averages are shown in parentheses) (from: US EPA, 1993)
Fig. 10. (contd).
Fig. 11. Annual average NO\textsubscript{2} concentration versus second high 1-h concentration at 214 stations in the USA in 1988.
(Second high 1-h values > 0.2 ppm are identified by state) CA = California; NY = New York; NJ = New Jersey; MA = Maine; NH = New Hampshire; OK = Oklahoma (US EPA, 1991)
and contribute to elevated NO\textsubscript{x} levels indoors. For example, Hollowell et al. (1977) reported high NO and NO\textsubscript{2} concentrations in a house where a vented forced-air gas-fired heating system was used. Elevated concentrations may also be a problem with malfunctioning vented appliances. Other data (e.g., Fortmann et al., 1984), however, suggest that fugitive emissions of NO\textsubscript{x} from vented appliances are small. The importance of unvented appliances to indoor NO\textsubscript{x} levels is well documented; this section focuses on emissions from such appliances.

### 3.4.1 Indoor sources

#### 3.4.1.1 Gas-fuelled cooking stoves

Several research programmes have investigated NO\textsubscript{x} emissions from stoves fuelled with natural and liquid petroleum gas (Himmel & DeWerth, 1974; Cote et al., 1974; Massachusetts Institute of Technology, 1976; Yamanaka et al., 1979; Traynor et al., 1982b; Cole et al., 1983; Caceres et al., 1983; Fortmann et al., 1984; Moschandreas et al., 1985; Cole & Zawacki, 1985; Tikalsky et al., 1987; Borrazzo et al., 1987). Most of these studies have included investigations of several other pollutants, including CO, aldehydes and unburned hydrocarbons. Table 12 lists average emission factors for range-top burners and for oven and broiler burners operated at maximum heat input rate. Data are shown for both well-adjusted blue flames and for poorly adjusted yellow flames. Each of the averages is based on the total number of stoves tested for that category, using data from the above studies. For top burners with blue flames, a total of 27 values are represented; for yellow flames, there are 23 values (24 for NO\textsubscript{x}). Averages for the oven and broiler burners represent 20 blue flame and 16 yellow flame values. Values are generally very similar for emissions from these two types of burners on the same stove. Overall, the results show that well-adjusted blue flames emit more NO but less NO\textsubscript{2} than poorly adjusted yellow flames. Emission factors from range-top burners are comparable to those from oven and broiler burners.

#### 3.4.1.2 Unvented gas space heaters and water heaters

The findings of several investigators (Thrasher & DeWerth, 1979; Traynor et al., 1983a, 1984b; Zawacki et al., 1986) are summarized in Table 13. The most significant result is the markedly lower emissions from heaters equipped with catalytic burners, radiant ceramic tile burners and improved-design steel
burners (radiant and Bunsen), compared to emissions from simpler convection designs using conventional cast-iron Bunsen burners. Equipping convective heaters with radiant tiles does not make much difference to emission levels, nor does the choice of natural gas or liquid petroleum gas fuel. Other studies by Billick et al. (1984), Zawacki et al. (1984) and Moschandreas et al. (1985) produced similar results.

### Table 12. Average emission factors for nitric oxide (NO), nitrogen dioxide (NO\(_2\)) and nitrogen oxides (NO\(_x\)) from burners on gas stoves

<table>
<thead>
<tr>
<th>Flame type</th>
<th>Factor for NO (µg/kJ)</th>
<th>Factor for NO(_2) (µg/kJ)</th>
<th>Factor for NO(_x) (µg/kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top burners</td>
<td>blue</td>
<td>20.0 ± 4.5</td>
<td>10.2 ± 3.1</td>
</tr>
<tr>
<td>Top burners</td>
<td>yellow</td>
<td>16.9 ± 4.5</td>
<td>15.0 ± 4.8</td>
</tr>
<tr>
<td>Ovens and broilers</td>
<td>blue</td>
<td>21.9 ± 6.3</td>
<td>7.23 ± 3.01</td>
</tr>
<tr>
<td>Ovens and broilers</td>
<td>yellow</td>
<td>19.8 ± 9.6</td>
<td>11.4 ± 5.7</td>
</tr>
</tbody>
</table>

3.4.1.3 Kerosene space heaters

The data presented in Table 14 show that emission factors of NO and NO\(_2\) for radiant kerosene heaters are generally much smaller than those for convective kerosene heaters. Emissions of NO from two-stage heaters are only slightly greater than those from radiant heaters, whereas emissions of NO\(_2\) are the lowest of the three heater types. Most of the emissions from radiant heaters are in the form of NO\(_2\) for convective heaters that are two-stage heaters, the emissions of NO and NO\(_2\) are of comparable magnitude. There are insufficient data to evaluate changes in emissions as kerosene heaters age. Other products, including particles, present in these emissions may also be of concern for their possible health effects.

3.4.1.4 Wood stoves

A number of studies have examined pollutant emissions from wood stoves. Some of these studies have developed emission
### Table 13. Summary of studies with unvented convective (C) and infrared (I) space heaters

<table>
<thead>
<tr>
<th>Type of heater</th>
<th>Number</th>
<th>Heat input (kJ/min)</th>
<th>NO emission (µg/kJ)</th>
<th>NO\textsubscript{2} emission (µg/kJ)</th>
<th>NO\textsubscript{X} emission (µg/kJ)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convective</td>
<td>5</td>
<td>86-661</td>
<td>24-47</td>
<td>2.3-7.3</td>
<td>39-77</td>
<td>Thrasher &amp; DeWerth (1979)</td>
</tr>
<tr>
<td>Convective</td>
<td>8</td>
<td>188-830</td>
<td>9.5-22</td>
<td>9.5-20</td>
<td>34-47</td>
<td>Traynor et al. (1983a)</td>
</tr>
<tr>
<td>Infrared</td>
<td>5</td>
<td>245-352</td>
<td>0.1-1</td>
<td>4.1-6.2</td>
<td>4.9-6.2</td>
<td>Traynor et al. (1984b)</td>
</tr>
<tr>
<td>Convective</td>
<td>4</td>
<td>335-526</td>
<td>17.8-28.7</td>
<td>10-18.3</td>
<td>40.1-57.5</td>
<td>Traynor et al. (1984c)</td>
</tr>
<tr>
<td>Infrared</td>
<td>5</td>
<td>264-334</td>
<td>0.005-1.7</td>
<td>1.6-4.8</td>
<td>2.7-5.7</td>
<td>Zawacki et al. (1986)</td>
</tr>
<tr>
<td>Convective</td>
<td>5</td>
<td>175-703</td>
<td>5.3-44.4</td>
<td>7.6-23.3</td>
<td>27.1-76.4</td>
<td>Zawacki et al. (1986)</td>
</tr>
</tbody>
</table>
Table 14: Average emission factors for nitric oxide (NO), nitrogen dioxide (NO\textsubscript{2}) and nitrogen oxides (NO\textsubscript{x}) from kerosene heaters

<table>
<thead>
<tr>
<th>Type of heater</th>
<th>Heat input rate (kJ/min)</th>
<th>Emission factor for NO (μg/kJ)</th>
<th>Emission factor for NO\textsubscript{2} (μg/kJ)</th>
<th>Emission factor for NO\textsubscript{x} (μg/kJ)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiant, new</td>
<td>144</td>
<td>0.45 ± 0.05</td>
<td>4.4 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>Leaderer (1982)</td>
</tr>
<tr>
<td>Radiant, new</td>
<td>113</td>
<td>0.08 ± 0.00</td>
<td>5.0 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Radiant, new</td>
<td>84.4</td>
<td>0</td>
<td>5.9 ± 0.3</td>
<td>5.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Convective, new</td>
<td>158</td>
<td>17 ± 0.7</td>
<td>7.0 ± 0.4</td>
<td>33 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Convective, new</td>
<td>97.9</td>
<td>12 ± 0.6</td>
<td>16 ± 0.3</td>
<td>33 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Convective, new</td>
<td>37.3</td>
<td>11 ± 0.9</td>
<td>17 ± 1.0</td>
<td>34 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Radiant, new</td>
<td>137</td>
<td>1.3 ± 0.7</td>
<td>4.6 ± 0.6</td>
<td>6.6 ± 1.3</td>
<td>Traynor et al. (1983b)</td>
</tr>
<tr>
<td>Radiant, 1 year old</td>
<td>111</td>
<td>2.1</td>
<td>5.1</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Convective, new</td>
<td>131</td>
<td>25 ± 0.7</td>
<td>13 ± 0.8</td>
<td>51 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Convective, 5 years old</td>
<td>94.8</td>
<td>11 ± 0.1</td>
<td>32 ± 2.6</td>
<td>49 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Radiant</td>
<td>110-200</td>
<td>-</td>
<td>-</td>
<td>13 ± 1.8</td>
<td>Yamanaka et al. (1979)</td>
</tr>
<tr>
<td>Convective</td>
<td>110-200</td>
<td>-</td>
<td>-</td>
<td>70 ± 6.8</td>
<td></td>
</tr>
</tbody>
</table>
factors based on concentrations in the flue gases; such information would be useful for assessing the contribution of wood stove emissions to ambient air quality. Very little information is available, however, on fugitive emissions from wood stoves into the indoor living space.

In a detailed literature survey, Smith (1987) reported that emissions of pollutants from wood stoves are highly variable, depending on the type of wood used, stove design, the way the stove is used and other factors. He reported emission factors for NO\textsubscript{2} and other pollutants for wood stoves used in developing countries. Many of these stoves are unvented, which results in excessive indoor concentrations as the combustion products are exhausted into the room. The major health concerns for wood fires without chimneys arise from pollutants other than NO\textsubscript{2}, such as particulate matter.

Traynor et al. (1984a) have studied wood stoves (three airtight and one non-airtight) used in a house. For each experiment, airborne concentrations of several pollutants were measured inside and outside the house during operation of one of the stoves. The results showed that all indoor and outdoor concentrations of NO and NO\textsubscript{2} were below 0.02 ppm. Moreover, indoor air concentrations of some other pollutants were high during use of the non-airtight stove. The airtight stoves had little influence on indoor concentrations of any pollutants. In another study, Traynor et al. (1982a) found elevated airborne concentrations of NO and NO\textsubscript{2} in three occupied houses during operation of wood stoves and a wood furnace. The concentrations were highly variable.

Because of the limited data, it is difficult to reach quantitative conclusions regarding the importance of wood stoves. However, the limited information available suggests that wood stoves are not a major contributor to indoor nitrogen oxide exposures. This is consistent with the small NO emission rates expected from the low temperature combustion processes characteristic of wood stoves.

3.4.1.5 Tobacco products

A number of studies have compared concentrations of NO\textsubscript{2} and other pollutants in houses with smokers and houses without smokers. In general, these studies have shown that concentrations are somewhat greater in the homes of smokers.

A few studies have reported emissions of NO\textsubscript{2} from cigarettes while sampling both sidestream and mainstream smoke together.
Woods (1983) reported 0.079 mg NO\textsubscript{2}/cigarette, while Moschandreas et al. (1985) listed emissions of 2.78 mg/cigarette for NO and 0.73 mg/cigarette for NO\textsubscript{2}. The National Research Council (1986) reported total NO\textsubscript{2} emissions of 100 to 600 \(\mu\)g/cigarette for mainstream smoke, with values 4 to 10 times greater for sidestream smoke. According to the report, virtually all of the emitted NO\textsubscript{2} is in the form of NO; once emitted, the NO is gradually oxidized to NO\textsubscript{2}. Thus environments containing cigarette smoke may have higher concentrations of both NO and NO\textsubscript{2} than environments without such smoke. The NO\textsubscript{2} concentration on trains travelling between Changchun and Harbin, China, was found to be related to the amount of cigarette smoking, which was greater on daytime trains than on night-time ones. On a one-way daytime train the average NO\textsubscript{2} concentration was 54 ppb (range, 37-84 ppb), whereas on a two-way night-time train it was 40.6 ppb (range, 30-59 ppb) (Du et al., 1992).

3.4.2 Removal of nitrogen oxides from indoor environments

A number of field studies of NO\textsubscript{2} levels in residences have reported that NO\textsubscript{2} is removed more rapidly than can be accounted for by infiltration alone (Wade et al., 1975; Macriess & Elkins, 1977; Oezkaynak et al., 1982, Traynor et al., 1982a; Ryan et al., 1983; Leaderer et al., 1986). Indoors, NO\textsubscript{2} is removed by infiltration/ventilation and by interior surfaces and furnishings. The removal of NO\textsubscript{2} by interior surfaces and furnishings and reactions occurring in air is often referred to as the reactive decay rate of NO\textsubscript{2}, and it can be a significant factor in the actual NO\textsubscript{2} levels measured in residences. Failure to account for the reactive decay rate can lead to a serious underestimation of emission rate measurements in chamber and test house studies and a serious overestimation of indoor concentrations when using emission rates to model indoor levels. The NO\textsubscript{2} reactive decay rate is typically determined by subtracting the decay of NO\textsubscript{2}, after a source is shut off, from that of a relatively non-reactive gas (e.g., CO, CO\textsubscript{2}, SF\textsubscript{6}, NO), which can be related to ventilation rates, expressed in room air changes per hour. The measured reactive decay rates in the above-mentioned field studies ranged from 0.1 to 1.6 air change times/hour. All studies noted that the reactive decay of NO\textsubscript{2} is as important and in some cases more important than infiltration in removing NO\textsubscript{2} indoors. Leaderer et al. (1986) monitored NO\textsubscript{2}, NO, CO and CO\textsubscript{2} continuously in seven houses over periods ranging from 2 to 8 days. They reported that the NO\textsubscript{2} decay rate was always greater than that due to infiltration alone and was highly variable among houses and among time periods within a house.
In an effort to identify the factors that control the NO\textsubscript{2} reactive decay rate, a number of small chamber (Miyazaki, 1984; Spicer et al., 1986), large chamber (Moschandreas et al., 1985; Leaderer et al., 1986) and test house studies (Yamanaka, 1984; Borrazzo et al., 1987b; Fortmann et al., 1987) have been conducted. The most extensive small chamber work was reported by Spicer et al. (1986), where 35 residential materials were screened for NO\textsubscript{2} reactivity in a 1.64-\text{m}^3 chamber and a limited number of the materials were tested for the impact of relative humidity on the reactivity rate. Fig. 12 shows the relative rates of NO\textsubscript{2} removal for the materials screened. The figure indicates that many of the materials used for building construction and furnishings are significant sinks for NO\textsubscript{2} and that their removal rate is highly variable. Many of the materials were found to reduce a significant proportion of the removed NO\textsubscript{2} to NO. In no cases was NO\textsubscript{2} re-emitted, although some materials emitted NO. The authors noted that the materials that removed NO\textsubscript{2} most rapidly fall in two categories: (1) porous mineral materials of high surface area; and (2) cellulosic material derived from plant matter. Higher relative humidities were found to enhance the removal rate for some materials (e.g., wool carpet), reduce the removal rate for some (e.g., cement block), and have little effect on others (e.g., wallboard). In a series of small (0.69 \text{m}^3) chamber studies (Miyazaki, 1984) reactive decay rates for NO\textsubscript{2} were found to vary as a function of material type and to increase with increasing surface area of the material, degree of stirring in the chamber, temperature and relative humidity. A saturation effect was noted on some of the carpets tested.

In a series of large chamber studies (34-\text{m}^3 chamber), Leaderer et al. (1986) evaluated the reactive decay rate of NO\textsubscript{2} as a function of material type, surface area of material, relative humidity and air mixing. The reactive decay rate was found to vary as a function of material surface roughness and surface area. Carpeting was found to be most effective in removing NO\textsubscript{2}, whereas painted wallboard was least effective. Increases in relative humidity were associated with increases in removal rates for all materials tested, but the slope was a shallow one. Of particular interest is the finding in this study that the degree of air mixing and turbulence was a dominant variable in determining the reactive decay rate for NO\textsubscript{2}. Moschandreas et al. (1985) evaluated six materials in a 14.5-\text{m}^3 chamber and found variations in decay rates according to material types and a positive impact of relative humidity on NO\textsubscript{2} decay rates in an empty chamber.
Fig. 12. Bar graph of NO\textsubscript{2} removal rate for various materials evaluated in a 1.64-m\textsuperscript{3} test chamber at 50% relative humidity (from: Spicer et al., 1986)
Yamanaka (1984), in assessing NO₂ reactive decay rates in a Japanese living room, found the decay to consist of both homogeneous and heterogeneous processes. The rates were found to vary as a function of surface property and sharply as a function of relative humidity. NO production during the decay was noted. In a test house study, Fortmann et al. (1987) noted that the NO₂ decay rate tends to decrease as the concentration increases. It is not clear whether this is due to surface saturation or second-order kinetics. This study also noted a sharp increase in NO levels during the NO₂ decay, indicating NO production as a result of the NO₂ decay. In a test house study conducted over a 7-month period, Borrazzo et al. (1987b) found that reaction rates for NO₂ in the test house were sensitive to the location in the house where they were measured. This indicates that reaction losses during transport of NO₂ from room to room in a house may be important.

Reactive decay of NO₂ associated with interior surface materials and furnishings is an important mechanism for removing NO₂ from the air within homes. Reactive decay rates for NO₂ vary as a function of the type and surface area of the material. The impact of relative humidity on the decay rate is unclear, with some studies showing a pronounced impact (Yamanaka, 1984), while others show only moderate or little impact (e.g., Spicer et al., 1986; Leaderer et al., 1986). The degree of air mixing or turbulence can have an important effect on the reactive decay rate. A by-product of NO₂ removal by materials may be NO production, and a saturation effect may occur for some materials. Reactive decay of NO₂ in residences is highly variable between residences, within rooms in a residence, and on a temporal basis within a residence. The large number of variables controlling the reactive decay rate make it very difficult to assess in large field studies through questionnaire or integrated air sampling.

### 3.5 Indoor concentrations of nitrogen oxides

Indoor concentrations of NO₂ are a function of outdoor concentrations, indoor sources (source type, condition of source, source use, etc.), infiltration/ventilation, air mixing within and between rooms, reactive decay by interior surfaces, and air cleaning or source venting.

#### 3.5.1 Homes without indoor combustion sources

Typical studies in homes without indoor sources of NO₂, summarized in Table 15, have reported concentrations lower than
Table 15. Average outdoor concentrations of nitrogen dioxide (NO₂) and average indoor/outdoor ratios in homes without gas appliances or unvented space heaters.

<table>
<thead>
<tr>
<th>Location</th>
<th>Housing type</th>
<th>Averaging time</th>
<th>Seasons</th>
<th>Number of homes</th>
<th>Average NO₂ concentration ((\mu g/m^3))</th>
<th>Indoor/outdoor ratios</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Outdoor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern California</td>
<td>Mixed</td>
<td>7 days</td>
<td>Summer</td>
<td>70</td>
<td>71.9</td>
<td>0.80 0.75</td>
<td>Southern California Gas Company 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>69</td>
<td>91.2</td>
<td>0.56 0.47</td>
<td></td>
</tr>
<tr>
<td>New Haven, CT</td>
<td>Single family unattached</td>
<td>14 days</td>
<td>Winter</td>
<td>60</td>
<td>13.2</td>
<td>0.56 0.55</td>
<td>Leaderer et al. 1986</td>
</tr>
<tr>
<td>Albuquerque, NM</td>
<td>Mixed</td>
<td>14 days</td>
<td>Winter 1</td>
<td>60</td>
<td>14.1</td>
<td>- 0.50</td>
<td>Marbury et al. 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter 2</td>
<td>56</td>
<td>19.6</td>
<td>- 0.32</td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>Mobile homes</td>
<td>7 days</td>
<td>Summer</td>
<td>45</td>
<td>25.9</td>
<td>0.61 0.54</td>
<td>Petreas et al. 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>23</td>
<td>44.6</td>
<td>0.27 0.26</td>
<td></td>
</tr>
<tr>
<td>Portage, WI</td>
<td>Mixed</td>
<td>7 days</td>
<td>Summer</td>
<td>47</td>
<td>15.2</td>
<td>0.91 0.72</td>
<td>Quackenboss et al. 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>47</td>
<td>17.2</td>
<td>0.65 0.45</td>
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</tr>
<tr>
<td>Tucson, AZ</td>
<td>Mixed</td>
<td>14 days</td>
<td>Summer</td>
<td>56</td>
<td>19.9</td>
<td>0.86 0.76</td>
<td>Quackenboss et al. 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spring/Autumn</td>
<td>41</td>
<td>25.5</td>
<td>0.71 0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>23</td>
<td>36.8</td>
<td>0.64 0.52</td>
<td></td>
</tr>
<tr>
<td>Boston, MA</td>
<td>Mixed</td>
<td>14 days</td>
<td>Summer</td>
<td>117</td>
<td>31.7</td>
<td>0.76 0.75</td>
<td>Ryan et al. 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autumn</td>
<td>117</td>
<td>37.5</td>
<td>0.43 0.40</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter/Spring</td>
<td>124</td>
<td>33.5</td>
<td>0.53 0.47</td>
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</table>
Table 15 (contd).

<table>
<thead>
<tr>
<th>Location</th>
<th>Housing typea</th>
<th>Averaging time</th>
<th>Seasons</th>
<th>Number of homes</th>
<th>Average NO2 outdoor concentration (μg/m³)</th>
<th>Indoor/outdoor ratios</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Central Texas</td>
<td>Single family unattached</td>
<td>5 days</td>
<td>Winter</td>
<td>9</td>
<td>53.8</td>
<td>0.47</td>
<td>Koontz et al. (1986)</td>
</tr>
<tr>
<td>Suffolk County, NY</td>
<td>Single family unattached</td>
<td>7 days</td>
<td>Winter</td>
<td>49</td>
<td>35.5</td>
<td>0.70</td>
<td>Research Triangle Institute (1990)</td>
</tr>
<tr>
<td>Onondago County, NY</td>
<td>Single family unattached</td>
<td>7 days</td>
<td>Winter</td>
<td>66</td>
<td>21.7</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Portage, WI</td>
<td>Single family unattached</td>
<td>7 days</td>
<td>Average over all seasons</td>
<td>25</td>
<td>12.8</td>
<td>0.65</td>
<td>0.51</td>
</tr>
<tr>
<td>Watertown, MA</td>
<td>Not given</td>
<td>3-4 days</td>
<td>November</td>
<td>18</td>
<td>37.0</td>
<td>0.65</td>
<td>0.51</td>
</tr>
<tr>
<td>Middlesbrough, UK</td>
<td>Not given</td>
<td>7 days</td>
<td>Winter</td>
<td>87</td>
<td>35.0</td>
<td>0.97</td>
<td>Goldstein et al. (1979)</td>
</tr>
<tr>
<td>Middlesbrough, UK</td>
<td>Not given</td>
<td>7 days</td>
<td>Winter</td>
<td>15</td>
<td>34.7</td>
<td>-</td>
<td>Melia et al. (1982a,b)</td>
</tr>
</tbody>
</table>

* Data from field studies of private residences in the USA and United Kingdom

a "Mixed" indicates a single family in an attached or unattached dwelling, condominium or apartment
outdoor levels due to removal from the air of NO₂ by the building envelope and interior surfaces. Thus indoor/outdoor concentration ratios are consistently less than unity. These homes provide some degree of protection from outdoor concentrations. Indoor/outdoor ratios vary considerably according to the season of the year, the lowest ratios occurring in the winter and highest occurring during the summer. Although urban concentrations are often highest in winter, this pattern in the indoor/outdoor ratio, attributed to seasonal differences in infiltration rates, NO₂ reactivity rates, the penetration factor and outdoor concentrations, can result in higher indoor concentrations in summer than in winter. The indoor-to-outdoor ratio for these homes does not appear to depend on geographical area, housing type or outdoor concentration. Results of monitoring in Portage, Wisconsin, USA, show that the presence of a gas stove contributes dramatically to the indoor NO₂ levels. Table 16, taken from the report of Quackenboss et al. (1986) and based on data collected in 1981 and 1982, clearly shows that gas stoves increase not only indoor concentrations but also the personal exposure of children.

<table>
<thead>
<tr>
<th>Season</th>
<th>Stove</th>
<th>Indoor Mean</th>
<th>Indoor SD</th>
<th>Outdoor Mean</th>
<th>Outdoor SD</th>
<th>Personal Mean</th>
<th>Personal SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>Gas</td>
<td>0.016</td>
<td>0.006</td>
<td>0.006</td>
<td>0.003</td>
<td>0.014</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Electric</td>
<td>0.007</td>
<td>0.003</td>
<td>0.006</td>
<td>0.003</td>
<td>0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>Winter</td>
<td>Gas</td>
<td>0.027</td>
<td>0.013</td>
<td>0.006</td>
<td>0.003</td>
<td>0.023</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Electric</td>
<td>0.005</td>
<td>0.003</td>
<td>0.009</td>
<td>0.003</td>
<td>0.008</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* From: Quackenboss et al. (1986); SD = standard deviation

3.5.2 Homes with combustion appliances

It is estimated that gas (natural gas and liquid propane) is used for cooking, heating water or drying clothes in about 45% of all homes in the USA (US Bureau of the Census, 1982) and in nearly
100% of homes in some other countries (e.g., the Netherlands). Gas appliances (gas cooker/oven, water heater, etc.) are the major indoor source category for indoor residential NO\textsubscript{2} by virtue of the number of homes with such sources. NO\textsubscript{2} concentrations in homes with gas appliances are higher than those without such appliances. Within this category, the gas cooker/oven and unvented heaters are by far the major contributors. Cookers and ovens are especially important sources when used inappropriately as a supplementary room heater. Average indoor concentrations (based on a 1- to 2-week measurement period) in excess of 100 µg/m\textsuperscript{3} have been measured in some homes with gas cookers (Table 17). Homes where gas cookers with pilot lights are used have higher NO\textsubscript{2} levels than homes that have gas cookers without pilot lights. Average NO\textsubscript{2} concentrations in homes with gas cookers/ovens exhibit a spatial gradient within and between rooms. Kitchen concentrations of NO\textsubscript{2} are higher than other rooms and a steep vertical concentration gradient in the kitchen has been observed in some homes, concentrations being highest nearest the ceiling. Average NO\textsubscript{2} concentrations are highest during the winter months and lowest during the summer months. This seasonal temporal gradient is attributed to differences in infiltration, appliance use, NO\textsubscript{2} reactivity rates and indoors and outdoor concentrations. The impact of gas appliance use on indoor NO\textsubscript{2} levels may be superimposed upon the background level resulting from outdoor concentrations. Only very limited data exist on short-term average (3 h or less) indoor concentrations of NO\textsubscript{2} associated with gas appliance use. These data suggest that short-term average concentrations of NO\textsubscript{2} are several times the longer-term average concentrations measured.

A wide variety of fuel types can be used for cooking and heating in different localities. These can produce various effects on indoor air quality. As an example, Table 18 gives data for indoor NO\textsubscript{2} concentrations measured at Lanzhou City, China, where coal and liquified gas were used in apartments and houses (Duan et al., 1992).

### 3.5.3 Homes with combustion space heaters

Unvented kerosene and gas space heaters are important sources of NO and NO\textsubscript{2} in homes, both because of the NO and NO\textsubscript{2} production rates of the heaters and the long periods of time that they are in use. The concentrations of NO emitted are usually several times higher than those of NO\textsubscript{2}. However, in the literature, indoor air concentrations of NO are frequently not reported.
<table>
<thead>
<tr>
<th>Location</th>
<th>Housing type</th>
<th>Averaging time (days)</th>
<th>Type of appliance</th>
<th>Season</th>
<th>No. of homes</th>
<th>Average measured NO₂ (μg/m³)</th>
<th>Indoor NO₂ due to source (μg/m³)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Outside Kitchen Bedroom Other</td>
<td>Outside Kitchen Bedroom Other Comment</td>
<td></td>
</tr>
<tr>
<td>Southern California</td>
<td>Mixed</td>
<td>7</td>
<td>Oven/range, ≤ pilot</td>
<td>Summer</td>
<td>147</td>
<td>75.3 91.6 68.4</td>
<td>31 12 - 1,2</td>
<td>Southern California Gas Company (1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spring</td>
<td>202</td>
<td>49.2 79.2 51.3</td>
<td>35 22 - 1,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>141</td>
<td>104 101.5 69</td>
<td>48 20 - 1,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oven/range, Winter</td>
<td>98</td>
<td>107</td>
<td>113 76</td>
<td>53 26 - 1,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oven/range, no pilot</td>
<td>Winter</td>
<td>98</td>
<td>97</td>
<td>74 53</td>
<td>20 7 - 1,2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water heater in home</td>
<td>Winter</td>
<td>21</td>
<td>92</td>
<td>59 50</td>
<td>11 11 - 1,2,3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wall furnace Winter</td>
<td>90</td>
<td>121</td>
<td>161 113</td>
<td>49 36 - 1,4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Floor furnace Summer</td>
<td>42</td>
<td>119</td>
<td>177 126</td>
<td>66 52 - 1,4</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>Housing type</td>
<td>Averaging time (days)</td>
<td>Type of appliance</td>
<td>Season</td>
<td>No. of homes</td>
<td>Average measured NO₂ (µg/m³)</td>
<td>Indoor NO₂ due to source (µg/m³)</td>
<td>Reference</td>
</tr>
<tr>
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<tr>
<td>New Haven, CT</td>
<td>Single family, unattached</td>
<td>14</td>
<td>Oven/range, ± pilot</td>
<td>Winter</td>
<td>42</td>
<td>14.8 44.7 27.6 30.4</td>
<td>36 20 22 1.5</td>
<td>Leaderer et al. (1986)</td>
</tr>
<tr>
<td>Albuquerque, NM</td>
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<td>Oven/range, ± pilot</td>
<td>Winter</td>
<td>82</td>
<td>19.1 33.1 41.9</td>
<td>- 24 31 1.5</td>
<td>Marbury et al. (1988)</td>
</tr>
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<td>California homes</td>
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<td>Winter</td>
<td>231</td>
<td>42.1 37.5</td>
<td>- 42 27 1.7</td>
<td>Petreas et al. (1988)</td>
</tr>
<tr>
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<td>Mixed</td>
<td>7</td>
<td>Oven/range, ± pilot</td>
<td>Summer</td>
<td>36</td>
<td>11.5 36.9 21.1 29.6</td>
<td>29 13 20 1.8</td>
<td>Quackenboss et al. (1986)</td>
</tr>
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<td>Tucson, AZ</td>
<td>Mixed</td>
<td>14</td>
<td>Oven/range, ± pilot</td>
<td>Winter</td>
<td>10</td>
<td>45.2 60.6 43.4 50.7</td>
<td>32 20 25 1.9</td>
<td>Quackenboss et al. (1986)</td>
</tr>
<tr>
<td>Location</td>
<td>Type</td>
<td>N</td>
<td>Time</td>
<td>Rating 1</td>
<td>Rating 2</td>
<td>Temperature 1</td>
<td>Temperature 2</td>
<td>Rating 3</td>
</tr>
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<td>----------</td>
<td>----------</td>
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<td>---------------</td>
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<tr>
<td>Boston, MA</td>
<td>Mixed</td>
<td>14</td>
<td>Summer</td>
<td>301</td>
<td>41.6</td>
<td>65.9</td>
<td>45.6</td>
<td>50.9</td>
</tr>
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<td>Autumn</td>
<td>277</td>
<td>43.7</td>
<td>74.3</td>
<td>47.5</td>
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<td>298</td>
<td>40.5</td>
<td>73.5</td>
<td>48.6</td>
<td>55.1</td>
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<td>Spring</td>
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<td>Central Texas</td>
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<td>Winter</td>
<td>22</td>
<td>34.6</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Suffolk Co., NY</td>
<td>Single</td>
<td>7</td>
<td>Winter</td>
<td>42</td>
<td>37.6</td>
<td>77.5</td>
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<td>Onondago Co., NY</td>
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<td>56</td>
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<td>62.6</td>
<td>0</td>
<td>50</td>
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<td>New York, NY</td>
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<td>122</td>
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<td>96</td>
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<td>71</td>
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<td>108</td>
<td>66</td>
<td>76</td>
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<td></td>
<td></td>
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<td>100</td>
<td>121</td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter 2</td>
<td>18</td>
<td>75</td>
<td>126</td>
<td>63</td>
<td>82</td>
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<td>Spring</td>
<td>13</td>
<td>95</td>
<td>121</td>
<td>82</td>
<td>99</td>
</tr>
</tbody>
</table>
### Table 17 (contd).

<table>
<thead>
<tr>
<th>Location</th>
<th>Housing type</th>
<th>Averaging time (days)</th>
<th>Type of appliance</th>
<th>Season</th>
<th>No. of homes</th>
<th>Average measured NO₂ (µg/m³)</th>
<th>Indoor NO₂ due to source (µg/m³)</th>
<th>Reference</th>
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</thead>
<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Outdoors</td>
<td>Kitchen</td>
<td>Bedroom</td>
</tr>
<tr>
<td>Portage, WI</td>
<td>Single family, unattached</td>
<td>7</td>
<td>Natural gas Oven/range, no pilot</td>
<td>All seasons</td>
<td>36</td>
<td>15.8</td>
<td>65.5</td>
<td>36.7</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watertown, MA</td>
<td>Not given</td>
<td>3-4</td>
<td>Gas cooking</td>
<td>Novemb.</td>
<td>60</td>
<td>37</td>
<td>74</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Decemb.</td>
<td>51</td>
<td>46</td>
<td>86</td>
<td>46</td>
</tr>
<tr>
<td>Netherlands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amet Enschede</td>
<td>Not given</td>
<td>7</td>
<td>Gas cooking</td>
<td>Autumn/ Winter</td>
<td>294</td>
<td>35</td>
<td>118</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Comment
| Location         | Environment | Season | Heating Method          | Year | Temperature | Month | PM₁₀ | PM₂·₅ | PM₁₀⁻₁₅ | PM₂·₅⁻₅₀ | PM₁₀⁻₁₅⁻₅₀ | 2.5 μg/m³ | 10 μg/m³ | Mode 50% | 95% |
|------------------|-------------|--------|-------------------------|------|-------------|-------|------|-------|---------|----------|-----------|-----------|----------|---------|
| Ede              | Not given   | Autumn/Winter | Gas cooking no pilot | 173  | 44          | 113   | 43   | 54   | 69      | 17       | 28        | 89        | 17       | 28      | Noy et al. (1984) |
| Vlagtwedde Rural area | 7 | Gas cooking no pilot | Water heaters | 162  | 28          | 107   | 24   | 51   | 90      | 7        | 34        |           |          |         |
| Rotterdam I Inner city | 7 | Gas cooking no pilot | Water heaters | 228  | 45          | 144   | 51   | 80   | 117     | 24       | 53        |           |          |         |
| Rotterdam II Inner city | 7 | Gas cooking no pilot | Water heaters | 102  | 45          | 143   | 64   | 73   | 117     | 37       | 46        | 9.17      |           |         |
| United Kingdom   | Not given   | Winter   | Gas cooking no pilot    | 428  | 35          | 213   | 58   | -    | 179     | 24       | -         | 1.15      | Goldstein et al. (1979) |
| Middlesbrough    | Not given   | Winter   | Gas cooking            | 183  | 34.7        | -     | 60   | 82.7  | -       | 39       | 61        | 1.16      | Mella et al. (1982a,b)  |
Table 17 (contd).

1. Background correction determined by multiplying: (a) the indoor/outdoor ratio for homes in the study with no indoor NO₂ sources for a given season; by (b) the outdoor NO₂ concentration measured for the home with sources; and subtracting the product from the indoor level measured in the house.

2. Homes containing forced air gas furnace. These homes are thought not to contribute significantly to indoor levels for this sample.

3. Homes with electric cooker/oven, forced air gas furnace, and gas water heater in home. Comparison is made with electric cooker/oven, forced air gas furnace, and gas water heater located outside home.

4. Homes have gas cooker/oven with source contribution calculated after correction of a gas cooker/oven. Values are background corrected with gas stove.

5. Living room or activity room.

6. Sampling was done over two different periods for the same houses within the same winter period.

7. Outdoor values were obtained from five locations, housing type, mobile home.

8. Other location in home; bedroom refers to average of levels in one or more bedrooms in house.

9. Other location in the main living room.

10. Other location is point nearest centre of home.

11. 48-h samples over 30 consecutive days.

12. Indoor/outdoor (I/O) ratio is assessed to be 0.6, 0.7, and 0.85 for the Winter, Spring/Autumn and Summer periods, respectively, for all locations, because no control home (no gas appliances) mean measurements were available. Using these I/O ratios, the impact of sources was calculated as footnote 1.

13. Each home was sampled six times over a 1-year period.

14. Outdoor levels are average for homes with or without gas appliances.

15. Outdoor levels were recorded at 75 locations in the general sampling area and were not home-specific. Bedroom levels were obtained for 107 of the 428 homes.

16. Outdoor levels were recorded at 82 locations in the general sampling area and were not home-specific. Outdoor levels were recorded at the beginning and end of the study.

17. Indoor/outdoor (I/O) ratio is assumed to be 0.6 for all locations, because no control home (no gas appliances) measurements were available. Using I/O ratio of 0.6, the impact of sources was calculated as in footnote 1.
### Table 18. Indoor concentration of NO₂ in Lanzhou city, China

<table>
<thead>
<tr>
<th>Type of residence</th>
<th>Average NO₂ concentration (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
</tr>
<tr>
<td>Apartment building with central heating, liquified gas for cooking</td>
<td>0.141</td>
</tr>
<tr>
<td>Apartment building without central heating, coal for cooking and heating</td>
<td>0.136</td>
</tr>
<tr>
<td>One-storey house, coal for cooking and heating</td>
<td>0.106</td>
</tr>
</tbody>
</table>

* From: Duan et al. (1992)

Field studies indicate that average residential concentrations (1- or 2-week average levels) exhibit a wide variation, depending primarily on the amount of heater use and the type of heater (Table 19). Under similar operating conditions, unvented gas space heaters appear to be associated with higher indoor NO₂ concentrations than kerosene heaters. Average concentrations in homes using unvented kerosene heaters have been found to be well in excess of 100 μg/m³. In one study (Leaderer et al., 1986), calculations of NO₂ concentrations in residences during kerosene heater use (in homes without gas appliances) indicate that approximately 50% of the homes have NO₂ concentrations above 100 μg/m³ and 8% above 480 μg/m³. A peak NO₂ concentration of 847 μg/m³ was measured over a 1-h period in a home during use of a kerosene heater.

A large field study (Koontz et al., 1986) of indoor NO₂ concentrations in Texas homes using unvented gas space heaters (most also had gas cookers) found that approximately 70% of the homes had average concentrations in excess of 100 μg/m³ and 20% had average concentrations in excess of 480 μg/m³. This study found that the indoor/outdoor temperature difference was the best indicator of average indoor NO₂ levels during the colder winter periods when heating demands are greatest.
Table 19. Two-week average nitrogen dioxide (NO₂) levels for homes in New Haven, Connecticut, USA, during winter, 1983

<table>
<thead>
<tr>
<th>Source category; location</th>
<th>NO₂ (µg/m³)</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>% above 100 µg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No kerosene heater or gas stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>144</td>
<td>13.2</td>
<td>5.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>House average</td>
<td>145</td>
<td>7.4</td>
<td>4.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Kitchen</td>
<td>147</td>
<td>7.6</td>
<td>3.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>146</td>
<td>7.3</td>
<td>3.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>145</td>
<td>7.3</td>
<td>8.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>One kerosene heater, no gas stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>95</td>
<td>12.9</td>
<td>4.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>House average</td>
<td>95</td>
<td>36.8</td>
<td>32.6</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Kitchen</td>
<td>96</td>
<td>39.1</td>
<td>35.5</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>96</td>
<td>38.5</td>
<td>36.6</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>95</td>
<td>31.9</td>
<td>30.8</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>No kerosene heater, one gas stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>42</td>
<td>14.8</td>
<td>4.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>House average</td>
<td>42</td>
<td>34.3</td>
<td>26.2</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Kitchen</td>
<td>42</td>
<td>44.7</td>
<td>31.4</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>42</td>
<td>30.4</td>
<td>24.8</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>42</td>
<td>27.8</td>
<td>25.1</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>One kerosene heater, one gas stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>18</td>
<td>14.5</td>
<td>5.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>House average</td>
<td>18</td>
<td>66.8</td>
<td>43.9</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Kitchen</td>
<td>18</td>
<td>74.5</td>
<td>52.0</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>18</td>
<td>57.4</td>
<td>36.6</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>18</td>
<td>68.5</td>
<td>56.5</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Two kerosene heaters, no gas stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>13</td>
<td>16.5</td>
<td>9.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>House average</td>
<td>13</td>
<td>69.5</td>
<td>36.0</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>Kitchen</td>
<td>13</td>
<td>73.0</td>
<td>31.7</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>13</td>
<td>73.6</td>
<td>44.3</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>13</td>
<td>67.8</td>
<td>44.9</td>
<td>23.1</td>
<td></td>
</tr>
</tbody>
</table>

104
Only limited data have so far been published on short-term peak indoor concentrations for homes with unvented gas space heaters, and no data are available on spatial variations or concentrations solely during the hours of heater operation.

No spatial gradient of NO$_2$ was found in homes with unvented kerosene space heaters, contrary to the strong spatial gradient noted for homes with gas appliances. This is probably due to the strong convective heat output and the long operating hours of the heaters, which result in rapid mixing within the homes.

Ferrari et al. (1988) conducted a study of air quality in homes with unvented space heaters in Sydney, Australia, over two winters. NO$_2$ concentrations were measured by both continuous (chemiluminescence with O$_3$ method) and passive monitors (badges and Palmes tubes). Concentrations of NO$_2$ exceeded 0.10 ppm (average concentration) in 85% of homes tested, and 0.16 ppm in 44% of homes. More than 10% of homes had average NO$_2$ concentrations exceeding 0.32 ppm, and the maximum recorded was greater than 0.5 ppm. Unvented gas space heaters are common in Sydney, and average use is about 3 h per night during the winter. As a result, an estimated 0.5 million residents are exposed to NO$_2$ concentrations exceeding 0.16 ppm for several hours per night during the colder months of the year.
Improper use of gas appliances (e.g., using a gas oven or stove to heat a living space) and improperly operating gas appliances or vented heating systems (e.g., out-of-repair gas cooker or improper operation of a gas wall or floor furnace) can be important contributors to indoor NO$_2$ concentrations, but few data are available to assess the magnitude of that contribution. Little or no field data exist that would allow for an assessment of the contributions of wood- or coal-burning stoves or fireplaces to indoor NO$_2$ concentrations, but such a contribution would be expected to be small. Cigarette smoking is expected to add relatively small amounts of NO$_2$ to homes (see also Tables 15 and 18).

In developing countries, biomass fuels (e.g., wood, biogas, animal dung, etc.) are much more widely used for home heating and/or cooking than in developed countries, these fuels often being burnt in open hearth fires or poorly vented appliances within indoor spaces of residential dwellings (WHO, 1992). As noted by Sims & Kjellström (1991), a very conservatively estimated 400 million people are affected by biomass smoke problems worldwide, mostly in rural areas of developing countries. A disproportionate number of women and young children are exposed, owing to the greater numbers of hours typically spent by them indoors and their involvement in cooking and other household chores. Increased NO$_2$ concentrations, as well as greater concentrations of carbon monoxide, suspended particulate matter (SPM) and volatile organic compounds (VOCs) are normally found in biomass smoke (Chen et al., 1990). Reviews of field studies in rural areas of developing countries indicate that exposure levels to biomass smoke components are usually rather high. Indoor concentrations for NO$_2$, for example, were found to fall within the range of 0.1 to 0.3 mg/m$^3$ in India, Nepal, Nigeria, Kenya, Guatemala and Papua New Guinea, as reported in reviews by WHO (1984) and Smith (1986, 1987). Similarly, Hong (1991) reported NO$_2$ concentrations in the range of 0.01 to 0.22 mg/m$^3$ resulting from indoor combustion of biogas in homes in Chengdu, Szechuan Province, China. Hong (1991) also reported NO$_2$ concentrations in the range of 0.02 to 0.16 mg/m$^3$ in other houses in Gansu Province, China, where dried cow dung was used as a fuel. The above NO$_2$ indoor air concentrations from biomass smoke should be compared with the WHO Air Quality Guidelines recommendation of 0.15 mg/m$^3$ for daily exposures to NO$_2$ (WHO, 1987).
3.5.4 Indoor nitrous acid concentrations

Recent studies have demonstrated that substantial concentrations of HNO$_2$ can be present inside residential buildings, especially when unvented combustion sources are used. HNO$_2$ is formed by the reaction of NO$_2$ with water on surfaces and the reaction is promoted by high humidity. HNO$_2$ may also be produced by other mechanisms, and this is the subject of active research. Brauer et al. (1993) found that HNO$_2$ can represent over 10% of the concentrations usually reported as NO$_2$.

3.5.5 Predictive models for indoor NO$_2$ concentration

Efforts to model indoor NO$_2$ levels have employed two distinct approaches: physical/chemical and empirical/statistical models.

The physical/chemical modelling approach has been used by numerous investigators in chamber, test house and small field studies (involving a small number of homes) to estimate emission rates of NO$_2$ from combustion sources (e.g., Traynor et al., 1982a; Leaderer, 1982; Moschandreas et al., 1984), to estimate reactive decay rates (e.g., Oezkaynak et al., 1982; Yamanaka, 1984; Leaderer et al., 1986; Spicer et al., 1986; Borrazzo et al., 1987a), to estimate the impact of ventilation and mixing on the spatial and temporal distribution of NO$_2$ (e.g., Oezkaynak et al., 1982; Traynor et al., 1982b; Borrazzo et al., 1987a), and to evaluate the applicability of emission rates determined under controlled conditions in estimating indoor concentrations of NO$_2$ (e.g., Traynor et al., 1982b; Borrazzo et al., 1987a). More recently, studies have reported the use of distributions of the input variables to the mass balance equation (emission rates, source use, decay rates, ventilation rates, etc.), determined from the published literature, to estimate distributions of indoor NO$_2$ levels for specific sources and combinations of sources (Traynor et al., 1987; Hemphill et al., 1987).

Prediction of indoor concentrations or concentration distributions of NO$_2$ in homes with combustion sources using physical/chemical (mass-balance) models requires accurate information for input parameters (e.g., emission rates). Although data are available for some of the input parameters under controlled experimental conditions, there are very limited data available concerning either the variability of such input parameters in actual homes or the factors that control variability (e.g., variability of emission or decay rates). Obtaining field
measurements or estimates of the inputs in large numbers of homes would be expensive and time-consuming. Such modelling efforts do, however, help to identify the potential range of indoor NO$_2$ concentrations, factors that may result in high levels, and the potential effectiveness of mitigation efforts.

Empirical/statistical models have been developed from large field studies that have measured NO$_2$ concentrations in residences and associated outdoor levels for time periods of a week or more. These have typically used questionnaires to obtain information on sources in the residences, source use, building characteristics (house volume, number of rooms, etc.), building use, and meteorological conditions. In some cases, additional measurements, including temperature have been recorded. Several investigators have attempted to fit simple regression models to their field study databases in an effort to determine whether the variations in NO$_2$ levels seen among houses can be explained by variations in questionnaire responses. The goal has been to see how well questionnaire information or easily available information (meteorological data) can predict indoor NO$_2$ levels. In most cases a linear model has been used, but several investigators have used log transformations of variables. These employ questionnaire responses and measured physical data (house volume, etc.) as independent variables and have met with moderate success. Linear regression models, with the exception of the Petreas et al. (1988) model, explain from 40 to 70% of the variations in residential NO$_2$ levels and typically have large standard errors associated with their estimates. Although log transformations of variables have always produced a higher percentage of explained variation due to the skewed distribution of the original variables, interpretation of the coefficients in a nonlinear model can require special attention.

Regression models developed from field studies employing questionnaires to explain variations in indoor levels of NO$_2$ have met with only moderate success.

Better information, through additional measurements and better questionnaire design, is needed on a range of factors if the statistical/empirical models are to be used to estimate indoor concentrations of NO$_2$ in homes without measurements. Factors include source type and condition, source use, contaminant removal (infiltration and reactive decay) and between and within room mixing.
3.6 Human exposure

To assess the health impact of exposure to nitrogen oxides, it is essential to conduct an accurate exposure assessment. Such data are of paramount importance for the definition of dose-effect and dose-response relationships. In fact, the risk to human health is not simply determined by indoor and outdoor concentrations of nitrogen oxides, but rather by the personal exposure of every individual. The integrated exposure is the sum of the individual exposures to oxides of nitrogen over all possible time intervals for all settings or environments. It requires, thus, the consideration of long-term average concentration level, variations and short-term exposures, as well as the activity patterns and personal and home characteristics of individuals (Berglund et al., 1994).

Exposure is a function of concentration and time. People spend various periods in different types of micro-environments with various concentration levels. On average, people spend about 90% of their time indoors (at home, work, school, etc.), about 5% in transit (Chapin, 1974), and 7% (range 3-12%) near smokers (Quackenboss et al., 1982). These values vary with the season, day of the week, age, occupation and other factors but it is decidedly important to predict indoor pollutant levels when total exposure is being estimated.

Adequate exposure assessment for NO$_2$ is particularly critical in conducting and evaluating epidemiological studies. Failure to measure or estimate exposures adequately and address the uncertainty in the measured exposures can lead to misclassification errors (Shy et al., 1978; Gladen & Rogan, 1979; Oezkaynak et al., 1986; Willett, 1989; Dosemeci et al., 1990; Lebret, 1990). Early studies comparing the incidence of respiratory illness in children living in homes with and without gas stoves used a simple categorical variable of NO$_2$ exposure, the presence or absence of a gas cooker. Such a dichotomous grouping can result in a severe non-differential misclassification error in assigning exposure categories. This type of error is likely to underestimate the true relationship and could possibly result in a null finding.

In assessing human exposure to NO$_2$ (and other oxides of nitrogen), averaging times chosen should account for the type of effect to be expected. With regard to NO$_2$, the principal biological responses include (a) relatively transient effects on respiratory function associated with acute, short-term (< 1 h) exposures, and (b) the likelihood of increased risk for respiratory disease in
children associated with frequently repeated short-term peak exposures and/or lower level long-term exposures.

Indirect and direct methods for personal exposure assessment are available. Indirect methods combine measures of concentrations at fixed sites in various types of micro-environments with information on where people have spent their time (time-activity patterns). Time-weighted average (TWA) exposure models have been developed to estimate total personal exposure (Fugas, 1975; Duan, 1982; Duan, 1991). The NO$_2$ exposure levels predicted from TWA exposure models have been shown to correlate closely with the exposure levels obtained by direct measurements of personal exposure (Nitta & Maeda, 1982; Quackenboss et al., 1986; Sega & Fugas, 1991). However, the large variation in NO$_2$ concentrations (distribution) within each type of micro-environment (because of variability in, for example, stove use, emission rates, ventilation frequencies, and the day-to-day and person-to-person variations in the use of time) decreases the accuracy of the predicted exposure and increases the risk for misclassification of the exposure.

Direct measurements of concentrations in the breathing zone of a person using personal passive exposure monitors provide time-integrated measurements of exposure for a certain period across the various micro-environments where a person spends time. It is important to collect exposure data over time intervals consistent with the expected effects. Effects from long-term, low-level exposure may be different from effects from short periods of high concentration (intermittent peak exposure). Intermittent peak exposure, which occurs during cooking on a gas stove, may be significant to total exposure and adverse health effects. If effects from peak exposure are to be considered in the exposure assessment, the sampling time must be short enough to detect these peak exposures. Such a short sampling time is possible with the more sensitive passive samplers and with conventional air monitors, such as chemiluminescence NO$_2$ monitors. However, direct methods of measuring personal exposure are relatively costly and time-consuming. Within-person and between-person variability, both in personal exposure and personal use of time, can be large.

Hence a sufficient number of personal exposure measurements must be collected for each person (repeated measurements), and a sufficient number of individuals must be sampled before the measurements can be considered to be representative. Personal
daily exposures have been shown to vary between individuals on the same day by a factor of up to about 15 in the urban area of Stockholm and between days for the same individual by a factor of up to 10 (Berglund et al., 1993).

A comparison of personal NO₂ exposures, as measured by Palmes diffusion tubes, and NO₂ exposures measured in residences had a correlation of 0.94 for a subsample of 23 individuals (Leaderer et al., 1986). Results of this comparison are depicted in Fig. 13 and show an excellent correlation between average household exposure and measured personal exposure.

It is important to note that indoor concentrations are strong predictors of personal exposure. In the case of homes with gas or electric stoves, personal exposure has been shown to be closely related to the household indoor average concentrations (Quackenboss et al., 1986; Harlos et al., 1987a).

Results of monitoring in Portage, Wisconsin, verify that the presence of a gas stove contributes dramatically to personal NO₂ exposure levels. Table 16, derived from the reports of Quackenboss et al. (1986) and based on data collected in 1981 and 1982, clearly shows that gas stoves increase not only indoor concentrations but also the personal exposure of children.

On the other hand, outdoor concentrations, even if measured outside each residence, have been found to be relatively poor predictors of personal exposure (Quackenboss et al., 1986; Leaderer et al., 1986). The association between personal exposure and outdoor levels of NO₂ is weakest during the winter for both gas and electric stove groups.

The only route of NO₂ exposure is inhalation. The dose is dependent on the inhalation volume and thus on physical activity, age, etc. Lung absorption of NO₂ is about 80-90% during rest and over 90% during physical activity (WHO, 1987).

Efforts have been made to find a sufficient biological marker for NO₂ exposure and dose. Increased urinary excretion of collagen and elastin (pulmonary connective tissue) breakdown products (including hydroxyproline, hydroxylysine and desmosine) has been suggested as a marker of diffuse pulmonary injury related to inhaled NO₂. A significant relationship between urinary hydroxyproline excretion and daily NO₂ exposure was found among housewives in Japan, but the hydroxyproline excretion fell
Fig. 13. Total personal exposure to NO\textsubscript{2} versus NO\textsubscript{2} levels in Connecticut (USA) residences (from: Leaderet al., 1986)
within the normal distribution for healthy people (Yanagisawa et al., 1986). The majority of the housewives were exposed to active or passive cigarette smoke, and this exposure was independently related to the excretion of hydroxyproline. Other investigators have not been able to substantiate the relationship between urinary hydroxyproline excretion and NO₂ exposure (Muelenaer et al., 1987; Adgate et al., 1992).

Measurements of the NO-haem protein complex in bronchoalveolar lavage (Maples et al., 1991) and of 3-nitrotyrosine in urine (Oshima et al., 1990) have been suggested as biological markers for NO₂ exposure. The work in progress to find a suitable biological marker for NO₂ exposure at levels found in the general environment is promising; however, no metabolite has yet proved to be sensitive or specific enough.

Personal exposure to air pollutants can be assessed by direct or indirect measures. Direct measures include biomarkers and use of personal monitors. No validated biomarkers for exposure are presently available for NO₂.

Studies using passive monitors to measure NO₂ exposures lasting one day to one week have been conducted in the USA (Dockery et al., 1981; Clausing et al., 1986; Leaderer et al., 1986; Quackenboss et al., 1986; Harlos et al., 1987; Schwab et al., 1990), in the Netherlands (Hoek et al., 1984), in Japan (Nitta & Maeda, 1982; Yanagisawa et al., 1984), and in Hong Kong (Koo et al., 1990). These studies generally indicate that outdoor levels of NO₂, although related to both personal levels and indoor concentrations, are poor predictors of personal exposures for most populations. Average indoor air residential concentrations (e.g., whole-house average or bedroom level) tend to be the best predictor of personal exposure, typically explaining 50 to 80% of the variation in personal exposures.

Indirect measures of personal exposure to NO₂ employ various degrees of micro-environmental monitoring and questionnaires to estimate an individual's or population's total exposure. One such model (Billick et al., 1991), developed from an extensive monitoring and questionnaire database, can estimate population exposure distributions from easily obtained data on outdoor NO₂ concentrations, housing characteristics and time-activity patterns. This model is proposed for use in evaluating the impact of various NO₂ mitigation measures. The model is promising, but has not yet been validated nor has associated uncertainty been characterized.
3.7 Exposure of plants and ecosystems

The sensitivity of plants to nitrogen oxides is determined both by their genetic characteristics and by environmental conditions.

The relation between exposure and uptake by plants depends on aerodynamic and stomatal resistance and thus increases with increasing light intensity, wind velocity and air humidity. After uptake, the response of a plant depends on its metabolic activity, and thus increases with poorer nutritional supply and lower temperature.

Moreover, the sensitivity of plants depends on the general air pollution situation. Emission of SO₂ is often combined with NOₓ, and these compounds act synergistically. Therefore, the impact of NOₓ may be higher in regions with elevated SO₂ concentrations. NOₓ forms part of photochemical smog. Although ozone is the most phytotoxic, the contribution of NOₓ to adverse effects on plants is not negligible.

For vegetation and ecosystems the impact of NOₓ is through its contribution to total nitrogen disposition rather than its direct toxicity. Thus, other nitrogen-containing pollutants have to be taken into consideration.

The dependencies of sensitivity, as summarized above, mean that wide variation exists in the vulnerability of different regions of the world.
4. EFFECTS OF ATMOSPHERIC NITROGEN COMPOUNDS (PARTICULARLY NITROGEN OXIDES) ON PLANTS

Effects of nitrogen on ecosystems are caused through deposition onto soil and foliar uptake of nitrogen in various forms. Total effects are often difficult to separate into component effects. This section, therefore, covers nitrogen inputs in all forms to ecosystems. Much of the research focuses on European ecosystems, where the majority of the research has been conducted. Here NH₃ deposition either dominates or is a major constituent of total nitrogen input. However, this is not true for other parts of the world. All effects of atmospheric nitrogen input, in whatever form, are included as indicators of more globally relevant effects on ecosystems but the reader should bear in mind local conditions of nitrogen input when assessing likely local consequences.

NOₓ, as used in this chapter, refers to the total nitrogen measured by chemiluminescence detectors; this is NO₂ following conversion to NO, and NO itself. Other nitrogen oxides may interfere to some extent in this method.

Elemental nitrogen (N₂) forms 80% of the atmosphere of the earth. This is equivalent to about 7.5 x 10⁶ kg above each hectare of the earth's surface. In unpolluted conditions a small fraction (1-13 kg nitrogen per ha per year) is converted by nitrogen-fixing microorganisms to biologically more active forms of nitrogen: NH₄⁺ and NO₃⁻. The natural deposition of nitrogen-containing atmospheric compounds other than N₂ is much less. The soil contains 5 times more nitrogen than the atmosphere, but weathering of rock is a negligible source of biologically active nitrogen. By denitrification (reduction of NO₃- to N₂ and to a lesser extent N₂O, NO and NH₃), 1-30 kg nitrogen per ha per year is recycled from the earth to the atmosphere.

Human activities, both industrial and agricultural, have greatly increased the amount of biologically active nitrogen compounds, thereby disturbing the natural nitrogen cycle. Various forms of nitrogen pollute the air, mainly NO, NO₂ and NH₃ as dry deposition and NO₃ and NH₄⁺ as wet deposition. Another contribution is from occult deposition (fog and clouds). There are many more nitrogen-containing air pollutants (e.g., N₂O₅, PAN, N₂O, amines) but these have not been considered in this chapter, either because their contribution to the total nitrogen deposition is considered to...
be small or because their concentrations are probably far below the
effect thresholds.

Transformations of nitrogen, as it moves from the atmosphere,
through ecosystems and back to the atmosphere, form the nitrogen
cycle. This is illustrated, together with anthropogenic sources of
nitrogen, in Fig. 14. The component processes affected by chronic
nitrogen deposition are indicated in Fig. 15.

Nitrogen-containing air pollutants can affect vegetation
indirectly, via chemical reactions in the atmosphere, or directly
after being deposited on vegetation, soil or water surfaces. The
indirect pathway is largely neglected in this chapter, although it
includes very relevant processes, and should be taken into account
when evaluating the entire impact of nitrogen-containing air
pollutants: NO and NO₂ are precursors for tropospheric ozone (O₃),
which acts both as a phytotoxin and a greenhouse gas. The effects
of O₃ will be discussed in a forthcoming Environmental Health
Criteria monograph. N₂O contributes to the depletion of
stratospheric O₃, resulting in increasing ultraviolet radiation. This
and other aspects of global climate change will be evaluated in a
WHO/WMO/UNEP document entitled “Climate and Health:
potential impacts of climate change”. The direct impact of
airborne nitrogen is due to toxic effects, eutrophication and soil
acidification. One effect of soil acidification is that aluminum
enters into solution, hence increasing its bioavailability. This
result in root damage. Aluminum toxicity will be discussed in a
further Environmental Health Criteria monograph.

Most biodiversity is found in (semi-)natural ecosystems, both
aquatic and terrestrial. Nitrogen is the limiting nutrient for plant
growth in many (semi-)natural ecosystems. Most of the plant
species from these (semi-)natural habitats are adapted to nutrient-
poor conditions, and can only compete successfully in soils with
low nitrogen levels (Chapin, 1980; Tamm, 1991). Ellenberg (1988b)
surveyed the nitrogen requirements of 1805 plant species from
Germany and concluded that 50% can compete successfully only
in habitats that are deficient in nitrogen. Furthermore, of the
plants threatened by increased nitrogen deposition, 75-80% are
indicator species for low-nitrogen habitats. When stratified by
ecosystem type, it is also clear that the trend of rare species
occurring with greater frequency in nitrogen-poor habitats is a
common phenomenon across many ecosystems (Fig. 16 and
Fig. 17). Plant species threatened by high nitrogen deposition are
common across many ecosystem types (Ellenberg, 1988b). The
Fig. 14. Schematic representation of the nitrogen cycle, emphasizing human activities that affect fluxes of nitrogen (from: National Research Council, 1978)
critical loads for nitrogen depend on (i) the type of ecosystem; (ii) the land use and management in the past and present; and (iii) the abiotic conditions (especially those which influence the nitrification potential and immobilization rate in the soil). The impact of increased nitrogen deposition upon biological systems can be the result of direct uptake by the foliage or uptake via the soil. The most relevant effects at the level of individual plants are injury to the tissue, changes in biomass production and increased
Fig. 16. Distribution of 2164 Central European plant species in the gradient of nitrogen indicator values (From: Ellenberg, 1988b)

a) "?" not known; "x" indifferent
"1" most pronounced nitrogen deficiency
"3" poor in nitrogen
"5" just sufficient in nitrogen
"7" more often found at places rich in nitrogen
"8" nitrogen indicator
"9" surplus nitrogen to polluted with nitrogen
"2", "4", "6" intermediate

b) Most of the threatened species can only compete on nitrogen-deficient stands (57 "potentially threatened" species not regarded)

c) The fraction of threatened species within the total of species in a given class of nitrogen indicator value diminishes with improved nitrogen supply. It remains constant from value "5" upwards (see above)
Fig. 17. Distribution of Central European plant species along a gradient of nitrogen indicator values across ecosystem types (From: Ellenberg, 1988b)

n = number of species
1 = most pronounced nitrogen deficiency
9 = surplus nitrogen polluted with nitrogen

susceptibility to secondary stress factors. At the vegetation level, this results in changes in competitive relationships between species and loss of biodiversity.
Effects on individual plants are discussed in section 4.1. The following natural ecosystems are treated in detail in section 4.2: freshwater ecosystems (shallow soft-water bodies, lakes and streams) and terrestrial ecosystems (wetlands and bogs, species-rich grasslands, heathlands and forests). Estuarine and marine systems are also considered.

Air quality guidelines refer to thresholds for adverse effects. Two different types of effect thresholds exist: critical levels and critical loads.

The critical level is defined as:

the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge.

The critical load is defined as:

a quantitative estimate of an exposure (deposition) to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge.

Generally, critical levels for nitrogen-containing air pollutants are expressed in terms of exposure (µg/m³ and exposure duration), while critical loads are expressed in terms of deposition (kg nitrogen per ha per year). Both critical level and load are intended to be set so as to protect vegetation, and can be converted into each other knowing the deposition velocity. Thus, it might seem to be superfluous to assess both critical levels and loads. However, with the currently accepted approach, critical levels and loads are more or less complementary: critical levels focus on effect thresholds for short-term exposure (1 year or less), while critical loads focus on safe deposition quantities for long-term exposure (10–100 years): critical levels are not aimed to protect plants completely against adverse effects. No-observed-effect concentrations (NOECs) are usually lower than critical levels. For instance, a critical level can be set at 5% yield reduction. Thus, owing simply to differences in definition, the critical level is generally higher than the critical load (Fig. 18b).

In current practice there are other differences between critical levels and loads: critical levels give details on individual compounds and focus on responses on plant level, while critical
loads cover all nitrogen-containing compounds and focus on the vegetation or ecosystem level. In other words: critical loads focus on functioning of the ecosystem, while critical levels focus on protection of the relatively sensitive plant species.

In the critical level concept, the different nitrogen-containing compounds are evaluated separately, because of their differences in phytotoxic properties, even when their load in terms of kg nitrogen per ha per year is the same (Ashenden et al., 1993). Another difference between critical level and critical load is that critical level considers the possibility of more- or less-than-additive effects (Wellburn, 1990), while in the critical load concept additivity of nitrogen-containing or acidifying compounds is presumed. Moreover, nitrogen-containing air pollutants have their impact not only because of their contribution to the nitrogen supply. Sometimes other effects seem to dominate. For instance, although occult deposition is generally small in terms of nitrogen deposition, it may be of great significance because of its ability to affect plant surfaces.

It was concluded for these reasons that both critical levels and loads are necessary within the scope of air quality guidelines for nitrogen-containing compounds.

Assessing effect thresholds is relatively simple in the case of toxic compounds with an exposure/response relationship which follows the usual sigmoid curve: the lowest exposure level that results in a response that is significantly different from the control treatment is the effect threshold. However, this approach is essentially invalid for exposure of nitrogen-limited vegetation to nitrogen-containing air pollutants. Nitrogen is a macro-nutrient and so each addition of nitrogen can result in a physiological response: growth stimulation gradually increases with higher exposure levels and changes in growth inhibition at higher levels (Fig. 18a). Moreover, depending on the definition of adverse effects, the status of the vegetation may not be optimal at background levels (Fig. 18b). These features complicate the assessment of effect thresholds for nitrogen-containing compounds. Nevertheless, in this chapter effect thresholds are presented, according to current practice.

4.1 Properties of NO\textsubscript{x} and NH\textsubscript{y}

In this section general information is initially presented on uptake, detoxification, metabolism and growth aspects. In the
Effects of Atmospheric Nitrogen Compounds on Plants

Fig. 18: Fictive exposure/response relationships for nitrogen-containing air pollutants.

a) Biomass production related to exposure to NH₃ or NOₓ

b) Fitness of a vegetation (e.g., expressed as health or species diversity) related to exposure (BG = natural background; CLE = critical level; CLO = critical load)

following subsections the data determining the critical levels for individual compounds and mixtures are discussed. The relevance of this information and possibilities for generalization are discussed in sections 4.1.8 and 4.1.9, where the critical levels are
estimated. Deposition on and emission from soils and vegetation is discussed in chapter 3.

4.1.1 Adsorption and uptake

The impact of a pollutant on plants is determined by its adsorption, rate of uptake (flux) and the reaction of the plants. Foliar uptake is probably dominant for NO, NO$_2$ (Wellburn, 1990) and NH$_3$ (Perez-Soba & Van der Eerden, 1993), while the pathway via soil and roots is the major route for nitrogen-containing pollutants in wet deposition.

The flux of the compounds from the atmosphere into the plant is a complicated process, which is highly dependent on the properties of both plant and compound and on environmental conditions. This is why deposition velocities proved to be highly variable (chapter 3).

The flux from the atmosphere to the leaf surface (and soil) depends on the aerodynamic and boundary layer resistances, which are determined by meteorological conditions and plant and leaf architecture. The flux from the leaf surface to the final site of reaction in the cell is determined by stomatal, cuticular and mesophyll resistance. The reaction of the plant to the nitrogen that arrives at the target site is dependent on the intrinsic properties of the plant and on its nutritional status, and again on environmental conditions.

The flux of atmospheric nitrogen through the soil is conditioned by properties of soil and vegetation and by meteorological conditions. The chemical composition of soil water, the rate of nitrification (NH$_4^+$ → NO$_3^-$), the preference of the plant for either NH$_4^+$ or NO$_3^-$, the root architecture and the metabolic activity of the plants play major roles in this uptake (Schulze et al., 1989).

Adsorption on the outer surface of leaves certainly takes place. Exposure to relatively high concentrations of gaseous NH$_3$ (180 µg/m$^3$) or NH$_4^+$ in rainwater (5 mmol/litre) damages the crystalline structure of the epicuticular wax layer of the needles of *Pseudotsuga menziesii* (Van der Eerden & Perez-Soba, 1992). NO$_2$ (Fowler et al. 1980) and NH$_4^+$ and NO$_3^-$ in wet and occult deposition can disturb leaf surfaces in several ways (Jacobson, 1991). The quantitative relevance of this effect for the field situations has not yet been shown in detail.
Uptake of \( \text{NH}_3 \) and \( \text{NO}_x \) is driven by the concentration gradient between atmosphere and mesophyll. It is generally directly determined by stomatal conductance and thus depends on factors influencing stomatal aperture. Although in higher plants uptake through the stomata strongly dominates, there are indications that penetration through the cuticle is not completely negligible. This has been demonstrated for \( \text{NO} \) and \( \text{NO}_2 \) (Wellburn, 1990). While stomata greatly influence the foliar uptake of aerial nitrogen compounds, many of these compounds subsequently alter stomatal aperture and the extent of further uptake. The nitrogen status of plants is also known to affect stomatal behaviour towards other environmental conditions such as drought (Ghashghaie & Saugier, 1989).

The flux of \( \text{NH}_3 \) into a plant appeared to be linearly related to the atmospheric concentration (Van der Eerden et al., 1991), there being no mesophyll resistance (Van Hove et al., 1989). This relation can become less than linear with high concentrations, e.g., some hundreds of \( \mu \text{g/m}^3 \) (Wollenheber & Raven, 1993). Mesophyll resistance is, however, probably more significant for \( \text{NO} \) and \( \text{NO}_2 \) (Capron et al., 1994).

There is increasing evidence that foliar uptake of nitrogen reduces the uptake of nitrogen by the roots (Srivastava & Ormrod, 1986; Perez-Soba & van der Eerden, 1993), although the driving mechanism is not yet clear.

In the presence of low concentrations plants can emit \( \text{NH}_3 \), rather than absorb it (chapter 3). \( \text{NO} \) and \( \text{N}_2\text{O} \) are emitted in significant quantities by the soil (chapter 3).

Rain, clouds, fog and aerosols always contain significant amounts of ions including \( \text{NH}_4^+ \) and \( \text{NO}_3^- \). In the past, foliar uptake of nitrogen from wet deposition was considered to be negligible, but recent research using \( ^{15}\text{N} \) and throughfall analysis shows that this path can contribute a high proportion of the total plant uptake (see Pearson & Stewart, 1993, and section 2.4). In general, cations (e.g., \( \text{NH}_4^+ \)) are more easily taken up through the cuticle than anions (e.g., \( \text{NO}_3^- \)). A substantial foliar uptake of \( \text{NH}_4^+ \) from rainwater has been measured in several tree species (Garten & Hanson, 1989). Lower plants, such as bryophytes and lichens do not have stomata and a waxy waterproof cuticle, and thus the potential for direct uptake of pollutants in the form of wet or dry deposition is greater. Epiphytic lichens are active absorbers of both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) (Reiners & Olson, 1984). Uptake
and exchange of ions through the leaf surface is a relatively slow process, and thus is only relevant if the surface remains wet for long periods.

4.1.2 Toxicity, detoxification and assimilation

One would expect a positive relationship between the solubility of a compound and its biological impact. NO is only slightly soluble in water, but the presence of other substances can alter its solubility. NO₂ has a higher solubility, while that of NH₃ is much higher.

Much information exists on mechanisms of toxicity, although it is sometimes confusing. NO₂, NO, HNO₂, and HNO₃ can be incorporated into nitrogen metabolism using the pathway: NO₂ → NO → (NH₃ → NH₄⁺) → glutamate → glutamine → other amino acids, amides, proteins, polyamines, etc. The enzymes involved include nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS). Glutamate dehydrogenase (GDH) plays a role in the internal cycling of NH₄⁺.

After exposure to NO₂, nitrate can accumulate for some weeks; accumulation of nitrite is rarely reported, although it is certainly an intermediate. Nitrite levels can be elevated for some hours due to the fact that NR activity is induced faster than that of NiR. In many cases storage of excess nitrogen has been found to be in the form of arginine (Van Dijk & Roelofs, 1988), which could last months or longer.

NO₂, NH₃ and NH₄⁺ are highly phytotoxic, and could well be the cause of adverse effects of nitrogen-containing air pollutants. Wellburn (1990) suggested that the free radical *N=O plays a role in the phytotoxicity of NO₂.

High concentrations can cause visible injury via lipid breakdown and cellular plasmolysis. At lower concentrations inhibition of lipid biosynthesis may dominate, rather than damage of existing lipids (Wellburn, 1990).

Raven (1988) assumed that the adverse effects of nitrogen-containing compounds are due to their interference with cellular acid/base regulation. They can influence cellular pH both before and after assimilation. Assimilation of most air pollutants, including NH₃, has been shown to result in production of protons (Wollenheber & Raven, 1993). Assimilation of nitrate and a high
buffer capacity can prevent the plant from being damaged by this acidification (Pearson & Stewart, 1993). If these adverse effects can effectively be counteracted, assimilation of nitrogen-containing compounds will result in growth stimulation.

Synergistic effects have been found in nearly all studies concerning SO₂ and NO₂ (Wellburn et al., 1981). Inhibition of NiR by SO₃₂⁻, resulting in the inability of the plant to detoxify nitrite, might be the cause of this interaction.

4.1.3 Physiology and growth aspects

When climatic conditions and nutrient supply allow biomass production, both NO₃ and NH₄ result in growth stimulation at low concentrations and growth reduction at higher concentrations. However, the exposure level at which growth stimulation turns into growth inhibition is much lower for NO₃ than for NH₄ (see Fig. 18a).

Foliar uptake of NH₃ generally results in an increase in photosynthesis and dark respiration, and in the amount of RUBISCO (ribulose 1,5-biphosphate carboxylase oxygenase) and chlorophyll. Some authors have shown that stomatal conductance increases and the stomata remain open in the dark, resulting in enhanced transpiration and drought sensitivity (Van der Eerden & Pérez-Soba, 1992). Most experiments with NO and NO₂ have been conducted with relatively high concentration levels (> 200 µg/m³). These experiments show inhibition of photosynthesis by both NO and NO₂, possibly additively (Capron & Mansfield, 1976). Inhibition by NO may be stronger than that of NO₂ (Saxe, 1986). The threshold for this response is well below the threshold for visible injury (Wellburn, 1990) and transpiration (Saxe, 1986). With lower (nearer to ambient) NO₂ concentrations, stimulation of photosynthesis may well occur. Both NO₃ and NH₄ generally cause an increase in shoot/root ratio. The specific root length and the amount of mycorrhizal infection can be reduced by both compounds. However, these alterations in root properties resemble general responses to increased nitrogen nutrient supply.

4.1.4 Interactions with climatic conditions

Evidence suggests that exposure of vegetation to NH₃ and to mixtures of NO₂ and SO₂ can influence the subsequent response to drought and frost stress. There is also evidence that environmental conditions can affect the response to NO₃ and to NH₄.
The foliar uptake of nitrogenous compounds in the form of wet and occult deposition is largely via the cuticle. Uptake and exchange of ions through the leaf surface is a relatively slow process, and thus is especially relevant if the surface remains wet for longer periods, e.g., in regions where exposure to mist and clouds is frequent.

The solubility of most gases, including NO, NO₂, and NH₃, rises as temperature falls, while the metabolic activity of plants and thus their detoxification capacity is lower. On the other hand, stomatal conductivity and thus the influx of gases generally falls as temperature falls.

Guderian (1988) proposed a lower critical level in winter than for the whole year, in acknowledgement of several results that indicate greater toxicity of NO₂ during winter conditions. For example, exposure of Poa pratensis in outdoor chambers to 120 µg/m³ inhibited growth during winter but had little effect or even stimulated growth in summer and autumn (Whitmore & Freer-Smith, 1982). Mortensen (1986) found that low light and non-injurious low temperature conditions can enhance the toxicity of NO₂. Capron et al. (1991) found that the depression relative to the control of net photosynthesis by 1250 µg NO/m³ plus 575 µg NO₂/m³ at 10 °C was three times, and at 5 °C was almost five times, that recorded at 20 °C. An interaction between NO₂ and temperature may also occur at lower realistic concentrations. This is suggested by the observation of nitrite accumulation at low temperatures during fumigation of Picea rubra with 38 µg NO₂/m³ plus 34 µg SO₂/m³ (Wolfenden et al., 1991). This temperature effect may play a role in combination with elevated concentrations of CO₂ as well: the stimulating effect of CO₂ on net photosynthesis was inhibited by NO₂ to a larger extent when applied at lower temperature (Capron et al., 1994). Observation of NH₃ injury to plants also indicates that this is greatest in winter (Van der Eerden, 1982).

In contrast with the view that NO₂ (and NH₃) injury is greater at low temperatures, Srivastava et al. (1975) found that inhibition by NO₂ of photosynthesis was greatest under optimal temperature and high light conditions, when stomatal conductance to the gas would be highest.

The exposure of plants to NO₂ and NH₃ may reduce their ability to withstand drought stress, owing to loss of control of transpiration by stomata and to an increase in the shoot/root ratio (Lucas, 1990; Atkinson et al., 1991; Fangmeijer et al., 1994).
4.1.5 Interactions with the habitat

Whether the atmospheric input of nitrogen has a positive or negative impact depends on the plant species and habitat. Based on experimental evidence, Pearson & Stewart (1993) hypothesized that species which are part of a climax vegetation on nutrient-poor acidic soils are often relatively sensitive to NO\textsubscript{2} and NH\textsubscript{3}. Morgan et al. (1992) found that NO\textsubscript{2} disrupted the NR activity to a greater extent in calcifuge than calcicole moss species. Ombrotrophic mires and other strongly nitrogen-limited systems may be especially prone to detrimental effects from input of nitrogen-containing air pollutants.

The assimilation of low concentrations of NO\textsubscript{2} and the incorporation into amino acids by NR (Morgan et al., 1992; Table 20) are indicators that this pollutant can contribute to the nitrogen budget of plants (Perez-Soba et al., 1994). The contribution of NO\textsubscript{2} to the nitrogen supply increases as root-available nitrogen is lowered (Okano & Totsuka, 1986; Rowland et al., 1987). Srivastava & Ormrod (1986) observed reduced ability to respond to a supply of nitrate to the roots when Hordeum vulgare was fumigated with NO\textsubscript{2}. Similarly, Perez-Soba & Van der Eerden (1993) found reduced uptake of NH\textsubscript{4} from the soil when Pinus sylvestris was fumigated with NH\textsubscript{3}. Although there is much evidence that nitrogen-containing air pollutants play a role in the nitrogen demand and nitrogen metabolism of the plant, Ashenden et al. (1993) found no obvious relationship between sensitivity to NO\textsubscript{2} and nitrogen preference, as indicated by Ellenberg (1985).

4.1.6 Increasing pest incidence

Any change in chemical composition of plants due to the uptake of nitrogenous air pollutants could alter the behaviour of pests and pathogens. Evidence indicates that, in general, NO\textsubscript{2} and NH\textsubscript{3} increase the growth rate of herbivorous insects (Dohmen et al., 1984; Fluckiger & Braun, 1986; Houlden et al., 1990; Van der Eerden et al., 1991). This may also apply to fungal pathogens (van Dijk et al., 1992).

4.1.7 Conclusions for various atmospheric nitrogen species and mixtures

4.1.7.1 NO\textsubscript{2}

In Table 20 the lowest effective exposure levels for NO\textsubscript{2} are given. Three different types of effects are considered:
• (bio)chemical: e.g., enzyme activity, consumption quality
• physiological: e.g., CO₂ assimilation, stomatal conductivity
• growth aspects: e.g., biomass, reproduction, stress sensitivity

Four exposure durations are used in this table. These are (including an indication of the exposure durations and the margins):

• short term (hours): < 8 h
• air pollution episodes (days): 8 h to 2 weeks
• growing season or winter season (months): 2 weeks to 6 months
• long term (years): > 6 months

To avoid the information being too selective, in each cell in this table a species is used only once. For each cell the three lowest effective concentrations and exposure durations are given; species and references are mentioned in footnotes. Exposure levels far higher than current levels measured in the field situation have not been considered.

The fact that not all cells in Table 20 are filled with information is because many of the experiments have been conducted with unrealistically high concentrations. The majority of observations mentioned in the table are on crops; several of these show growth stimulation. Most of the responses on a biochemical level deal with enhanced NR activity, which shows that the plants are capable of assimilating the NO₂. A general effect threshold as derived from Table 20 would be substantially higher if enhanced NR and biomass production of crops were not assumed to be an adverse effect. However, growth stimulation is often considered an adverse effect in most types of natural vegetation. Moreover, Pearson & Stewart (1993) assumed detoxification of NH₃ and NO₂ to be a potentially adverse effect, because it contributes to cellular acidification, which can not always be counteracted.

4.1.7.2 NO

In Table 21 the lowest effective exposure levels for NO are given.

Most research into the effects of nitric oxide has been based on glasshouse crops, particularly the tomato (*Lycopersicon esculentum*). Virtually all experiments deal with photosynthesis or enzymatic reactions and a few growth aspects were measured. The
### Table 20. Lowest exposure levels (in μg/m³) and durations at which NO₂ caused significant effects

<table>
<thead>
<tr>
<th>Aspect</th>
<th>(Bio)chemical</th>
<th>Physiological</th>
<th>Growth aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term</td>
<td></td>
<td></td>
<td>200 (130); 104 h/week; 7 months&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>120-500; 9.5 months&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>122; 37 weeks&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Growing season or winter</td>
<td>50; 39 days&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120; 22 days&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10-43; 130 days&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>125; 140 days&lt;sup&gt;c&lt;/sup&gt;</td>
<td>190 (65); 105 h in 15 days&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55-75; 62 days&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>940; 19 days&lt;sup&gt;d&lt;/sup&gt;</td>
<td>in 15 days&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190-190 (28-33); 120 h in 40 days&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Air pollution episodes</td>
<td>140; 1 day&lt;sup&gt;e&lt;/sup&gt;</td>
<td>375 (165); 35 h in 5 days 190; 3 days&lt;sup&gt;g&lt;/sup&gt;</td>
<td>375; 2 weeks&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>150; 7 days&lt;sup&gt;f&lt;/sup&gt;</td>
<td>375 (165); 35 h in 5 days&lt;sup&gt;f&lt;/sup&gt;</td>
<td>100 (25);</td>
</tr>
<tr>
<td></td>
<td>65; 1 day&lt;sup&gt;f&lt;/sup&gt;</td>
<td>in 5 days&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20 h in 5 days&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Short term</td>
<td>7500; 6 h&lt;sup&gt;i&lt;/sup&gt;</td>
<td>940; 1 h&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2000-3000; 3.5 h&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7500; 4 h&lt;sup&gt;j&lt;/sup&gt;</td>
<td>850; 7 h&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1100; 1.5 h&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> If the fumigation was not continuous an average value has been estimated and quoted in parentheses (calculated assuming 10 μg/m³ during the periods in which the fumigation was switched off).

<sup>b</sup> Pinus sylvestris; changes in amino acid composition, with no physiological changes (Nåsholm et al., 1991)

<sup>c</sup> Lolium perenne; increase in GDH activity (Wellburn et al., 1981)

<sup>d</sup> Lycopersicum esculentum; decrease in nitrate content of the leaves (Taylor & Eaton, 1966)

<sup>e</sup> Picea rubens; increase in amino acid composition; with no physiological changes (Norby et al., 1989)

<sup>f</sup> Pinus sylvestris; increase in GDH activity (Wingale et al., 1987)

<sup>g</sup> Several bryophyte species; increase in NR activity (Morgan et al., 1992)

<sup>h</sup> Zea mays; increase in NR activity (Yoneyama et al., 1979)

<sup>i</sup> Vicia faba; change in amino acid composition (Ito et al., 1984)

<sup>j</sup> Betula sp; increased water loss (Neighbour et al., 1988)

<sup>k</sup> Phaseolus vulgaris; reversible increase in dark respiration (Sandhu & Gupta, 1989)

<sup>l</sup> Glycine max; increase in photosynthesis (Sabarathnam et al., 1988a,b)

<sup>m</sup> Phaseolus vulgaris; increase in transpiration (Ashenden, 1979)

<sup>n</sup> Glycine max; enhanced dark respiration (Sabarathnam et al., 1988b)

<sup>o</sup> Vicia faba; reversible structural damage on cellular level (Wellburn et al., 1972)

<sup>p</sup> Pisum sativum; emission of stress ethylene (Melthorn & Wellburn, 1987)

<sup>q</sup> Medicago sativa, Avena sativa; inhibition of photosynthesis (Hill & Bennet, 1970)

<sup>r</sup> Several grass species; reduction in shoot growth (Whitmore & Mansfield, 1983)

<sup>s</sup> Citrus sinensis; increased fruit drop (Thompson et al., 1970)

<sup>t</sup> Polystichum fomosum and 3 fern species; injury and changes in growth (Ashenden et al., 1990; Bell et al., 1992)

<sup>u</sup> Brassica napus and Hordeum vulgare; growth stimulation (resp.: Adaros et al., 1991a,b)
Table 20 (contd).

- Phaseolus vulgaris; increase in total dry matter, not in yield (Bender et al., 1991)
- Raphanus sativus; growth stimulation (Rumeola & Palmer, 1987)
- Helianthus annuus; reduction in net assimilation rate (Okano et al., 1985b)
- Pinus strobus; slight needle necrosis in 2 of 8 clones (Yang et al., 1983)
- Nicotiana tabacum; leaf necrosis (Bush et al., 1962)

Table 21. Lowest exposure levels (in mg/m$^3$) at which NO caused significant effects

<table>
<thead>
<tr>
<th>(Bio)chemical</th>
<th>Physiological</th>
<th>Growth aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing season</td>
<td>44; 21 days$^b$</td>
<td>625; 16 days$^a$</td>
</tr>
<tr>
<td></td>
<td>500; 28 days$^c$</td>
<td>500; 16 days$^a$</td>
</tr>
<tr>
<td>Air pollution episodes</td>
<td>375; 8 days$^f$</td>
<td>1250; 4 days$^d$</td>
</tr>
<tr>
<td></td>
<td>44; 8-24 h$^e$</td>
<td>125; 20 h$^f$</td>
</tr>
<tr>
<td></td>
<td>1875; 18 h$^f$</td>
<td>1250; 5 days$^d$</td>
</tr>
<tr>
<td>Short term</td>
<td>188; 7 h$^g$</td>
<td>750; 1 h$^i$</td>
</tr>
<tr>
<td></td>
<td>500; 3 h$^h$</td>
<td>2500; 10 min$^i$</td>
</tr>
<tr>
<td></td>
<td>1875; 20 min$^h$</td>
<td>1250; 5 days$^d$</td>
</tr>
</tbody>
</table>

* If the fumigation was not continuous an average value has been estimated and quoted in parentheses (calculated assuming 10 mg/m$^3$ during the periods in which the fumigation was switched off).
* Four bryophyte species; inhibition of nitrate-induction of NR (Morgan et al., 1992)
* Lycopersicon esculentum; induction of NR (Wellburn et al., 1980)
* Lactuca sativa; induction of NR (Bestford & Hand, 1989)
* Ctenidium molluscum (bryophyte); inhibition of NR (Morgan et al., 1992)
* Capsicum annum; reduction in NR activity (Murray & Wellburn, 1980)
* Pisum sativum; increase in ethylene release (Mehlhorn & Wellburn, 1987)
* Lycopersicon esculentum; induction of NR (Wellburn et al., 1980)
* Eight indoor ornamental species; inhibition of photosynthesis (Saxe, 1986)
* Lycopersicon esculentum; inhibition of photosynthesis (Capron & Mansfield, 1989)
* Avena sativa & Medicago sativa; inhibition of photosynthesis (Hill & Bennet, 1970)
* Lactuca sativa; inhibition of photosynthesis (Capron, 1989)
* Lycopersicon esculentum; inhibition of photosynthesis (Mortensen, 1986)
* Lycopersicon esculentum; reduction in plant mass (Capron et al., 1991)
* Lycopersicon esculentum; reduction in plant mass (Anderson & Mansfield, 1979)
* Lycopersicon esculentum; reduction in plant mass (Bruggink et al., 1988)
Effects of Atmospheric Nitrogen Compounds on Plants

Existing data are rather difficult to interpret since controlled fumigation with NO inevitably results in some oxidation to NO₂. Thus atmospheres will contain a mixture of the oxides. There is growing interest in the distinct properties and effects of NO and NO₂, and the mechanisms of their cellular action probably differ (Wellburn, 1990). The exchange properties of NO and NO₂ over vegetation (personal communication by D. Fowler to the IPCS) and single plants (Saxe, 1986) appear quite different. Their effects are also contrasting, and there is clearly some dispute over which oxide is the most toxic. Earlier studies of the inhibition of photosynthesis found NO to act more rapidly than NO₂ (at several ppm) but to cause less overall depression of the photosynthetic rate (Hill & Bennet, 1970). More recent photosynthetic studies by Saxe (1986), using similar concentrations, found NO to be considerably more toxic than NO₂. There is very little information on contrasting effects of the two oxides at low concentrations, but this also adds weight to the suggestion that NO is biologically more toxic. In her studies of NR in bryophytes, Morgan et al. (1992) discovered that exposure to NO initially inhibited NR while NO₂ induced activity. At present, however, there is insufficient knowledge across a range of species to establish separate critical levels for NO and NO₂, and studies using a wider variety of vegetation are urgently required.

4.1.7.3 NH₃

The lowest effective exposure levels for NH₃ are given in Table 22.

The toxicity of NH₃ during very short exposure periods has been tested for the purpose of evaluating accidental releases during transport or industrial processes. The estimated critical level for 10 min is (100 ppm) (personal communication by Lee & Davison to the IPCS). This type of exposure is out of the context of this monograph. Several cells in Table 22 could not be filled; the majority of quoted effects are on biomass production and tissue injury. It is clear that the data in this table are not random; nearly all of the information originating from one Dutch research group. Only a few pollution climates were considered. The results may be representative for mild oceanic climates, but probably not for cold climates with dark winters: toxicity of NH₃ increases with lower temperature and lower light intensity. The effects of NH₃ need to be studied with more plant species and under more climatic conditions in order to obtain critical levels with sufficient potential for generalization.
Table 22. Lowest exposure levels (in $\mu g/m^2$) at which $NH_3$ caused significant effects

<table>
<thead>
<tr>
<th></th>
<th>(Bio)chemical</th>
<th>Physiological</th>
<th>Growth aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long term</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growing season</td>
<td>50; 6 months&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53; 9 months&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>25; 1 year&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>or winter</td>
<td>53; 8 months&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>35; 15 months&lt;sup&gt;mm&lt;/sup&gt;</td>
</tr>
<tr>
<td>Air pollution</td>
<td>100; 6 weeks&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50; 6 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60; 2 months&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>episodes</td>
<td>60; 14 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>20; 90 days&lt;sup&gt;dd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>180; 13 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>30; 23 days&lt;sup&gt;dd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2000; 24 h&lt;sup&gt;e&lt;/sup&gt;</td>
<td>213; 5 days&lt;sup&gt;dd&lt;/sup&gt;</td>
<td>120; 11 days&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>213; 5 days&lt;sup&gt;dd&lt;/sup&gt;</td>
<td></td>
<td>1000; 2 weeks&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300; 3 days&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Short term</td>
<td></td>
<td>30 000; 1 h&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000; 2 h&lt;sup&gt;ii&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000; 6 h&lt;sup&gt;ii&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> If the fumigation was not continuous an average value has been estimated and quoted in parentheses (calculated assuming 10 $\mu g/m^3$ during the periods in which the fumigation was switched off).

<sup>b</sup> Species of Violia canina alliance; imbalanced nutrient status (Dueck & Elderson, 1992)

<sup>c</sup> Deschampsia flexuosa; change in amino acid composition (Van der Eerden et al., 1993)

<sup>d</sup> Pinus sylvestris; increased GS activity (Pérez-Soba et al., 1990)

<sup>e</sup> Pseudotsuga menziesii; imbalanced nutrient status (Van der Eerden et al., 1992)

<sup>f</sup> Lycopersicum esculentum; increase of $NH_3$ in the dark (Van der Eerden, 1982)

<sup>g</sup> Lolium perenne; 30% of N in the plant is derived from foliar uptake (Voeltenheber & Raven, 1993)

<sup>h</sup> Pinus sylvestris; increased loss of water after two weeks of desiccation (Dueck et al., 1990)

<sup>i</sup> Populus sp.; increase in stomatal conductance in leaves; increase in mesophyll conductance and maximum photosynthetic rate at a slightly higher exposure level (Van Hove et al., 1992)

<sup>j</sup> Loliurn perenne; significant impact acld/base regulation and nutrients status

<sup>k</sup> Pseudotsuga menziesii; erosion of wax layer (Thijse & Baas, 1990; the authors have some doubts about the causality of this effect (personal communication)

<sup>l</sup> Calluna vulgaris; reduction in survival rate after winter (Dueck, 1990)

<sup>m</sup> Amica montana; reduced survival after winter and flowering (Van der Eerden et al., 1991)

<sup>n</sup> Field exposure during winter; median concentration; severe injury of several conifer species (Van der Eerden, 1982)

<sup>o</sup> Viola canina; Agrostis capillaris; 50% growth stimulation of the shoot (not of the roots) (Van der Eerden et al., 1991)

<sup>p</sup> Racomitrium lanuginosum; chlorosis (Van der Eerden et al., 1991)

<sup>q</sup> Hypnum jutlandicum; chlorosis (Van der Eerden et al., 1991)
Effects of Atmospheric Nitrogen Compounds on Plants

Table 22 (contd).

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidium sativum</td>
<td>Reduction in dry weight (Van Hout &amp; Prinz, 1976)</td>
</tr>
<tr>
<td>Several horticultural crops</td>
<td>Leaf injury</td>
</tr>
<tr>
<td>Various deciduous trees</td>
<td>Leaf injury (Ewert, 1979)</td>
</tr>
<tr>
<td>Brassica sp., Helianthus sp.</td>
<td>Leaf injury (Benedict &amp; Breen, 1955)</td>
</tr>
<tr>
<td>Rosa sp.</td>
<td>Leaf injury (Garber, 1935)</td>
</tr>
</tbody>
</table>

4.1.7.4 $\text{NH}_4^+$ and $\text{NO}_3^-$ in wet and occult deposition

$\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{H}^+$ make up about half of the ionic composition of rain, clouds, fog, and aerosols. The impact of the acidity of rain and clouds has received much attention in recent years (Jacobson, 1991). This is not the case with other compounds in wet deposition, although their relevance is recognized. At the same pH, Cape et al. (1991) found a much greater effect of sulfuric acid than of nitric acid, indicating that the impact of acid rain is not only through protons, but also through anions.

There is an abundance of information on the effects of $\text{NH}_4^+$ in soil solution. However, threshold concentrations for $\text{NH}_4^+$ in the soil (e.g., Schenk & Wehrman, 1979) cannot be considered a critical level for rainwater quality, because the type of exposure and response is completely different.

Wet deposition containing $\text{NH}_4^+$ can reduce frost tolerance (Cape et al., 1990) and induce leaching of $\text{K}^+$ and other cations (Roelofs et al., 1985). It is not yet clear whether this type of ion exchange can have deleterious effects on its own in the field situation.

Currently, too few quantitative data on the effects of nitrogen-containing wet and occult deposition are available for critical levels for this group of compounds to be derived.

4.1.7.5 Mixtures

A polluted atmosphere generally consists of a cocktail of compounds, but certain combinations are more frequent. Because of their role in the formation of tropospheric $\text{O}_3$, simultaneous co-occurrence of relatively high levels of $\text{O}_3$ and $\text{NO}$ are rarely observed, while sequential co-occurrences are much more frequent.
Nitrogen Oxides (Kosta-Rick & Manning, 1993). If burning of fossil fuels results in emission of SO$_2$, this is often combined with emission of NO$_x$.

a) SO$_2$ plus NO$_2$

Synergism has been found in nearly all studies concerning this combination, with only few exceptions (Kuppers & Klump 1988; Murray et al., 1992). Based on data presented by Whitmore (1985), for *Poa pratensis* the effect threshold for combinations of SO$_2$ and NO$_2$ in equal concentrations when expressed in ppm, is in the range of 1.2-2.0 ppm·days (Fig. 19). This threshold applies to effects by combinations of SO$_2$ and NO$_2$; the effects of single exposures were not assessed in this study. However, it is reasonable from other references to expect synergism, and thus to include this threshold in Table 23, in which combined effects are summarized. Another threshold for combinations of SO$_2$ and NO$_2$ was defined by Van der Eerden & Duym (1988) (Fig. 20; Table 23).

![Fig. 19. Dose response curve, from combined results of two experiments, showing the effects of mixtures of SO$_2$ and NO$_2$ on growth of *Poa pratensis*. Plants were exposed to 7 ppb (control) 40 ppb (+), 70 ppb (×) or 100 ppb (●) for periods of 4 to 50 days (Whitmore, 1985).](image-url)
b) SO₂ plus NH₃

Adsorption of either NH₃ or SO₂ on leaf surfaces is enhanced by the presence of the other compound (Van Hove et al., 1989).

Fig. 20. Threshold surface for combined effects of SO₂ and NO₂. Exposure levels above the surface are potentially toxic (Van der Eerden & Duym, 1988)
### Table 23. Lowest exposure levels at which NO\textsubscript{2} increases the effect of SO\textsubscript{2}, O\textsubscript{3}, or SO\textsubscript{2} plus O\textsubscript{3}.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Biochemical</th>
<th>Physiological</th>
<th>Growth aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term</td>
<td>150-190: 9 months(^1)</td>
<td>220: 50 weeks(^2)</td>
<td>19: 10-41 weeks(^3)</td>
</tr>
<tr>
<td>Growing season</td>
<td>55-75: 34 days(^4)</td>
<td>135: 28 days(^5)</td>
<td>10-43: 130 days(^6)</td>
</tr>
<tr>
<td>or winter</td>
<td>135: 28 days(^5)</td>
<td>30: 38 days(^7)</td>
<td>30: 43 days(^8)</td>
</tr>
<tr>
<td>Air pollution episodes</td>
<td>80: 2 weeks(^9)</td>
<td>75: 1 day(^10)</td>
<td></td>
</tr>
<tr>
<td>Short term</td>
<td>153: 1 h(^\text{a})</td>
<td>325: 1 h(^\text{m})</td>
<td>400: 1 h(^\text{n})</td>
</tr>
</tbody>
</table>

\(^{1}\) If the fumigation was not continuous an average value has been estimated and quoted in parentheses (calculated assuming 10 \(\mu\)g/m\(^3\) during the periods in which the fumigation was switched off).
\(^{2}\) Phaseolus vulgaris; inhibition of parts of nitrogen metabolism when exposed sequentially with O\textsubscript{3} (100-120 \(\mu\)g/m\(^3\); 8 h/day).
\(^{3}\) Lolium perenne; decrease in proline content during winter hardening when applied in combination with SO\textsubscript{2} at 188 \(\mu\)g/m\(^3\) (Davison et al., 1987).
\(^{4}\) Lolium perenne; less negative osmotic potential during winter hardening when applied in combination with SO\textsubscript{2} at 188 \(\mu\)g/m\(^3\) (Davison et al., 1987).
\(^{5}\) Phaseolus vulgaris; inhibition of photosynthesis when in combination with SO\textsubscript{2} (215 \(\mu\)g/m\(^3\)); without SO\textsubscript{2} inhibition at 760 \(\mu\)g/m\(^3\) (Kentner et al., 1990).
\(^{6}\) Several crops; growth stimulation by NO\textsubscript{2} turns into a reduction in synergism with sequential treatment with O\textsubscript{3} (160-200 \(\mu\)g/m\(^3\); 8 h/day) (Reneickes & Palmer, 1987).
\(^{7}\) Six tree species; reduced plant growth in combination with SO\textsubscript{2} (280 \(\mu\)g/m\(^3\)), both antagonism and synergism (Freer-Smith, 1984).
\(^{8}\) 10 grass species were tested in combination with SO\textsubscript{2} (27 \(\mu\)g/m\(^3\)). Three species showed growth stimulation. Reduced growth was found at higher concentrations. Interactions with acidic mist and with O\textsubscript{3} were found (Ashenden et al., 1993).
\(^{9}\) Poa pratensis; inhibition of biomass production; in combination with SO\textsubscript{2} (42 \(\mu\)g/m\(^3\)) for 38 days; the longest exposure period used in the experiments. Calculated from data from Whitmore (1985), assuming synergism and a critical level for SO\textsubscript{2} plus NO\textsubscript{2} of 1.2 ppm-days (Whitmore, 1985).
\(^{10}\) Brassica napus and Hordeum vulgare; antagonism (and rarely synergism) with O\textsubscript{3} (6-44 \(\mu\)g/m\(^3\); 8 h/day) and SO\textsubscript{2} (9-33 \(\mu\)g/m\(^3\), continuously); enhanced yield turns into reduction (Edwards et al., 1990).
\(^{11}\) Plantago major; reduced shoot dry weight synergism with SO\textsubscript{2} (50 \(\mu\)g/m\(^3\)) and O\textsubscript{3} (60 \(\mu\)g/m\(^3\), 8 h/day) (Mool, 1984).
\(^{12}\) Poa pratensis; inhibition of biomass production; in combination with SO\textsubscript{2} (110 \(\mu\)g/m\(^3\)) for 2 weeks (the upper margin of the exposure period of this cell in the table; the shortest fumigation in this survey was 20 days. Calculated from data from Whitmore (1985), assuming synergism and a critical level for SO\textsubscript{2} plus NO\textsubscript{2} of 1.2 ppm-days (Whitmore, 1985).
Effects of Atmospheric Nitrogen Compounds on Plants

Table 23 (contd).

m Critical level for NO\(_2\), assuming SO\(_2\) to be present at 70 μg/m\(^3\); considered to be a critical level for a 24-h mean (UNECE, 1994) (Van der Eerden & Duym, 1988)

* Lycopersicon esculentum; reduction in plant mass if in combination or preceded by O\(_3\) (160 μg/m\(^3\); 1 h) (Goodyear & Ormrod, 1988).

Interactive physiological effects have been found as well (Dueck, 1990; Dueck et al., 1990; Dueck & Elderson, 1992). Currently, there is far too little information on this combination to quantify this interaction.

c) NO plus NO\(_2\)

When activated charcoal has been used as filter material in NO\(_2\) fumigation experiments, NO must have been present as well, because activated charcoal has virtually no capacity to absorb NO. In those studies, responses must have been due to NO\(_2\) plus NO. Although the toxicity of NO was often considered to be much less than that of NO\(_2\), currently the two compounds are assumed to be equally toxic and to act additively. However, Wellburn (1990) and others have stated that NO is more toxic, and Saxe (1994) showed that the variation in sensitivity amongst species is different for the two compounds. This supports the suggestion of Wellburn that the mechanism of toxicity is different.

For the purpose of deriving critical levels, the assumption of additivity may result in an underestimation. However, there are not enough data to quantify this.

d) Mixtures with O\(_3\)

The combination NH\(_3\) plus O\(_3\) has rarely been studied. No statistically significant interactions have been found as yet, but in one study the threshold for leaf injury was higher in the presence of NH\(_3\) (Van der Eerden et al., 1994). The combination NO\(_2\) plus O\(_3\) has been studied more frequently, but the responses differed considerably between experiments and species. Additivity or antagonism was found by Runeckles & Palmer (1987), Adaros et al. (1991a,b), and Bender et al. (1991). Synergism was reported by Ito et al. (1984), Runeckles & Palmer (1987) and Kosta-Rick & Manning (1993).
The combination of \( \text{SO}_2 \) plus \( \text{O}_3 \) plus \( \text{NO}_2 \) has also been studied. Again the responses varied between plant species and experiment. Antagonism, additivity and synergism have all been found (Kosta-Rick & Manning, 1993).

e) Mixtures with elevated \( \text{CO}_2 \)

Generally, an increased supply of \( \text{CO}_2 \) to crops results in an enhanced biomass production. The responses of native species are more variable but are also frequently positive. This growth stimulation is limited by a deficiency of other nutrients. Nitrogen can be one such limiting factor, and for this reason a nitrogen fertilizer such as \( \text{NH}_3 \) and possibly low \( \text{NO}_2 \) concentrations could be hypothesized to have a more-than-additive relationship with \( \text{CO}_2 \). However, as yet there is no experimental evidence for this. Van der Eerden et al. (1994) and Perez-Soba et al. (1994) found stimulation of photosynthesis and growth by both \( \text{NH}_3 \) and \( \text{CO}_2 \), but not by a combination of these two compounds.

Effects of the combination of \( \text{NO}_2 \) and \( \text{CO}_2 \) have not yet been studied within the scope of global climate change. But some relevant information could be gained from the literature dealing with \( \text{CO}_2 \) enrichment in glasshouses. When the flue gases of the heating system are used as a \( \text{CO}_2 \) source, \( \text{NO}_2 \) (in which NO is dominant) becomes a major contaminant. The fertilizing effect of elevated \( \text{CO}_2 \) can largely disappear in the presence of \( \text{NO}_2 \) (Anderson & Mansfield, 1979; Saxe & Voight Christensen, 1984; Mortensen, 1985; Bruggink et al., 1988; Capron et al., 1994).

The \( \text{CO}_2 \), \( \text{NH}_3 \) and \( \text{NO}_2 \) concentrations used in combination in these experiments were relatively high and therefore cannot be used in the critical level assessment. More experiments with lower concentrations are required.

Table 23 indicates, surprisingly, that the effective long-term exposures are generally higher than those of shorter duration. However, long-term responses (more than half a year) have rarely been studied. Therefore, the information on effects over growing season periods may be much more representative of long-term effects.

A study included in a report by UNECE (1994) used 21 \( \mu \text{g} \text{SO}_2/\text{m}^3 \) and 11 \( \mu \text{g} \text{NO}_2/\text{m}^3 \) over the entire growing season and found synergism in reducing biomass production of \( \text{Pisum sativum} \) and \( \text{Spinacea oleracea} \). Similar results were found for \( \text{Hordeum} \)
vulgare and Brassica oleracea, when fumigation was conducted for 120–190 days with 30–40 μg SO₂/m³ and 30–50 μg NO₂/m³. This study cannot be used for the assessment of critical levels because it has not yet been published, but it indicates that lower levels of the two pollutants than those quoted in Table 23 can influence plant responses.

4.1.8 Appraisal

Table 24 shows the former air quality guidelines for NO₂ and some other critical levels assessed in the same period. Fig. 21 summarizes the results given in Tables 20 to 23. In this figure curves are drawn to estimate critical levels according to current practice, known as the "envelope" approach. After having plotted all effective exposure levels in a graph of concentration and exposure time, a curve is drawn just below the lowest effective exposures. Critical levels can be derived from this curve. Fig. 21 shows that more experiments with exposure periods of 0.5 to 5 days are required to give a more solid basis for the estimation of critical levels of 24 h.

<table>
<thead>
<tr>
<th>Concentration (μg/m³)</th>
<th>Exposure time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>4 h</td>
<td>WHO (1987)</td>
</tr>
<tr>
<td>30*</td>
<td>annual mean</td>
<td>WHO (1987)</td>
</tr>
<tr>
<td>800</td>
<td>1 h</td>
<td>Guderian (1988)</td>
</tr>
<tr>
<td>60</td>
<td>growing season</td>
<td>Guderian (1988)</td>
</tr>
<tr>
<td>40</td>
<td>winter</td>
<td>Guderian (1988)</td>
</tr>
</tbody>
</table>

* SO₂ and O₃ not higher than 30 μg/m³ and 60 μg/m³, respectively

A second approach to arrive at critical levels is the statistical model of Kooijman (1987). Based on the variation in sensitivity between species, critical levels are calculated taking into account the number of tested species and the level of uncertainty (Van der Eerden et al., 1991). The second approach is better, but only part of the available data is suitable for this approach.
Fig. 21. Graphical presentation of the data presented in Tables 20 to 23. The curve in each graph is drawn below the lowest effective exposure levels. From this curve an annual mean and 1 day mean is estimated, indicated by dashed lines. Black triangles refer to growth aspects, black dots to physiological responses and open circles to biochemicals effects. Black squares show the WHO air quality guidelines of 1987 (WHO, 1987). Fig. a: NO; Fig. b: NO₂; Fig. c: NH₃; Fig. d: NO₂ in combination with SO₂ or O₃.
Tables 20 to 23 show that some new relevant information has appeared. Comparing the data of Table 20 with those of Table 21 (Fig. 21a and 21b), it appears that NO\textsubscript{2} has slightly higher effect thresholds than NO. However, this probably reflects the separate attention paid to these compounds, rather than any difference in toxicity. It is now obvious that the toxicity of NO cannot be ignored, and it should be included in the guidance values. The consideration of NO and NO\textsubscript{2} together (leading to a guidance value for NO\textsubscript{N}) seems the best way of evaluating the impact of NO. However, future research should evaluate the specific phytotoxic properties of the individual compounds and their combinations.

It is not yet possible to discriminate in the critical level assessment between separate types of vegetation, such as crops, plantation forests, natural forests and other natural vegetation. A 1-h average for NO\textsubscript{2} of 800 μg/m\textsuperscript{3} to prevent acute damage (Table 24) is probably too high. A critical level for NO\textsubscript{2} of around 300 μg/m\textsuperscript{3} would be better. A critical level of 95 μg/m\textsuperscript{3} as a 4-h mean, as proposed in the previous WHO guidelines (WHO, 1987), is still realistic, but not very practical. If critical levels for short periods (e.g., 1 or 8 h) should be defined, it is probably necessary to separate day- and night-time exposures. A critical level for a 24-h mean is more practical, as this is relevant for both peak concentrations of several hours and air pollution episodes of several days.

For the growing season and winter half year, Guderian (1988) suggested critical levels of 60 and 40 μg/m\textsuperscript{3}, respectively. From Table 20 it can be seen that the critical level of 60 μg/m\textsuperscript{3} can cause substantial growth stimulation rather than reduction. Within the context of air quality guidelines, this type of response must be regarded as potentially adverse because, for instance, of its influence on competition within the natural vegetation. From current knowledge it is evident that 60 μg/m\textsuperscript{3} is too high to prevent growth stimulation. In addition, the critical level of 30 μg/m\textsuperscript{3} for an annual mean, given in the 1987 WHO guidelines, will almost certainly not protect all plant species. However, for crops, where growth stimulation is rarely an adverse effect, this could be acceptable if secondary effects are negligible. The experimental basis for the WHO air quality guidelines of 1987 was relatively poor, but evidence is increasing that these are certainly not unrealistically low. Not even all direct adverse effects are eliminated by these levels (Adaros et al., 1991a,b; Bender et al., 1991; Ashenden et al., 1993). Thus, the updated guidelines/guidance values should be lower than the ones of 1987.
A long-term critical level for NO₂ of 10 μg/m³, especially to avoid eutrophication of nutrient-poor vegetation, was proposed by Guderian (1988) and Zierock et al. (1986). The basis for this proposal was the work of Lee et al. (1985) and Press et al. (1986), who found reduced growth of *Sphagnum cuspidatum* in regions with an annual mean concentration of 38 and 11 μg/m³, respectively, compared to the growth in another region with 4 μg/m³ after 140 days of exposure. However, Lee et al. (1985) also showed that the poor growth of *Sphagnum* was more closely related to the excessively high concentrations of nitrate and ammonium ions in bog water rather than to the concentration of NO₂ alone. Thus, this information could well be used to assess water quality guidelines, but is not very useful as a basis for air quality guidelines.

**4.1.8.1 Representativity of the data**

Critical levels for adverse effects of NH₃ on plants were estimated using the model of Kooijman (Van der Eerden et al., 1991). To protect 95% of the species at P < 0.05, a 24-h critical level of 270 and an annual mean critical level of 8 μg/m³ were estimated. With the graphical approach the 24-h average was a little lower and the annual mean somewhat higher (13 and 200 μg/m³, respectively; Fig. 21).

On the basis of a review by Cape (1994), critical levels for H⁺ and NH₄⁺ were adopted for locations where ground-level cloud is present for more than 10% of the time and where calcium and magnesium concentrations in rain or cloud do not exceed H⁺ and NH₄⁺ concentrations (mainly high elevation areas in cold climate zones): 300 μmol NH₄⁺/litre as an annual mean (UNECE, 1994).

There remains considerable deficiency in the amount and scope of experimentally derived information on which to base air quality guidelines. This results from the fact that most experiments have been performed under conditions that cannot directly be compared to outdoor circumstances. In most experiments, only primary effects such as photosynthesis and biomass production were evaluated, and rarely secondary effects such as alteration of stress tolerance or competitive ability. The plant species chosen in most experiments were crops, although evidence suggests that some native species are relatively more sensitive. For instance, lower plants such as bryophytes and lichens are not protected by a waxy waterproof cuticle and do not have the potential to close stomata. Furthermore, Pearson & Stewart (1993) suggested that plants species from nutrient-poor acidic soils are more sensitive.
Further work, employing low concentrations of NH$_3$ and NO$_x$ (especially NO) in different climates, is urgently required. It is not realistic to screen for all likely growth and physico-chemical effects in the majority of species in order to arrive at general effect thresholds. Selections must be made on the basis of an understanding of differences in sensitivity between species. However, an obvious mechanistic explanation for sensitivity differences is not yet available. For instance, there appears to be no relationship between the sensitivity to NO$_2$ and the nitrogen preference (Ellenberg, 1985; Ashenden et al., 1993). Sensitivity classifications for some tens of species have been made for NO$_2$ and NH$_3$ (e.g. US EPA, 1978; Taylor et al., 1987), but it appears difficult to extend predictions very far beyond those examined. The hypotheses of Raven (1988) and Pearson & Stewart (1993) should be studied in more detail in laboratory experiments and field studies, as they could result in an efficient selection criterium for further screening.

An attempt to determine the global situation regarding nitrogen-containing compounds is being made. The assumption that all deposited nitrogen-containing compounds (which is part of the critical load concept) act additionally in their impact on vegetation is poorly based on experimental results and is probably not valid for the short term.

Generalizations and simplifications have to be made to arrive at conclusions that are applicable in environmental policy making, but this should be done with great care. Mechanistic simulation models can become a powerful tool for making general predictions on the basis of various air pollution experiments (Van de Geijn et al., 1993). However, sufficient knowledge of biochemical and physiological mechanisms to incorporate the impact of air pollution on vegetation into these models is still lacking. This applies especially to natural vegetation where stress sensitivity and competition are key factors.

Many gaps in understanding the impact of nitrogen-containing air pollution on vegetation still exist, and this is a good reason to use a safety factor in determining critical levels and loads. However, currently there is still no broadly accepted approach to quantify such a safety factor.

4.1.9 General conclusions

The sum of information on gaseous NH$_3$ and on NH$_4^+$ in wet and occult deposition is still too limited to arrive at air quality
guidelines, as they should have a broad applicability. The critical levels for NH₃ and NH₄⁺ are probably only valid for temperate oceanic climatic zones (see sections 4.1.7.3, 4.1.7.4 and 4.1.8).

In most studies with NO and NO₂, no significant effects were found at levels below 100 μg/m³, but several relevant exceptions exist. NO₂ altered the response to O₃ generally with a less-than-additive interaction. In combination with SO₂, NO₂ acted more-than-additively in most cases. With CO₂ and with NO, no interaction and thus additivity were generally found. The lowest effective concentration levels of NO₂ are about equal for NO₂ alone and in combination with O₃ or SO₂, although, generally, at concentrations near to its effect threshold NO₂ causes growth stimulation if it is the only pollutant, while in combination with SO₂ and/or O₃ it results in growth inhibition.

To include the impact of NO, a critical level for NO₂ instead of one for NO₂ is proposed, assuming that NO and NO₂ act in an additive manner. A strong case can be made for the provision of critical levels for short-term exposures, but currently there are insufficient data to provide these with sufficient confidence. Current evidence exists for a critical level of around 75 μg/m³ for NO₂ as a 24-h mean.

The critical level for NO₂ (NO and NO₂, added in ppb and expressed as NO₂ in μg/m³) is 30 μg/m³ as an annual mean. At concentrations slightly above this critical level, growth stimulation will be the dominant effect if the ambient conditions allow growth and NO₂ is the only pollutant. This stimulation may be combined with a minor increase in sensitivity to biotic and abiotic stresses. In cases where biomass production is a positive effect, e.g., in agriculture and plantation forests, the critical level can be higher. Current knowledge is insufficient to arrive at critical levels for these systems.

The critical level can be converted into deposition quantities. With deposition velocities of 3 and 0.3 mm/second for NO₂ and NO respectively (see section 3.2.2 and Table 5), the annual deposition corresponding to a NO₂ concentration of 30 μg/m³ is 4.8 kg/ha when half of the NO₂ is NO₂ and 8.3 kg/ha when all is NO₂. This indicates that at a concentration near to its critical level the contribution of NO₂ to the nitrogen demand is negligible for fertilized crops but not for natural vegetation (see section 4.2).
4.2 Effects on natural and semi-natural ecosystems

4.2.1 Effects on freshwater and intertidal ecosystems

In this section the effects of atmospheric nitrogen deposition on freshwater and intertidal ecosystems are evaluated. The effects of increased emissions of nitrogen compounds with respect to eutrophication are examined in order to establish ecosystem guidelines for nitrogen deposition. The ecological effects of nitrogen deposition are reviewed for (i) shallow softwater lakes and (ii) lakes and streams.

4.2.1.1 Effects of nitrogen deposition on shallow softwater lakes

In the lowlands of western Europe, soft water is often found on sandy soil which is poor in calcium carbonate or almost devoid of it. The water is poorly buffered and the concentrations of calcium in the water layer are very low. The water bodies are shallow and fully mixed, with periodically fluctuating water levels. They are mainly fed by rain water and thus are oligotrophic. These softwater ecosystems are characterized by plant communities from the phytosociological alliance Littorellion (Schoof-van Pelt, 1973; Wittig, 1982; Roelofs, 1986; Vöge, 1988; Arts, 1990). The stands of these communities are characterized by the presence of rare and endangered isoetids, such as *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes lacustris*, *Echinodorus* species, *Luronium natans* and many other softwater macrophytes. These softwater bodies are now almost all within nature reserves and have become very rare in western Europe. A strong decline in amphibians has also been observed in these water bodies (Leuven et al., 1986).

The effects of nitrogen pollutants on these softwater bodies have been intensively studied in the Netherlands both in field surveys and experimental studies. Field observations on about 70 softwater bodies (with well-developed isoetid vegetation in the 1950s) showed that the water, in which these macrophytes were still abundant in the early 1980s, was poorly buffered (alkalinity of 50–500 μeq/litre), slightly acidic (pH=5–6) and very poor in nitrogen (Roelofs, 1983; Arts et al., 1990). The softwater sites where these plant species had disappeared could be divided into two groups. In 12 of the 53 softwater sites, eutrophication, resulting from nutrient-enriched water, seemed to be the cause of the decline. In this group of non-acidified water bodies, plant species, such as *Myriophyllum alterniflorum*, *Lemma minor* or
Riccia fluitans had become dominant. High concentrations of phosphate and ammonium ions were measured in the sediment. In some of the larger water bodies no macrophytes were found, as a result of dense plankton bloom. In the second group of lakes and pools (41 out of 53) another development had taken place: the isoetid species were replaced by dense stands of Juncus bulbosus or aquatic mosses such as Sphagnum cuspidatum or Drepanocladus fluitans. This clearly indicates acidification of the water in recent decades, probably caused by enhanced atmospheric deposition. In the same field study it was shown that the nitrogen levels in the water were higher in ecosystems where the natural vegetation had disappeared, compared with ecosystems where the Littorellion stands were still present (Roelofs, 1983). This strongly suggests the detrimental effects of atmospheric nitrogen deposition in these softwater lakes.

Several ecophysiological studies have revealed the importance of (i) inorganic carbon status of the water as a result of intermediate levels of alkalinity, and (ii) low nitrogen concentrations for the growth of the endangered isoetid macrophytes. Furthermore, almost all of the typical softwater plants had a relatively low potential growth rate. Increased acidity and higher concentrations of ammonium ion in the water clearly stimulated the development of Juncus bulbosus and submerged mosses such as Sphagnum and Drepanocladus species (Roelofs et al., 1984; Den Hartog, 1986). Cultivation experiments confirmed that the nitrogen species involved (ammonium or nitrate ions) differentially influenced the growth of the studied species of water plants. Almost all of the characteristic softwater isoetids developed better when nitrate was added instead of ammonium, whereas Juncus bulbosus and aquatic mosses (Sphagnum & Drepanocladus) were clearly stimulated by ammonium (Schuurkes et al., 1986). The importance of ammonium for the growth of these aquatic mosses was also reported by Glime (1992).

The effects of atmospheric deposition have been studied in small-scale softwater systems during a 2-year treatment with different artificial rainwaters. Acidification, without airborne nitrogen input (using sulfuric acid), did not result in a mass growth of Juncus bulbosus, and a diverse isoetid vegetation remained present. However, after increasing the nitrogen concentration in the precipitation (as ammonium sulfate), similar changes to those seen in field conditions were observed, i.e. a dramatic increase in the dominance of Juncus bulbosus, of submerged aquatic mosses and of Agrostic canina (Schuurkes et al., 1987). It became obvious that the observed changes occurred
because of the effects of ammonium sulfate deposition, leading to both eutrophication and acidification. The increased levels of ammonium in the system directly stimulated the growth of plants such as Juncus bulbosus, whereas the surplus ammonium would be nitrified in this water (pH \( \approx 4.0 \)). During this nitrification process, \( \text{H}^+ \) ions are produced, which increases the acidity of the system. The results of this study clearly demonstrated that the changes in composition of the vegetation had already occurred after a 2-year treatment with \( \geq 19 \text{ kg nitrogen per ha per year} \). A reliable critical load for nitrogen deposition in these shallow softwater lakes is thus most likely to be below 19 kg nitrogen per ha per year and probably between 5 to 10 kg nitrogen per ha per year. This value is supported by the observation that the greatest decline in the species composition of the Dutch Litorellion communities has coincided with nitrogen loads of around 10-13 kg nitrogen per ha per year (Arts, 1990).

4.2.1.2 Effects of nitrogen deposition on lakes and streams

There is ample evidence that an increase of acidic and acidifying compounds in atmospheric deposition had resulted in recent acidification of lakes and streams in geologically sensitive regions of Scandinavia, western Europe, Canada and the USA (Hultberg, 1988; Muniz, 1991). This acidification is characterized by a decrease in pH and acid neutralizing capacity and by increases in concentrations of sulfate, aluminium, and sometimes nitrate and ammonium. It has been shown since the 1970s, using various approaches (field surveys, laboratory studies, whole-lake experiments), that these changes have had major consequences for plant and animal species (macrofauna, fishes) and for the functioning of these aquatic ecosystems.

The critical loads of acidity (from \( \text{SO}_4^2- \) and \( \text{NO}_3^- \)) for aquatic ecosystems, based on steady-state water chemistry models, were published by the UN Economic Commission for Europe (UNECE) in 1988 and 1992. These models incorporate both sulfur and nitrogen acidity, and critical loads are calculated on the basis of: (i) base cation deposition; (ii) internal alkalinity production or base cation concentrations; and (iii) nitrate leaching from the water system. The calculated critical loads are thus site-specific (sensitive areas or not) and also depend on the local hydrology and precipitation (for full details, see Hultberg (1988), Henriksen (1988) and Kämari et al. (1992)). The critical loads of nitrogen acidity (kg nitrogen per ha per year) for the most sensitive lakes and streams are:

149
In many areas with high water alkalinity and/or high base cation deposition, the values of the critical load for nitrogen acidity are much higher than those for sensitive freshwaters. At present, the possible effects of nitrogen eutrophication by ammonia/ammonium or nitrate deposition are not incorporated in the establishment of critical loads for nitrogen, except for shallow softwater lakes (see section 4.2.1.1). This is because primary production in almost all aquatic ecosystems is limited by phosphorus availability, and thus nitrogen enrichment has been considered unimportant in this respect (Moss, 1988). This certainly holds for those aquatic ecosystems considered above, where the critical load with respect to acidifying effects are certainly more relevant than the effects of nitrogen eutrophication. It is, however, to be expected that the following aquatic ecosystems are sensitive to nitrogen enrichment: (i) alpine lakes; (ii) water with low background nitrogen; and (iii) humic lakes (Kämäri et al., 1992). The effects of nitrogen eutrophication (including ammonia/ammonium) in these water bodies need further research and should be taken into account in future critical loads determinations for nitrogen. At present it is not possible to present reliable critical loads for nitrogen eutrophication in these aquatic ecosystems. An overview of critical loads for nitrogen in aquatic ecosystems is given in section 8.2.2.

4.2.2 Effects on ombrotrophic bogs and wetlands

In this section the effects of atmospheric nitrogen deposition in (semi-)natural wetlands are evaluated. The effects of enhanced nitrogen inputs are considered for: (i) ombrotrophic (raised) bogs; (ii) fens; and (iii) intertidal fresh- and saltwater marshes. A common feature of all these systems is the anaerobic nature of their waterlogged soils, characterized by low redox potential, high concentrations of toxic reduced substances and high rates of denitrification (Gambrell & Patrick, 1978; Schlesinger, 1991).
4.2.2.1 Effects on ombrotrophic (raised) bogs

Ombrotrophic ("rain-nourished") bogs, which receive all their nutrients from the atmosphere, are particularly sensitive to airborne nitrogen loads. These bogs are systems of acidic wet areas and are very common in the boreal and temperate parts of Europe. Because of the anaerobic conditions, decomposition rates are slow, favouring the development of peat. In western Europe and high northern latitudes, typical plant species include bog-mosses (*Sphagnum* species), sedges (*Carex, Eriophorum*) and heathers (*Andromeda, Calluna and Erica*). Insectivorous plant species (e.g., *Drosera*) are especially characteristic of these bogs; they compensate for low nitrogen concentrations by trapping and digesting insects (Ellenberg, 1988b).

Clear effects of nitrogen eutrophication have been observed in Dutch ombrotrophic bogs. The composition of the moss layer in the small remnants of the formerly large bog areas has markedly changed in recent decades as nitrogen loads have increased to 20–40 kg nitrogen per ha per year (especially as NH$_4$/$\text{NH}_3$). The most characteristic species (*Sphagnum*) are replaced by the more nitrophilous mosses (Greven, 1992). A national survey of Danish ombrotrophic bogs has shown a decline of the original bog vegetation together with an increase of more nitrogen-dependent species in areas with high ammonia deposition (> 25 kg ammonium nitrogen per ha per year (Aaby, 1990).

The effects of atmospheric nitrogen deposition on ombrotrophic bogs have also been intensively studied in the United Kingdom (Lee et al., 1989; Lee & Studholme, 1992). Many characteristic *Sphagnum* species are now largely absent from affected ombrotrophic bog areas in the United Kingdom, such as the southern Pennines in England. Atmospheric nitrogen deposition has more than doubled in these areas to around 30 kg nitrogen per ha per year, compared with areas of healthy *Sphagnum* growth. In contrast to the situation in continental western Europe, most of the nitrogen deposition in the United Kingdom is of nitrogen oxides, although the importance of ammonia/ammonium deposition is also increasing in the United Kingdom (Fowler et al., 1980; Sutton et al., 1993). Several studies on bogs in the United Kingdom have shown that increased supplies of nitrogen are rapidly absorbed and utilized by bog-mosses (*Sphagnum*), reflecting the importance of nitrogen as a nutrient and its scarcity in unpolluted regions (Woodin et al., 1985; Woodin & Lee, 1987). The high nitrogen loadings are, however,
supraoptimal for the growth of many characteristic *Sphagnum* species, as demonstrated by restricted development in growth experiments and transplantation studies between clean and polluted locations. In areas with high nitrogen loads, such as the Pennines, the growth of *Sphagnum* is in general less than in unpolluted areas (Lee & Studholme, 1992). After transplantation of *Sphagnum* from an unpolluted site to a bog in the southern Pennines, a rapid increase in plant nitrogen content from around 12 to 20 mg/g dry weight was observed (Press et al., 1986). A large increase in arginine in the shoots of these bog-mosses was also found after application of nitrogen. In field experiments in northern and southern parts of Sweden, nitrogen was found to be the limiting factor for the growth of *Sphagnum*. However, other nutrients, especially phosphorus, may become secondarily limiting to plant growth when nitrogen inputs reach a threshold (Aerts et al., 1992).

A further possible effect of the increased nitrogen content of *Sphagnum* is an increased decay rate of the peat, as nitrogen content strongly influences decomposition rates (Swift et al., 1979). The decay rate of *Sphagnum* peat in Swedish ombrotrophic bogs has been studied along a gradient of nitrogen deposition (Hogg et al., 1994). The results of this short-term decay experiment indicated that the decomposition rate of *Sphagnum* peat is more influenced by the phosphorus content of the material than by its nitrogen content, although some relation with nitrogen supply has been observed. Further evidence is necessary to evaluate the long-term effects of enhanced nitrogen supply on the decay of peat.

All these studies strongly indicate the detrimental effects of atmospheric nitrogen on the development of the bog-forming *Sphagnum* species. However, enhanced nitrogen deposition can influence the competitive relationships in nutrient-deficient vegetation such as bogs. The effects of the supply of extra nitrogen on the population ecology of *Drosera rotundifolia* has been recently studied in a 4-year experiment in Swedish ombrotrophic bogs (Redbo-Torstensson, 1994). It was demonstrated that experimental applications of more than 10 kg nitrogen (as NH₄NO₃) per ha per year clearly affected the population of this insectivorous species: the establishment of new individuals and the survival of the plants significantly decreased in the vegetation treated with extra nitrogen. This decrease in the total population density of the characteristic bog species *Drosera* was not caused by toxic effects of nitrogen, but by enhanced
competition for light with tall species such as *Eriophorum* and *Andromeda*, which responded positively to the increased nitrogen inputs.

On the basis of the United Kingdom and Scandinavian studies, it has become clear that increased nitrogen loads strongly affect ombrotrophic bog ecosystems, especially because of the high nitrogen retention capacity and closed nitrogen cycling. The growth of bog-mosses is reduced, directly by nitrogen and indirectly by a changed competitive relationship between the prostrate dominants (e.g., *Eriophorum*) and the subordinate plant species. A reliable critical load for nitrogen in these ombrotrophic bogs is 5–10 kg nitrogen per ha per year, although additional long-term studies with enhanced nitrogen (both nitrogen oxides and ammonia/ammonium) are necessary to validate this figure.

### 4.2.2 Effects on mesotrophic fens

Fens are wetland ecosystems that are typical of alkaline to slightly acidic habitats in many countries. The alkalinity is due to groundwater draining from surrounding rocks or sediments that are relatively rich in calcium carbonate. Most of these fen ecosystems are characterized by rare and endangered plant species. The effects of nitrogen enrichment upon mesotrophic fens have been intensively studied in the Netherlands (Verhoeven & Schmitz 1991; Koerselman & Verhoeven, 1992). These fen ecosystems are characterised by many *Carex* species and are rich in forbs (e.g., *Pedicularis palustris*; orchids). Most of these Dutch fen ecosystems are managed as hay meadows, with removal of the plant material further restricting nutrient levels, and are now nature reserves.

A considerable increase of tall graminoids (grass or *Carex* species), with a somewhat higher potential growth rate, was observed after experimentally adding nitrogen to three Dutch fen ecosystems (Vermeer, 1986; Verhoeven & Schmitz, 1991). This increase caused a significant decrease in the diversity of subordinate plant species. In one of the Dutch fen sites investigated, which had a long history of hay making, it has been shown that phosphorus deficiency was also a major factor in the productivity of the system, since there was a high output of phosphorus from the ecosystem with the hay (Verhoeven & Schmitz, 1991; Koerselman & Verhoeven, 1992). Using the results of fertilization trials and nutrient budget studies in these fen ecosystems (Koerselman et al., 1990; Koerselman & Verhoeven, 1992), with their relatively closed nitrogen cycle, it seems
reasonable to establish a critical load of 20-35 kg nitrogen per ha per year, based upon the output of the nitrogen from the fen system via normal management. In some fen ecosystems, the critical nitrogen load based on the change in diversity may be substantially higher, because of the limitation of productivity by phosphorus (Egloff, 1987; Verhoeven & Schmitz, 1991). In this situation, however, the risks of nitrogen losses to surface water or groundwater will increase because of phosphorus limitation, and this effect should be taken into account in critical load evaluation. High rates of denitrification could also influence the establishment of critical loads for these fen ecosystems, and this aspect needs further investigation.

4.2.2.3 Effects on fresh- and saltwater marshes

In the wetland ecosystems discussed above, the nitrogen cycle is more closed than that of intertidal marshes. The data on atmospheric nitrogen inputs to the nitrogen cycling in intertidal fresh- and saltwater marshes (with large prostrate graminoids as species of Spartina, Typha and Carex) have been reviewed by Morris (1991). It has become evident that nitrogen inputs to these marsh ecosystems via surface water (well above 100 kg nitrogen per ha per year) are much higher than the atmospheric loading. In non-tidal freshwater marshes, it has been demonstrated in many studies that denitrification is very high with a very large output of nitrogen from the ecosystem (Morris, 1991). Because of the combined effect of these processes, atmospheric nitrogen deposition is of only minor importance for these marshes, and it is not useful to establish a critical load for airborne nitrogen to these systems. In his review Morris (1991) formulated a critical load for atmospheric nitrogen in wetland ecosystems of around 20 kg nitrogen per ha per year. It is more appropriate to make a distinction for different types of wetlands, as shown above. An overview of the critical loads for wetlands is given in chapter 8.

4.2.3 Effects on species-rich grasslands

Almost all of the research on the effects of atmospheric deposition on terrestrial vegetation has focused on ecosystems (e.g., forest, heathland and bogs) involving poorly buffered acidic soils. Semi-natural grasslands with traditional agricultural use have also been an important part of the landscape in western and central Europe, and contain, or used to contain, many rare and endangered plant and animal species. A number of these grasslands have been set aside as nature reserves in several European
Effects of Atmospheric Nitrogen Compounds on Plants

countries (Ellenberg, 1988b; Woodin & Farmer, 1993). These semi-natural grasslands, which are of conservation interest, are generally nutrient-poor because of long agricultural use with low levels of manure and the removal of plant growth by grazing or hay making. The vegetation is characterized by many low growing species and is of nutrient-poor soil status (Ellenberg, 1988b). Although these grasslands are nowadays rare, the proportion of endangered plant and animal species in these communities is very high (Van Dijk, 1992). Many experiments have shown that application of artificial fertilizer (nitrogen, phosphorus and potassium) changes these grasslands into tall, species-poor stands, dominated by a few highly productive crop grasses (Van Den Bergh, 1979; Willems, 1980; Van Hecke et al., 1981). To maintain a large diversity of species, addition of fertilizer has to be avoided. It is thus to be expected that these species-rich grasslands will be affected by increased atmospheric nitrogen input (Wellburn, 1988; Liljelund & Torstensson, 1988; Ellenberg, 1988b).

Many semi-natural grassland types are present in western and central Europe. Most of these grasslands belong to the so-called neutral grasslands (Molinio-Arrhenateretea; moist to moderately dry), to the dry calcareous grasslands (Festuca-Brometea) or to the acid grasslands on very poor soils (Nardetalia). Detailed descriptions have been given by Ellenberg (1988b) and Van Dijk (1992). To obtain critical loads for nitrogen for all these grasslands, it would be essential to study the effects of nitrogen eutrophication in a representative range within these communities. Detailed data are, however, scarce. Therefore, the results of an integrated research programme on nitrogen eutrophication in Dutch calcareous grasslands are used as a target study in this chapter to obtain (i) information on observed changes in this type of grassland caused by enhanced nitrogen input, and (ii) a reliable estimation of the critical load for nitrogen in these well-buffered non-acidic grasslands. The results of this calcareous grassland study will be discussed in respect to other semi-natural grasslands.

4.2.3.1 Effects of nitrogen on calcareous grasslands

Calcareous grasslands are communities on limestone. The subsoils consist of various kinds of limestone with high contents of calcium carbonate (> 90%), covered by shallow well-buffered rendzina soils (A/C-profiles; pH of the top soil is 7-8 with a calcium carbonate content of around 10%). The depth of the soil varies between 10 and 50 cm and the availability of nitrogen and phosphorus is low. The grasslands are generally found on slopes
with limestone in the subsoil and a deep groundwater table. Plant productivity is low, and the peak standing crop is in general between 150 and 400 g/m². The canopy of the vegetation is open and low (10-20 cm). Calcareous grasslands are among the most species-rich plant communities in Europe and contain a large number of rare and endangered species. The area of these semi-natural grasslands has decreased substantially in Europe during the second half of this century (Wolkinger & Plank, 1981; Ratcliffe, 1984). Some remnants have become nature reserves in several European countries. To maintain the characteristic calcareous vegetation a specific management is needed to prevent their natural succession towards woodland (Wells, 1974; Dierschke, 1985). The calcareous grasslands in the Netherlands are mown in autumn with removal of the hay (Bobbink & Willems, 1987).

a) Nitrogen enrichment and vegetation composition

The effects of nitrogen enrichment in Dutch calcareous grasslands on vegetation composition have been investigated in two field experiments (Bobbink et al., 1988; Bobbink, 1991). Either potassium (100 kg per ha per year), phosphorus (30 kg per ha per year) or nitrogen (100 kg per ha per year), as well as a complete fertilization (nitrogen, phosphorus and potassium), were applied for 3 years to study the long-term effects on vegetation composition. Nitrogen was given as ammonium nitrate and was applied to two nature reserves with opposite aspects (north and south). Total above-ground biomass increased considerably, as expected, after three years of nitrogen, phosphorus and potassium fertilization. In the experiments where the nutrients were applied individually, a moderate increase in above-ground dry weight was only seen with nitrogen addition: (about 330 g/m² compared with about 210 g/m² in the untreated plots). The dry weight distribution of the species was significantly affected by nutrient treatments. In the nitrogen-treated vegetation, the dry weight of the grass species *Brachypodium pinnatum* was about 3 times higher than in the control plots. Nitrogen application also resulted in a drastic reduction of the biomass of forb species (including several Dutch Red List species) and of the total number of species. The observed decrease in species diversity in the nitrogen-treated vegetation was not caused by nitrogen toxicity, but by the change in vertical structure of the grassland vegetation. The growth of *Brachypodium* was strongly stimulated and its overtopping leaves reduced the light within the vegetation. It overshadowed the other characteristic species and growth of these species declined rapidly (Bobbink et al., 1988; Bobbink, 1991). Stimulation of *Brachypodium* growth
and a substantial reduction in species diversity were observed following application of nitrogen to a 5-year permanent plot study using a factorial design (Willems et al., 1993).

Many characteristic lichens and mosses have also disappeared in recent years from European calcareous grasslands (During & Willen, 1986). This has been caused partly by the indirect effects of extra nitrogen inputs, as shown experimentally by Van Tooren et al. (1990). Data on the effects of nitrogen eutrophication on the species-rich fauna of calcareous grassland are not available. However, it is very likely that the diversity of animals, especially of insects, will also be reduced when tall grasses are strongly dominating the vegetation, because of the decreasing abundance of many herbaceous flowering species which act as host or forage plants.

b) Nitrogen enrichment and nutrient storage in calcareous grasslands

The seasonal distribution of nutrients after nitrogen fertilization in spring (120 kg nitrogen as ammonium nitrate) has been studied with the repeated harvest approach (Bobbink et al., 1989). It resulted in a significantly increased peak standing crop of *Brachypodium*. This grass proves to have very efficient nitrogen uptake and very efficient withdrawal from its senescent shoots into its well-developed rhizome system. *Brachypodium* can benefit from the extra nitrogen redistributed to the below-ground rhizomes by enhanced growth in the next spring. The distribution of nitrogen has also been quantified in 3-year fertilization experiments. *Brachypodium* greatly monopolized (> 75%) the nitrogen storage in both the above-ground and below-ground compartments of the vegetation with increasing nitrogen availability (Bobbink et al., 1988; Bobbink, 1991).

Nitrogen cycling and accumulation in calcareous grassland can be significantly influenced by two major outputs from the system: (i) leaching from the soil; and (ii) removal with management regimes. Nitrogen losses by denitrification in dry calcareous grasslands are low (< 1 kg nitrogen per ha per year), owing to the well-drained soil conditions (Mosier et al., 1981). Ammonium and nitrate leaching has been studied in Dutch calcareous grasslands by Van Dam et al. (1992). Both the water fluxes and the solute fluxes at different soil depths have been measured over 2 years in untreated vegetation and in calcareous grassland vegetation sprayed at 2-weekly intervals with ammonium sulfate (50 kg nitrogen per ha per year). The nitrogen leaching from the untreated vegetation was very low (0.7 kg nitrogen per ha per
and amounted to only 2% of the total atmospheric deposition of nitrogen. After the spraying with ammonium sulfate, nitrogen leaching increased significantly to 3.5 kg nitrogen per ha per year, although this figure was also a very small proportion (4%) of the nitrogen input in this vegetation (Van Dam et al., 1992). It is thus evident that calcareous grassland ecosystems retain nitrogen almost completely in the system. This is caused by a combination of enhanced plant uptake (Bobbink et al., 1988; Bobbink, 1991) and increased immobilization in the soil organic matter (Van Dam et al., 1992).

### 4.2.3.2 Critical loads for nitrogen in calcareous grasslands

The most important output of nitrogen from calcareous grassland is via exploitation or management. The annual nitrogen removal in the hay varies slightly between years and sites, but in general between 17 and 22 kg nitrogen per ha is removed from the system under normal management conditions in the Netherlands (Bobbink, 1991; Bobbink & Willems, 1991). The ambient nitrogen deposition in Dutch calcareous grasslands, as determined by Van Dam (1990), is high (35–40 kg nitrogen per ha per year) and is nowadays the major nitrogen input to the system. Legume species (Leguminosae) also occur in calcareous vegetation, and form an additional nitrogen input owing to the nitrogen-fixing microorganisms in their root nodules (about 5 kg nitrogen per ha per year).

The nitrogen mass balance of Dutch calcareous grasslands is summarized in Table 25. It is obvious that calcareous grasslands now significantly accumulate nitrogen (16–26 kg per ha per year) in the Netherlands. A critical nitrogen load has been determined with a mass balance model, because of the lack of long-term addition experiments with low nitrogen loads. Assuming a critical long-term immobilization rate for nitrogen of 0–6 kg nitrogen per ha per year, the critical nitrogen load can be derived by adding the nitrogen fluxes due to net uptake, denitrification and leaching, corrected for the nitrogen input by fixation. In this way, 15–25 kg nitrogen per ha per year is considered as nitrogen critical load for this ecosystem. Nitrogen cycling within the system (between plants and soil) is not used for this calculation.

In calcareous grassland in England, addition of nitrogen stimulated the dominance of grasses in most cases (Smith et al., 1971; Jeffrey & Pigott, 1973). In these studies, the application of 50–100 kg nitrogen per ha per year resulted in a strong dominance
Effects of Atmospheric Nitrogen Compounds on Plants

Table 25. Nitrogen mass balance (kg nitrogen per ha per year) for dry calcareous grassland in the Netherlands

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
<th></th>
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<tbody>
<tr>
<td>Atmospheric deposition</td>
<td>35-40</td>
<td>Harvest</td>
</tr>
<tr>
<td>Nitrogen fixation</td>
<td>5</td>
<td>Denitrification</td>
</tr>
<tr>
<td>Soil leaching</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>40-45</td>
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</tbody>
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of the grasses Festuca rubra, F. ovina or Agrostis stolonifera. However, Brachypodium and Bromus erectus, the most frequent species in calcareous grassland in continental Europe, were absent from these sites. Following a survey of data from a number of conservation sites in southern England, Pitcairn et al. (1991) concluded that Brachypodium had expanded in the United Kingdom during the last 100 years. They considered that much of the early spread could be attributed to a decline in grazing pressure but that the more recent spread had, in some cases, taken place despite grazing or mowing, and could be related to nitrogen inputs. However, a study of chalk grassland at Parsonage Downs (United Kingdom) showed no substantial change in species composition over the twenty years between 1970 and 1990, a period when nitrogen deposition is thought to have increased significantly (Wells et al., 1993). Brachypodium was present in the sward but had not expanded as in the Dutch grasslands. In a linked experimental study, applications of nitrogen to eight forbs and one grass (Brachypodium) at levels of 20, 40 and 80 kg nitrogen per ha per year for two years did not result in Brachypodium becoming dominant.

Apart from the Dutch studies, the effects of enhanced nitrogen inputs have been little investigated in continental European calcareous grasslands. Some data from a recent fertilization experiment at the alvar grasslands, a thin-soiled vegetation over flat limestone, on the Swedish island Öland, suggest that the vegetation hardly responds to applications of nitrogen or phosphorus (Sykes & Van der Maarel, 1991; personal communication by Van der Maarel). Only irrigation in combination with
EHC 18: Nitrogen Oxides

nutrients has caused an increase in grasses. This is probably due to the low annual precipitation in this area (400–500 mm).

Increased nitrogen availability is probably of major importance in many European calcareous grasslands. An increased availability of nitrogen is indicated by enhanced growth of some tall grasses, especially stress-tolerant species, which have a slightly higher potential growth rate and efficient nitrogen utilization. It clearly depends on the original species composition, as to which of the grass species will increase following enhanced nitrogen inputs. Furthermore, the difference between the Dutch and United Kingdom results may reflect differences in management; the impacts of grazing in the United Kingdom grasslands could offset any competitive advantage the grasses may have obtained from additional nitrogen inputs. The critical load for nitrogen in these calcareous grasslands could be influenced by management; long-term studies involving additional nitrogen input with various management schemes are needed to quantify these aspects.

4.2.3.3 Comparison with other semi-natural grasslands

Productivity in grasslands is strongly influenced by nutrients, as shown in many agricultural studies (e.g. Chapin, 1980). It is also well-known that large amounts of fertilizer (nitrogen, phosphorus and potassium) alter almost all grassland types into tall, species-poor swards dominated by a few highly productive crop grasses (e.g. Bakelaar & Odum, 1978; Van Den Bergh, 1979; Willems, 1980; Van Hecke et al., 1981). Most of these species-rich grasslands are deficient in nitrogen or phosphorous, and thus characterized by plant species of nutrient-poor habitats. It is thus likely that these grasslands are sensitive to nitrogen eutrophication from the atmosphere (Wellburn, 1988; Ellenberg, 1988b). Thus, it is also important to establish critical loads for nitrogen in the species-rich grasslands, although data from experiments with nitrogen application in these semi-natural grasslands are scarce.

Increased nitrogen availability can also affect other semi-natural grasslands, although experimental evidence is quite scarce. A classical study into the effects of nutrients on neutral grasslands is the Park Grass experiment at Rothamsted, England, which has been running since 1856 (Williams, 1978). After application of nitrogen as ammonium sulfate or sodium nitrate (48 kg nitrogen per ha per year), the vegetation became very poor in species and dominated by grasses such as Holcus lanatus or Agrostis sp. The effects of nutrients in dry and wet dune grasslands (1% calcium
Effects of Atmospheric Nitrogen Compounds on Plants

carbonate) on sandy soils have been studied at Braunton Burrows (Devon, England) by Willis (1963). Nutrients were applied over 2 years (6 x 40 kg nitrogen per ha per year) using a factorial design for nitrogen and phosphorus. Nitrogen proved to be the most important nutrient in stimulating the growth of some grass species (Festuca rubra and Poa pratensis). This enhanced growth reduced significantly the abundance of many small plants such as prostrate phanerogamic species, mosses and lichens (Willis, 1963). In this coastal area with low nitrogen deposition (currently about 10 kg nitrogen per ha per year) the vegetation of dune grasslands is at present still species-rich, whereas in many Dutch dune grasslands with higher nitrogen loading (20-30 kg nitrogen per ha per year) certain grasses have increased and it has become a problem to maintain diversity. Recent studies of the response of mesothrophic grasslands in the United Kingdom have shown that additions as small as 25 kg per ha per year can lead to changes in species diversity after several years of fertilizer additions and that changes take place more rapidly at higher rates of addition (Mountford et al., 1994). This indicates that many of these semi-natural grasslands are also sensitive to nitrogen eutrophication and that the critical loads are likely to be of the same magnitude or slightly higher (20-30 kg nitrogen per ha per year) than in calcareous grasslands.

Many other semi-natural grassland types occur, especially in the montane-subalpine regions, containing a large proportion of the biodiversity of the area. However, data are too scarce to establish reliable load for these grasslands, although it may be expected that: (i) most of these grassland are sensitive to nitrogen; and (ii) the critical load for nitrogen is probably lower than for lowland (calcareous) grasslands. The presented critical loads for species-rich grasslands are summarized in section 8.2.2.

4.2.4 Effects on heathlands

Various types of plant communities have been described as heath, but the term is applied here to plant communities where the dominant vegetation is small-leaved dwarf-shrubs forming a canopy of 1 m or less above soil surface. Grasses and forbs may form discontinuous strata, and there is frequently a ground layer of mosses or lichens (Gimingham et al., 1979; De Smidt, 1979). Dwarf-shrub heathlands occur in various parts of the world, especially in montane habitats, but are widespread in the atlantic and sub-atlantic parts of Europe. In these parts of the European continent, natural heathland is limited to a narrow coastal zone.
Inland lowland heathlands are man-made (semi-natural), although they have existed for several centuries. Lowland heaths are widely dominated by the Ericaceae, especially Calluna vulgaris in the dry heathlands and Erica tetralix in the wet heathlands (Gimingham et al., 1979). In these heaths, development towards woodland has been prevented by mowing, burning, sheep grazing and sod removal.

Until the beginning of this century, the balance of nutrient input and output was in equilibrium in the lowland heathlands of western Europe (De Smidt, 1979; Gimingham & De Smidt, 1983). The original land use implied a regular, periodic removal of nutrients from the ecosystems via grazing and sod removal (Heil & Aerts, 1993). Sod removal was practised less systematically in many Scandinavian and Scottish heathlands (Gimingham & De Smidt, 1983). Here Calluna has been renewed by burning at regular intervals, varying from 4-6 years in southern Sweden to 15-20 years in western Norway (Nilsson, 1978; Skogen, 1979).

The original land use of the lowland heathland ceased in the early 1900s and the area occupied by this community decreased markedly all over its distribution area (Gimingham, 1972; De Smidt, 1979; Ellenberg, 1988b). Dwarf-shrub heathlands may be divided into four categories according to broad differences in habitat: (1) dry heathlands; (2) wet heathlands; (3) montane and (4) arctic-alpine heathlands.

4.2.4.1 Effects on inland dry heathlands

During recent decades many lowland heathlands in western Europe have become dominated by grass species. An evaluation, using aerial photographs, has shown that more than 35% of Dutch heathland has been altered into grassland (Van Kootwijk & Van der Voet, 1989). In recent years, similar changes have been observed in SW Norway, which has the largest local emission of ammonia as well as the heaviest nitrogen input through long-range deposition in Norway (Anonymous, 1991). It has been suggested that nitrogen eutrophication might be a significant factor in this transition to grasslands. Field and laboratory experiments confirm the importance of nutrients, especially in the early phase of heathland development (Heil & Diemont, 1983; Roelofs 1986; Heil & Bruggink, 1987; Aerts et al., 1990). However, Calluna can compete successfully with the grasses, even at high nitrogen loading, if its canopy remains closed (Aerts et al., 1990). Apart from the changes in competitive interactions between Calluna and the grasses, heather beetle plagues and nitrogen accumulation in...
Effects of Atmospheric Nitrogen Compounds on Plants

the soil are important factors in the changing lowland heaths. Furthermore, evidence is growing that frost sensitivity of the dominant dwarf-shrubs may also be affected by increasing nitrogen inputs.

Heathland canopies have a strong filtering effect on air pollutants, a varying canopy structure being an important factor. For sulfur and nitrogen it has been shown that bulk deposition accounts for only about 35–40% of total atmospheric input (Heil et al., 1987; Bobbink et al., 1992b). Total atmospheric deposition of nitrogen is 30–45 kg nitrogen per ha per year in the heathland sites in the eastern part of the Netherlands. More than 70% of the total nitrogen input is deposited as ammonium or ammonia (Bobbink et al., 1992b; Bobbink & Heil, 1993). Although data for nitrogen inputs in other European lowland heathlands are missing, it is very likely that in many European agricultural regions nitrogen deposition has increased in recent years (Asman, 1987; Buijsman et al., 1987).

In Calluna heathland, outbreaks of the chrysomelid heather beetle (Lochmaea suturalis) occur frequently. These beetles feed exclusively on the green parts of Calluna. The closed Calluna canopy is opened over large areas and the interception of light by Calluna decreases strongly (Berdowski, 1987, 1993). Thus the growth of the under-storey grasses (Deschampsia or Molinia) is enhanced significantly. In general insect grazing is affected by the nutritive value of the plant material, and the nitrogen content is especially important in this respect (Crawley, 1983). Experimental applications of nitrogen to heathland vegetation cause the concentrations of this element in the green parts of Calluna to increase (Heil & Bruggink, 1987; Bobbink & Heil, 1993). It is, therefore, very likely that the frequency and intensity of heather beetle outbreaks are stimulated by increased atmospheric nitrogen input in Dutch heathland. This hypothesis is supported by the observations of Blankwaardt (1977), who reported that from 1915 onwards heather beetle outbreaks were observed in the Netherlands with an interval of about 20 years, whereas in the last 15 years the outbreaks have occurred with a periodicity of less than 8 years. It has also been observed that during a heather beetle outbreak Calluna plants are more severely damaged in nitrogen-fertilized vegetation (Heil & Diemont, 1983). In a rearing experiment with larvae of the heather beetle, Brunsting & Heil (1985) demonstrated that the growth of the larvae was increased by higher nitrogen concentrations in the leaves of Calluna. Van der Eerden et al. (1990) studied the effects of ammonium sulfate
on the growth of heather beetle after a outbreak of the beetle in vegetation artificially sprayed under a cover. They found no significant effect of the treatments on total number or on biomass of the first stage larvae. However, the development of subsequent larval stages was accelerated by the application of ammonium sulfate in the artificial rain: the percentage of third stage larvae increased by 20%, compared with larvae in the control treatment. Furthermore, heather beetle larvae were put on Calluna shoots taken from plants which had been fumigated with ammonia in open-top chambers (12 months; 4 to 105 μg/m²) (Van der Eerden et al., 1991). After 7 days the larvae were counted and weighed. Both the mass and the development rate of the larvae clearly increased with increasing concentrations of ammonia. The heather beetle has recently been found in SW Norway and it is expanding its territory. It is probably an important cause of Calluna death in this region (Hansen, 1991). It can be concluded that nitrogen inputs influence outbreaks of heather beetle, although the exact relationship between both processes needs further research.

In the past Dutch inland heathlands were grazed by flocks of sheep and sods were generally removed at intervals of 25-50 years (De Smidt, 1979). Nowadays these heathlands are mostly managed by mechanical sod removal, which can restore the heathland vegetation if a seed bank of the original heathland species is still present (Bruggink, 1993). The increase in organic matter and in the amounts of nitrogen in the system during secondary succession is well known (Begon et al., 1990). It was shown in the 1970s that during secondary heathland succession the above-ground and below-ground biomass and the amount of litter increase (Chapman et al., 1975; Gimingham et al., 1979). It is likely that changes in nitrogen accumulation will have occurred due to the increase in atmospheric deposition.

Berendse (1990) performed a detailed study on the accumulation of organic matter and of nitrogen during the secondary succession after sod removal in the Netherlands. He found a large increase in plant biomass, soil organic matter and total nitrogen storage in the first 20 to 30 years after sod removal. Furthermore, it was demonstrated that nitrogen mineralization was low during the first 10 years (about 10 kg nitrogen per ha per year), but increased considerably over the next 20 years to 50-110 kg nitrogen per ha per year. Regression analysis of the total nitrogen storage versus the years after sod removal revealed an annual nitrogen increase in the system of about 33 kg nitrogen per ha per year (probably somewhat lower in the early years and higher in
later years) (Berendse, 1990). These values are in good agreement with measured nitrogen deposition in Dutch heathlands in the late 1980s (Bobbink et al., 1992b).

Thus, the organic matter in the soil increases rapidly after sod removal, which removes almost all of the soil organic matter. However, this process is accelerated by the enhanced dry matter production and litter production of the dwarf shrubs caused by the extra nitrogen inputs. Nitrogen accumulation in the system also increases. Hardly any nitrogen disappears from the system because nitrate leaching to deeper layers is only of minor importance in Dutch heathlands, as shown by De Boer (1989) and Van Der Maas (1990). Nitrogen availability from atmospheric inputs, in addition to mineralization, is within a relatively short period (about 10 years) high enough to stimulate the transition of heathland to grassland, especially after the opening of the heather canopy by secondary causes.

It has been demonstrated that frost sensitivity of some tree species increases with increasing concentrations of air pollutants (e.g. Aronsson, 1980; Dueck et al., 1991). This increase in frost sensitivity is sometimes correlated with enhanced nitrogen concentrations in the foliage of the trees. Long-term effects of air pollutants on the frost sensitivity of Calluna and Erica are to be expected because of (i) the evergreen growth form of these species and (ii) the increasing content of nitrogen in the leaves of Calluna, associated with increased nitrogen deposition in the Netherlands and Norway (Heil & Bruggink, 1987; Hansen, 1991). It has been suggested that damage to Calluna shoots in the successive severe winters of the mid-1980s was at least partly caused by the increased frost sensitivity. Investigations into the effects of air pollutants on the frost sensitivity of heathland species outside the Netherlands started in the early 1990s (Hansen, 1991; Uren, 1992).

The effects of sulfur dioxide, ammonium sulfate and ammonia upon frost sensitivity in Calluna have been studied by Van der Eerden et al. (1990). After fumigation with sulfur dioxide (90 μg/m² for 3 months), increased frost injury in Calluna was only found at temperatures that seldom occur in the Netherlands (lower than -20 °C). Fumigation with ammonia of Calluna plants in open-top chambers over a 4-7 month period (100 μg/m³) revealed that frost sensitivity was not affected in autumn (September or November), whereas in February, just before growth started, frost injury increased significantly at -12 °C (Van der Eerden et al., 1991). These authors also studied the frost
sensitivity of *Calluna* vegetation sprayed with six different levels of ammonium sulfate (3–91 kg nitrogen per ha per year). The frost sensitivity increased slightly, although significantly, after 5 months in vegetation treated with the highest level of ammonium sulfate (400 μmol/litre; 91 kg nitrogen per ha per year), compared with the control treatments. However, frost sensitivity of *Calluna* decreased again two months later and no significant effects of the ammonium sulfate application upon frost hardiness were seen at that time. Thus, high levels of ammonia or ammonium sulfate seem to increase the frost sensitivity of *Calluna* plants, although the significance of this phenomenon is still uncertain at ambient nitrogen inputs. The relation between frost sensitivity and nitrogen input has not yet been sufficiently quantified to use it for a precise assessment of critical loads in this respect.

It has been shown above that atmospheric nitrogen is the trigger for changes of lowland dry heathlands into grass swards in the Netherlands. Lowland dry heathlands in the United Kingdom do not show consistent patterns over the past 10 to 40 years. Pitcairn et al. (1991) assessed changes in abundance of *Calluna* in three heaths in East Anglia (eastern England) over recent decades. All three heaths showed a decline in *Calluna* and an increase in grasses. The authors concluded that increases in nitrogen deposition was at least partly responsible for the changes, but also noted that the management had changed. A wider assessment of heathlands in SE England showed that in some cases *Calluna* had declined and subsequently been invaded by grasses while other areas were still dominated by dwarf shrubs (Marrs, 1993). This clearly stresses the importance of management for the maintenance of dwarf shrubs in heathlands. A simulation model, which integrates processes such as atmospheric nitrogen input, heather beetle outbreak, soil nitrogen accumulation, sod removal and competition between species, has been used to establish the critical loads of nitrogen deposition in lowland dry heathlands (Heil & Bobbink, 1993a,b). The model has been calibrated with data from field and laboratory experiments in the Netherlands. As an indicator of the effects of atmospheric nitrogen, the proportion and increase of grasses in the heathland system are used. Atmospheric nitrogen deposition has varied between 5 and 75 kg nitrogen per ha per year in steps of 5–10 kg nitrogen during different simulations. From these simulations, the value for the critical load of nitrogen for the changes from dwarf shrubs to grasses was 15–20 kg nitrogen per ha per year.
4.2.4.2 Effects of nitrogen on inland wet heathlands

The western European lowland heathlands of wet habitats are dominated by the dwarf shrub *Erica tetralix* (Ellenberg, 1988b) and are generally richer in plant species than the dry heathlands. In recent decades a drastic change in species composition of Dutch wet heathlands has been observed. Nowadays, many wet heathlands that were originally dominated by *Erica* have become monospecific stands of the grass *Molinia*. Together with *Erica* almost all of the rare plant species have disappeared from the system. It has been hypothesized that this change has been caused by atmospheric nitrogen eutrophication.

Competition experiments using micro-ecosystems have clearly shown that *Molinia* is a better competitor than *Erica* at high nitrogen availability. After 2 years of application of nitrogen (150 kg per ha per year), the relative competitive strength of *Molinia* compared with *Erica* doubled (Berendse & Aerts, 1984). A 3-year field experiment with nitrogen application in Dutch lowland wet heathland (around 160 kg nitrogen per ha per year) also indicated that *Molinia* is able to outdo *Erica* at high nitrogen availability (Aerts & Berendse, 1988). In contrast to the competitive relations between *Calluna* and the grasses, *Molinia* can outdo *Erica* without opening of the dwarf shrub canopy. This difference is caused by the lower canopy of *Erica* (25-35 cm), compared with *Calluna*, and the tall growth form of *Molinia*, which can overgrow and shade *Erica* if enough nitrogen is available. It is in this respect also important that heather beetle plagues do not occur in wet heathlands and that no frost damage has been observed in this community.

It has been demonstrated that in many Dutch wet heathlands the accumulation of litter and humus has led to increased nitrogen mineralization (100–130 kg nitrogen per ha per year) (Berendse et al., 1987). In the first 10 years after sod removal the annual nitrogen mineralization is very low, but afterwards it increases rapidly. The leaching of accumulated nitrogen from wet heathlands is extremely low (Berendse, 1990). The observed nitrogen availabilities are high enough to change *Erica*-dominated wet heathlands into monocots of *Molinia*.

Berendse (1988) developed a wet heathland model to simulate carbon and nitrogen dynamics during secondary succession. He incorporated in this model the competitive relationships between *Erica* and *Molinia*, the litter production from both species, soil
nitrogen accumulation and mineralization, leaching, atmospheric nitrogen deposition and sheep grazing. He simulated the development of lowland wet heathland after sod removal, because almost all of the Dutch communities are already strongly dominated by *Molinia* and it is impossible to expect changes in this situation without drastic management. Using the biomass of *Molinia* with respect to *Erica* as an indicator, his results suggested 17-22 kg nitrogen per ha per year as the critical load for the transition of lowland wet heathland into a grass-dominated sward (Berendse, 1988). The decrease in endangered wet heathland forbs is partly caused by the overshadowing by *Molinia*, but some species had already disappeared from wet heathlands before the increase of *Molinia* started. The critical load for this decline is probably lower than the given values and is discussed in section 4.2.4.4.

4.2.4.3 Effects of nitrogen on arctic and alpine heathlands

Semi-natural *Calluna* heathlands are found in the lowlands along the Norwegian coast to 68°N and show distinct plant gradients in the south-north direction, from coast to inland and from lowland to upland areas (Fremstad et al., 1991). In central parts of western Norway the plant composition changes at an altitude of about 400 m, above which alpine species occur regularly in the heaths. At this altitude oceanic upland *Calluna* and *Erica* heaths merge into alpine heaths, which are naturally occurring, non-anthropogenic communities. Some oligotrophic alpine heaths also contain *Calluna*, but most heaths in Fennoscandia and in European parts of Russia are dominated by other ericoid species (*Vaccinium* spp., *Empetrum nigrum* s. lat., *Arctostaphylos* spp., *Loiseleuria procumbens*, *Phyllodoce caerulea*, *Betula nana*, *Juniperus communis* and *Salix* spp.). Many heath types have a more or less continuous layer of mosses and lichens. Related heaths are found in alpine regions in the British Isles, in Iceland, in southernmost Greenland, in northern Russia, and on siliceous rocks in the Alps (Grabherr, 1979; Elvebakk, 1985; Ellenberg, 1988b).

Alpine and arctic habitats have many ecological characteristics in common, although the climatic conditions are more severe in the arctic regions than in most alpine regions. The growing season is short (3-3.5 months in the low arctic zone), air and soil temperatures are low, winds are frequent and strong, and the distribution of plant communities depends on the distribution of snow during winter and spring. Most alpine and all arctic zones are influenced by frost activity or solifluction, except for soils in
the low alpine and hemiarctic zones, where podzolic soils are found. Decomposition of organic matter and nutrient cycling are slow, and a large amount of the nitrogen input is stored in the soil in forms which can not be used by plants (Chapin, 1980). The low nutrient availability limits primary production. Most species are adapted to a strict nitrogen economy and their nitrogen indicator values are generally low (Ellenberg, 1979).

Barsdate & Alexander (1975) investigated the nitrogen balance of an arctic area in Alaska. The most important sources of nitrogen were nitrogen fixation (75%) and ammonia in precipitation (22%). Most of the nitrogen input is retained in living biomass, and very little is leached from the soil. Denitrification is also low, partly due to nutrient deficiency. Nitrogen metabolism as such does not seem to be inhibited by low soil temperatures (Haag, 1974). Nitrogen fixation in arctic habitats has been studied in bacteria, soil algae, lichens and legume species (Leguminosae) (Novichkova-Ivanova, 1971). Blue-green algae (cyanobacteria) are especially important in this respect, either as free-living species, species associated with mosses or phycobionts in lichens (e.g. Peltigera, Nephroma and Stereocaulon). The rate of nitrogen fixation depends on temperature and moisture, and thus varies through the year (Alexander & Schnell, 1973).

It is to be expected that arctic and alpine communities are sensitive to increased atmospheric nitrogen input, because nitrogen retention is very efficient, although primary production is also strongly regulated by factors other than nitrogen (temperature, moisture) (Tamm, 1991). The effects of increased nitrogen availability on alpine/tundra vegetation have been studied in several fertilizer experiments. In most experiments full nitrogen, phosphorus and potassium fertilizer was used, although sometimes nitrogen was applied separately. The following effects of nitrogen addition have been observed:

- In alpine and arctic vegetation, nitrogen is quickly absorbed by phanerogamic species and incorporated into their tissues. The increase in nitrogen contents differs for graminoids, deciduous and evergreen species (Summers, 1978; Shaver & Chapin, 1980; Lechowicz & Shaver, 1982; Karlsson, 1987).

- Phanerogamic plant species respond to nitrogen application in different ways: increased growth and biomass, enhanced number of tillers, more flowers and changes in phenology (Henry et al., 1986).
• Some phanerogamic plant species are damaged or even killed at high doses of nitrogen fertilizer (Henry et al., 1986).

• Changes in species cover and composition are likely when nitrogen is applied for a longer period of time (5-10 years).

All these studies concentrated on effects on phanerogamic plant species; little information is available on the effects of nitrogen on cryptogams. Many authors, however, stress that nitrogen fixation probably will decrease when atmospheric deposition increases in arctic and alpine ecosystems. In forest studies it has already been shown that Cladonia spp. and some mosses are very sensitive to nitrogen addition. The suggested critical load for arctic and alpine heaths (5-15 kg nitrogen per ha per year) is lower than that for lowland heathland (15-20 kg nitrogen per ha per year).

4.2.4.4 Effects on herbs of matgrass swards

In recent decades, in addition to the transition from dwarf-shrub-dominated to grass-dominated heathlands, a reduced species diversity in these ecosystems has been observed. Species of the acidic Nardetalia grasslands and related dry and wet heathlands seem to be especially sensitive. Many of these herbaceous species (e.g., Arnica montana, Antennaria dioica, Dactylorhiza maculata, Gentiana pneumonanthe, Genista pilosa, Genista tinctoria, Lycopodium inundatum, Narthecium ossifragum, Pedicularis sylvatica, Polygala serpyllifolia and Thymus serpyllum) are declining or have even become locally extinct in the Netherlands. The distribution of these species is related to small-scale, spatial variability of the heathland soils. It has been suggested that atmospheric deposition has caused such changes (Van Dam et al., 1986). Dwarf shrubs as well as grass species are nowadays dominant in the former habitats of these endangered species.

Enhanced nitrogen fluxes into nutrient-poor heathland soil leads to an increased nitrogen availability in the soil. However, most of the deposited nitrogen in western Europe originates from ammonia/ammonium deposition and may also cause acidification as a result of nitrification. Whether eutrophication or acidification or a combination of both processes is important depends on pH, buffer capacity and nitrification rates of the soil. Roelofs et al. (1985) found that, in dwarf-shrub-dominated heathland soils, nitrification is inhibited at pH 4.0-4.2 and that ammonium accumulates while nitrate decreases to almost zero at these or lower pH values. Furthermore, nitrification has been observed in
the soils from the habitats of the endangered species, owing to its somewhat higher pH and higher buffer capacity. In soils within the pH range of 4.1-5.9, the acidity produced is buffered by cation exchange processes (Ulrich, 1983). The pH will drop when calcium is depleted, and this may cause the decline of those species that are generally found on soils with somewhat higher pH. To study the pH effects on root growth and survival rate, hydroculture experiments have been conducted over 4-week periods with several of the endangered species (*Arnica, Antennaria, Viola, Hieracium pilosella* and *Gentiana*) and with the dominant species (*Molinia* and *Deschampsia*) (Van Dobben, 1991). The dominant species indeed have a lower pH optimum (3.5 and 4.0, respectively) than the endangered species (4.2-6.0). However, the endangered species could survive very low pH without visible injuries during this short experimental period.

The pH decrease may indirectly result in an increased leaching of base cations, increased aluminium mobilization and thus enhanced aluminium/calcium (Al/Ca) ratios of the soil (Van Breemen et al., 1982). Furthermore, the reduction of the soil pH may inhibit nitrification and result in ammonium accumulation and consequently increased NH$_4$/NO$_3$ ratios. In a recent field study the characteristics of the soil of several of these threatened heathland species have been compared with the soil characteristics of the dominant species (*Calluna vulgaris, Erica tetralix* and *Molinia caerulea*) (Houdijk et al., 1993). Generally the endangered species grow on soil with higher pH, lower nitrogen content, and lower Al/Ca ratios than the dominant species. The NH$_4$/NO$_3$ ratios were higher in the dwarf-shrub-dominated soils than in the soil of the endangered species. Fennema (1990, 1992) has demonstrated that soil from locations where *Arnica* is still present had a higher pH and lower Al/Ca ratio than soil of former *Arnica* stands. However, he found no differences in total soil nitrogen or NH$_4$/NO$_3$ ratios. Both these studies indicate that high Al/Ca ratios or even increased NH$_4$/NO$_3$ ratios are associated with the decline of these species. However, no significant effects of Al and Al/Ca on growth rates have been observed in hydroculture experiments in which the effects of Al and Al/Ca ratios on root growth and survival rate were studied (Van Dobben, 1991). Comparable experiments of Pegtel (1987) with *Arnica* and *Deschampsia* and Kroeze et al. (1989) with *Antennaria, Viola, Filago minima*, and *Deschampsia* showed similar results. However, results of a hydroculture experiment with *Arnica* showed that this species is very sensitive to enhanced Al/Ca ratios at intermediate or low nutrient levels (De Graaf, 1994). Pot experiments have indicated
that increased NH₄/NO₃ ratios lead to decreased health of *Thymus*. Hydroculture experiments with this plant species confirmed that increased NH₄/NO₃ ratios affected the cation uptake (Houdijk, 1993). In a pot experiment *Thymus*, planted on acid heathland soil and on artificially buffered heathland soil, was sprayed with 0, 15 and 150 kg nitrogen (as ammonium) per ha per year during 6 months (Houdijk et al., 1993). In this relatively short period, a deposition of 15 kg nitrogen (as ammonium) per ha per year on the acid soil did not lead to ammonium accumulation in the soil. As a result of nitrification, soil pH decreased faster than in the absence of ammonium deposition. At the highest deposition (150 kg nitrogen (as ammonium) per ha per year), nitrification rates in the acid heathland soils were too low to prevent ammonium accumulation, and increased NH₄/NO₃ ratios probably caused the decline of *Thymus*. Only in the artificially buffered soils with higher pH were nitrification rates high enough to balance ammonium and nitrate. *Thymus* plants on these soils were healthy despite very high total nitrogen contents.

At present, however, there is too little information available on these rare heathland and acidic grassland species to formulate a critical load for nitrogen. The observation that these heathland species generally disappear before dwarf shrubs are replaced by grasses leads to the assumption that their critical load is lower than the critical load for the transition to grasses (< 15–20 kg nitrogen per ha per year) and probably between 10 and 15 kg nitrogen per ha per year. An overview of the critical loads in heathlands is given in section 8.2.2.

**4.2.5 Effects of nitrogen deposition on forests**

**4.2.5.1 Effects on forest tree species**

The growth of the vast majority of the forest tree species in the Northern hemisphere was until recently limited by nitrogen. In forestry, nitrogen fertilizers were used to increase wood production (Tamm, 1991). An increase in the supply of an essential nutrient, including nitrogen, will stimulate tree growth; the initial impact of enhanced nitrogen deposition will, therefore, be a fertilizer effect. However, continued high inputs of nitrogen produces negative effects on tree growth (Chapin, 1980). Until the mid-1980s, almost all of the research on forest decline focused on acidification, but it has now become evident that enhanced nitrogen deposition may also be important in recent forest decline.
The effects of high atmospheric nitrogen input are very complex (Wellburn, 1988; Pitelka & Raynal, 1989; Heij et al., 1991; Pearson & Stewart, 1993). Chronic nitrogen deposition may result in nitrogen saturation, when enhanced nitrogen inputs no longer stimulate tree growth, but start to disrupt ecosystem structure and function, and increased amounts of nitrogen are lost from the ecosystem in leachate (Agren, 1983; Aber et al., 1989; Tamm, 1991). The nitrogen input at which saturation occurs depends on a number of factors including the amount of deposition, vegetation type and age (see chapter 3), soil type and management history. The following indirect processes, besides the direct effect of gaseous pollutants on the shoots, are important:

- **Soil acidification, due to nitrification of ammonium.** This process leads to accelerating leaching of base cations and, in poorly buffered soils, to increased dissolution of aluminium, which can damage fine roots development and mycorrhizas, and thus reduce nutrient uptake (Ulrich, 1983; Ritter, 1990).

- **Eutrophication.** Whether ammonium will accumulate in soil or not is strongly dependent upon the nitrification rate and the deposition levels (Boxman et al., 1988). In addition to an initial growth stimulation and changes in root/shoot ratio, ammonium accumulation will lead to an imbalance of the nutritional state of the soil and concomitantly of the trees (Roelofs et al., 1985; Van Dijk & Roelofs, 1988; Schulze et al., 1989; Boxman et al., 1991). Accumulation of nitrates in the ecosystem may also lead to eutrophication. As a consequence of all these processes, the health of the trees declines and their sensitivity to drought, frost, insect pests and to pathogens can increase markedly (Wellburn, 1988). These phenomena may also play a secondary, but certainly not unimportant, role in the dieback of forest trees and have also been reviewed.

Although many tree species occur in natural forest ecosystems, almost all studies on air pollution have concentrated on a few forestry tree species from acidic, nutrient-poor soils. Most of these species are conifers (*Picea, Pinus* and *Pseudotsuga* spp.) and the following section concentrates on the long-term soil-mediated effects on these trees. Available data on broad-leaved species (*Fagus, Quercus*) are also considered. Long-term effects of nitrogen eutrophication on the composition of the tree layer in natural forests may be expected but have not yet been quantified. Soil acidification *per se* has only been briefly reviewed, because
the critical load for acidity and tree growth is well established (Nilsson & Grennfelt, 1988; Downing et al., 1993).

\textbf{a) Soil-mediated changes in nutritional status of forest tree species}

It has been shown that in areas with high ammonia/ammonium deposition, ammonium accumulates in acid forest soils with little or no nitrification. Van Dijk & Roelofs (1988) found ammonium ion accumulation in damaged \textit{Pinus} and \textit{Pseudotsuga} stands receiving 60-100 kg nitrogen per ha per year, although the pH of the soil was the same as that in healthy stands. This build-up of ammonium ion leads to increased ratios of ammonium to base cations (Roelofs et al., 1985; Boxman et al., 1988), a reduction of base cation uptake and, eventually, nutritional problems. Using soil columns with different ammonium sulfate spraying treatments, critical ratios of excess ammonium to base cations have been determined (Boxman et al., 1988). The nutritional problems of the coniferous species studied have been found above values of 5, 10 and 1, respectively, for the NH$_4$/K, NH$_4$/Mg and Al/Ca ratios in soil solution. In soil with zero or a low nitrification rate, 10-15 kg nitrogen per ha per year is a reliable critical load to prevent critical ammonium to cation ratios, whereas in base-cation-rich soil with moderate to high nitrification rates the critical loads obtained are higher (20-30 kg nitrogen per ha per year).

The nutritional status of the coniferous trees studied, after enhanced nitrogen inputs, is affected by both ammonium accumulation and soil acidification. Base cation concentrations in the soil are reduced by leaching, whereas base cation uptake by plants is reduced by excess of ammonium and of aluminium. Furthermore, root growth is decreased (see later). Laboratory, greenhouse and field measurements in the Netherlands, Germany and southern Sweden (Van Dijk & Roelofs, 1988; Van Dijk et al., 1989, 1990, 1992a; Hofmann et al., 1990; Schulze & Freer-Smith, 1991; Boxman et al., 1991, 1994; Ericsson et al., 1993) have shown that the complex of factors just noted produce severe deficiencies of magnesium and potassium in coniferous trees. Most of these studies were in areas, or involved experiments, with large inputs (> 40-100 kg nitrogen per ha per year).

The magnesium and phosphorus concentrations in leaves of oak trees (\textit{Fagus sylvatica}), a common deciduous tree in Europe, decreased significantly from 1984 to 1992 in permanent plots in NW Switzerland. Furthermore, the magnesium concentrations in
the leaves of young *Fagus sylvatica* decreased significantly within a 4-year period of fertilizer application at ≥ 25 kg nitrogen per ha per year (Flückiger & Braun, 1994). In Sweden, suboptimal concentrations of magnesium and potassium in *Fagus* leaves were found in areas with the highest nitrogen deposition (Balsberg-Pählsson, 1989) and addition of nitrogen enhanced nutritional imbalance in a 120-year-old *Fagus* stand (Balsberg-Pählsson, 1992). It is thus clear that this deciduous tree species is also sensitive to nutritional imbalance induced by enhanced nitrogen supply.

Base cations are also lost from the canopy by increased leaching, linked to high amounts of atmospheric deposition (Wood & Bormann, 1975; Roelofs et al., 1985; Bobbink et al., 1992b). As a result of high nitrogen inputs, the organic nitrogen concentration in the needles of conifers has increased significantly to supraoptimal levels (Van Dijk & Roelofs, 1988; De Kam et al., 1991). Concentrations of nitrogen-rich free amino acids, especially arginine, have significantly increased in the needles with high nitrogen concentration (> 1.5% nitrogen in *Picea abies*) (Hällgren & Näsholm, 1988; Pietila et al., 1991; Van Dijk et al., 1992) and in *Fagus* leaves (Balsberg-Pählsson, 1992).

Although there is clear evidence that high NH₃/NH₄ loads produce adverse changes in the nutritional status and the growth of the investigated coniferous and broad-leaved trees, it is difficult to obtain a critical load for nitrogen from these studies, because of the complexity of the ecosystem. A quite reliable critical load for nitrogen deposition on beech tree health is around 15-20 kg nitrogen per ha per year, as demonstrated in the Swiss studies (Flückiger & Braun, 1994).

The results of the EC nitrogen saturation study (NITREX), which incorporates long-term experiments in both clean and nitrogen-polluted areas and whole ecosystem manipulation of nitrogen inputs, are providing important evidence on the effects of nitrogen deposition on tree health and ecosystem health. Atmospheric deposition of nitrogen was reduced from 40 to 2 kg nitrogen per ha per year in a nitrogen-saturated *Pinus sylvestris* stand in the Netherlands (Boxman et al., 1994, 1995). Throughfall water was intercepted with a roof and replaced by clean throughfall water from 1989 onwards. In the clean plot a quick response of the soil solution chemistry was observed. The nitrogen concentrations in the upper soil and the fluxes of this element through the soil profile decreased. As a result, base cation
leaching and the ratios of ammonium to various cations also decreased; potassium and magnesium concentrations in the needles increased significantly. The needle nitrogen concentrations were only slightly reduced in the “clean” situation, but they were significantly lower than in the needles of the control plots. The concentration of arginine decreased significantly in the needles of the trees from the clean throughfall plot. Furthermore, tree growth became higher after 4 years of clean throughfall than in control plots with high nitrogen deposition. No changes in the mycorrhizal status or in the undergrowth have so far been observed (Boxman et al., 1994, 1995). This study clearly demonstrates the detrimental effects of enhanced atmospheric nitrogen deposition on the nutritional balance of coniferous trees.

b) Nitrogen deposition and tree susceptibility to frost, drought and pathogens

It has been suggested by several authors that sensitivity of trees to secondary stress factors is increased by high nitrogen loading (Wellburn, 1988; Pitelka & Raynal, 1989). In field fertilizer applications it is often observed that tree growth starts earlier in the season, which may increase damage by late frost. Furthermore, it has been shown, after nutrient applications, that frost damage to *Pinus sylvestris* increases considerably at needle nitrogen concentrations above 1.8% (Aronsson, 1980), although other fertilizer studies have demonstrated reverse effects, i.e. improved nitrogen status of the plants diminishes frost damage (De Hayes et al., 1989; Klein et al., 1989; Cape et al., 1991).

Only few data are available with respect to frost damage in direct relation to airborne nitrogen deposition. After exposure to NH₃ and SO₂, *Pinus sylvestris* saplings became more frost sensitive (< -10 °C) than control plants (Dueck et al., 1990). Dueck et al. (1990) also determined the frost sensitivity of *Pinus sylvestris* growing in areas with low ammonia/ammonium pollution (approximately 4 μg NH₃/m³) and in highly polluted areas (40 μg NH₃/m³). Surprisingly, the frost sensitivity was not higher in the polluted area than in the other investigated sites, and was sometimes even lower. After experimental treatment with ammonia (53 μg NH₃/m³) the growth of the trees had increased, indicating that the observed change in frost sensitivity might have occurred as a result of changes in physiology and nutrient imbalance.
The effects of simulated acid mist containing sulfate, ammonium, nitrate and H⁺ on the frost sensitivity of Picea rubens has been studied (Sheppard et al., 1993; Sheppard, 1994). There was a strong correlation between the application of sulfate-containing mist and an increase in frost sensitivity, but no such correlation was seen after treatment with ammonium or nitrate ions. Sulfur compounds clearly affect the frost sensitivity of coniferous trees, but this effect may be a consequence of the nutritional status (nitrogen, base cations) of the trees (Sheppard, 1994). It is concluded that the effects of increased nitrogen inputs on frost sensitivity remain uncertain. Insufficient research has been carried out to use the results for assessment of a critical load.

The water uptake of coniferous tree species may be affected by increasing nitrogen deposition, owing to an increase in shoot-to-root ratio and a reduction in fine-root length. Indeed, the health of many tree species in the regions of the Netherlands with high nitrogen deposition was particularly poor in the dry years in the mid-1980s, but improved again during the subsequent normal years (Heij et al., 1991). Many authors have mentioned a negative impact of high nitrogen supply on the development of fine roots and mycorrhiza, although positive effects have also been described (Persson & Ahlstrom, 1991).

Van Dijk et al. (1990) applied 0, 48, 480 kg nitrogen (as ammonium sulfate) per ha per year to young Pinus sylvestris, Pinus nigra and Pseudotsuga menziesii in a pot experiment. After seven months the coarse root biomass had not changed, but the fine root biomass decreased by 36% at the highest nitrogen application. In parallel, a 63% decrease in mycorrhizal infection at the highest nitrogen application was found. In the Dutch EC nitrogen saturation study, the fine root biomass and the number of root tips of Pinus sylvestris increased after reduction of the current nitrogen deposition to pre-industrial levels, indicating restricted root growth and nutrient uptake capacity at the ambient nitrogen load of about 40 kg nitrogen per ha per year (Boxman et al., 1994, 1995).

In a hydroculture experiment with Pinus nigra at pH=4.0, Boxman et al. (1991) found an increase in coarse/fine root ratio after increasing the ammonium concentration to 5000 μM. Furthermore, a clear relation was found between the nitrogen content of the fine roots and mycorrhizal infection (as measured as the number of dichotomously branched roots). In a hydroculture experiment Jentschke et al. (1991) found, however, that 2700 μM
Nitrate had hardly any effect on the mycorrhizal development of *Picea abies* seedlings inoculated with *Lactarius rufus*. Ammonium at 2700 μM only had a slight negative effect on mycorrhizal development, whereas a reduction in root growth was recorded. In a pot experiment with *Picea abies*, Meyer (1988) found optimal mycorrhizal development when the mineral nitrogen content of the soil was 40 mg nitrogen/kg dry soil, while at 350 mg nitrogen/kg dry soil a 95% reduction in mycorrhizal development was found. In this study no correlation was found with the soil pH. Alexander & Fairly (1983) found, after fertilizer application to a 35-year-old *Picea sitchensis* stand with 300 kg nitrogen (as ammonium sulfate) per ha, a 15% reduction in mycorrhizal development in the second year after application. Termorshuizen (1990) applied 0 to 400 kg nitrogen ha per year either as ammonium or nitrate to young *Pinus sylvestris* inoculated with *Paxillus involutus* in a pot experiment. Above application rates of 10 kg nitrogen per ha per year there was a decrease in the amount of mycorrhizal root tips and the number of sclerotia.

In addition to the above-mentioned data for coniferous trees, it had been shown that the shoot-to-root ratios of young *Fagus sylvatica* trees, grown in containers with acid forest soil, increased significantly from about 1 to between 2 and 3 after a 4-year experimental application of nitrogen (25 kg nitrogen per ha per year or more) (Flückiger & Braun, 1994).

It is thus likely that enhanced nitrogen inputs affect drought sensitivity through changes in shoot to root ratios, number of fine roots and the ectomycorrhizal infection of the roots. However, the data are too few to use for the assessment of a critical load of nitrogen, based upon this aspect of reduced tree health.

There may also be significant effects of fungal pathogens or insect pests associated with increasing nitrogen deposition. The foliar concentrations of nitrogen increased markedly in tree needles or leaves in experiments with nitrogen additions, and also in forest sites with high atmospheric nitrogen loading (Roelofs et al., 1985; Van Dijk & Roelofs, 1988; Balsberg-Pålsson, 1992). Animal grazing generally increases with increasing palatability of the leaves or shoots. Nitrogen is of major importance for the palatability of plant material, and this certainly holds for insect grazing (Crawley, 1983). Secondary plant chemicals, e.g., phenolics, are important for increased resistance of plants. The total amount of phenolics in *Fagus* leaves in a 120-year stand decreased by more than 30% after fertilizer application of about
45 kg nitrogen per ha per year, compared with the control treatment (Balsberg-Pahlsson, 1992). An ecologically important relation between nitrogen enrichment and insect pests has been quantified for lowland heathland (Brunsting & Heil, 1985; Berdowski, 1993, see section 4.1) but not, so far, for forest ecosystems.

From 1982 to 1985 an epidemic outbreak of the pathogenic fungus \textit{Sphaeropsis sapinea} was observed in coniferous forest (mainly \textit{Pinus nigra}) in the Netherlands. This greatly affected whole stands, and was especially severe in the south-east part of the Netherlands, where there was high airborne nitrogen deposition (Roelofs et al., 1985). Van Dijk et al. (1992) showed that there was a significantly higher foliar nitrogen concentration in the infected stands, together with higher soil ammonium levels, than in the uninfected stands. Most of the additional nitrogen in the needles of the affected stands was stored as nitrogen-rich free amino acids, especially arginine. Proline concentrations were also higher in the infected trees, indicating a relation with water stress (Van Dijk et al., 1992).

The effects of \textit{Sphaeropsis} have also been studied by De Kam et al. (1991). Two-year-old plants of \textit{Pinus nigra} were grown for 3 years in pots and given five treatments of ammonium sulfate (very low to about 300 kg nitrogen per ha per year), in combination with two levels of potassium sulfate. The 5-year-old plants were then inoculated with \textit{Sphaeropsis}. The bark necroses were much more frequent in the plants treated with ammonium sulfate than in the controls. Effects of ammonium sulfate upon fungal damage were even observed at an addition of 75 kg nitrogen per ha per year, but were very significant in the plants treated with 150 kg nitrogen per ha per year. After potassium addition the number of necroses caused by the fungus was greatly reduced (De Kam et al., 1991).

In beech forests in NW Switzerland, a significant positive correlation has been found between the nitrogen/potassium ratios in the leaves and necroses caused by the beech cancer \textit{Nectria ditissima} (Flückiger & Braun, 1994). These authors also experimentally inoculated \textit{Fagus sylvatica} trees at different applications of nitrogen with this beech cancer and observed increased dieback of new leaves and shoots. Furthermore, the infestation of \textit{Fagus sylvatica} with beech aphids (\textit{Phyllaphis fagi}) was also affected by the nitrogen availabilities. The degree of infestation with the aphid increased significantly with enhanced
EHC 188: Nitrogen Oxides

leaf nitrogen/potassium ratios (Flückiger & Braun, 1994). Although evidence for nitrogen-mediated changes in susceptibility to fungal pests and insect attacks has until now been based upon observations of only few species, it is obvious that trees became more susceptible to these attacks with increasing nitrogen enrichment and this may play a crucial role in the dieback of some forest stands.

A critical load for nitrogen had been established at 10-15 kg nitrogen (at no or low nitrification) to 20-30 kg nitrogen per ha per year in highly nitrifying soils, based upon nutritional imbalance of coniferous species (Boxman et al., 1988). Recent evidence of Fagus sylvatica tree health in acidic forests indicated a critical load of 15-20 kg nitrogen per ha per year, based upon both field and experimental observations. Elevated nitrogen deposition can seriously affect tree healthy via a complex web of interactions (e.g. susceptibility to frost and drought). Pathogens may play an important role in tree decline, but at this moment it is not possible to combine the observed processes and effects to an overall value for a critical load of nitrogen for tree health.

4.2.5.2 Effects on tree epiphytes, ground vegetation and ground fauna of forests

a) Effects on ground-living and epiphytic lichens and algae

The effects of SO$_2$ as an acidifier on epiphytic lichens have been extensively studied (Insarova et al., 1992; Van Dobben, 1993). SO$_2$ was previously the dominant airborne pollutant, and it has been shown that most (epiphytic) lichens are more negatively affected by acidity than by nitrogen compounds (except NO$_2$). Most lichens have green algae as photobionts and are affected by acidity but not by nitrogen. Some of them even react positively to nitrogen (Insarova et al., 1992). However, 10% of all lichen species in the world have cyanobacteria (blue-green algae) as the photobiont. These cyanobacterial lichens are negatively affected by acidity, and also by nitrogen. Most of the NW European lichens with cyanobacteria live on the soil surface or are tree epiphytes. The most pollution-sensitive lichens are among them and they are threatened by extinction in NW Europe. This is probably the result of increased nitrogen deposition, which inhibits the functioning of the cyanobacteria. In the Netherlands, for example, all cyanobacterial lichens that were present at the end of the 19th century are now absent. In Denmark, 96% of the lichens with cyanobacteria are extinct or threatened. Furthermore,
the cyanobacterial lichens appear frequently on the Red List of the European Union countries (Hallingbäck, 1991).

Very few data exist to establish a critical load for nitrogen for these lichens with blue-green algae. Nohrstedt et al. (1988) investigated the effects of nitrogen application (as ammonium nitrate or calcium nitrate) on ground-living lichens (*Peltigera aphthosa* and *Nephroma arcticum*) with blue-green algae as photobionts. The plots were treated once or three or four times with 120, 240 or 360 kg nitrogen per ha. After a short period all *Peltigera* and *Nephroma* lichens were eliminated and even 19 years later no recolonization had occurred. However, it is impossible to transform these very high doses to critical loads. The effects of air pollutants on lichens are usually related to concentrations in the air or in the precipitation. It is probably more relevant to relate the effects of nitrogen on cyanobacterial lichens to deposition than to concentrations. For tree epiphytes stemflow is most relevant, whereas for ground-living lichens throughfall will be more important. Although much research is still needed, it has been suggested that a load of 5-15 kg nitrogen per ha per year is already critical for the growth of these cyanobacterial lichens (Hallingbäck, 1991). These lichens may be the most sensitive components of some forest ecosystems and thus determine the critical load for these systems.

Free-living green algae, especially of the genus *Pleurococcus* (*Protococcus* and *Demococcus* are synonyms), are strongly stimulated by enhanced nitrogen deposition. They cover practically all outdoor surfaces which are not subject to frequent desiccation in regions with high nitrogen deposition, such as in the Netherlands and in Denmark. The thickness and the colonization rate of spruce needles by green algae has been investigated in the Swedish Environmental Monitoring Programme (Brakenhielm, 1991). The Swedish data show that these algae do not colonize spruce needles in regions with a total deposition (throughfall) lower than about 5 kg nitrogen per ha per year. In areas with deposition above 20 kg nitrogen per ha per year, the green algal cover of the needles is so thick and the algae colonize so early that they may impede the photosynthesis of the spruce trees.

b) Effects on forest ground vegetation

In the Netherlands the forest vegetation of a site in the central part of the country was investigated in 1958 (with about 20 kg nitrogen per ha per year) and in 1981 (with about 40 kg nitrogen per ha per year).
per ha per year). All lichens had disappeared during this period and a considerable increase in Deschampsia flexuosa and Corydalis claviculata was found. A large representative sample test (n=2000), covering about 90% of the Dutch forests, revealed in the mid-1980s that among the 40 most common forest plants were: Galeopsis tetrahit, Rubus species, Deschampsia flexuosa, Dryopteris caelatissima, Molinia caerulea, Poa trivialis, and Urtica dioica (Dirkse & Van Dobben, 1989; Dirkse, 1993). In Sweden, Quercus robur stands in two geographical areas with different nitrogen deposition were compared with special emphasis on nitrogen indicator species (Tyler, 1987). The stands were quite comparable except for the nitrogen inputs: 6-8 kg nitrogen per ha per year and 12-15 kg nitrogen per ha per year, respectively. In the stand with the highest deposition, the soil solution was more acidic, probably due to acidic deposition as well (+ 10 kg sulfur per ha per year), and it was estimated that acidification of the soil has accelerated during the last 30 to 50 years. The following species were more common in the most polluted site Urtica dioica, Epilobium angustifolium, Rubus idaeus, Stellaria media, Galium aparine, Aegopodium podagraria and Sambucus spp. Thus, both in Sweden and the Netherlands, species indicative of nitrogen enrichment became common (Ellenberg, 1988b).

Comparable observations were reported by Falkengren-Grerup (1986) and by Falkengren-Grerup & Eriksson (1990), who examined the changes in soil and vegetation in Quercus and Fagus stands in southern Sweden. They concluded that the exchangeable base cations were reduced and that aluminium had doubled over the past 35 years. They also found a decrease in soil pH, with a disappearance of several species when pH dropped below a threshold. In spite of soil acidification some species had increased in cover, and the most plausible explanation seemed to be increased nitrogen deposition, which was about 15-20 kg nitrogen per ha per year in southern Sweden and which had doubled since 1955. A marked increase in cover was found for Lactuca muralis, Dryopteris filix-max, Epilobium angustifolium, Rubus idaeus, Melica uniflora, Aegopodium podagraria, Stellaria holostea and S. nemorum, some of these species being nitrogen indicators. Despite soil acidification, acid-tolerant species (Deschampsia flexuosa, Mainthemum bifolium and Luzula pilosa) did not increase. A distinct decrease was observed for Dentaria bulbifera, Pulmonaria officinalis and Polygonatum multiflorum. Furthermore, Rosen et al. (1992) found a significant positive correlation between the increase of Deschampsia flexuosa cover in the last 20 years in the Swedish forests and the pattern of nitrogen deposition.
In a large semi-natural Fagus-Quercus forest in NE France, about 50 permanent vegetation plots were investigated in 1972 and 1991. The changes in species composition on calcareous soils and in moderately acidic habitats were followed. During the study period a significant increase in nitrophilous ground flora was observed in the high-pH (6.9) stands. This indicated that at this location (with ambient deposition of 15–20 kg nitrogen per ha per year) there was a distinct effect of increasing nitrogen availability (Thimonier et al., 1994).

From 1968 to 1985, three sites in a 30-year-old Pinus sylvestris forest in Lisselbo (central Sweden) were annually fertilized with 0, 20, 40 and 60 kg nitrogen per ha per year (as NH₄NO₃ plus ambient deposition of 10 kg nitrogen per ha per year). The original ground vegetation consisted of Calluna vulgaris, Vaccinium vitis-idea, V. myrtillus, Cladonia spp., Cladina spp., and the mosses Dicranum spp., Pleurozium spp. and Hylocomium spp. The first changes were observed within 8 to 15 years and after about 20 years the experimental plots were compared and statistically analysed. The original species disappeared at nitrogen applications above 20 kg (plus ambient deposition) nitrogen per ha per year and were replaced by Epilobium augustifolium, Rubus idaeus, Deschampsia flexuosa, Dryopteris carthusiana and the moss Brachythecium oedipodium (Dirkse et al., 1991; Van Dobben, 1993). In another experiment at Lisselbo the combined effects of acidification (addition of H₂SO₄, pH=2.0) and nitrogen addition (0 and 40 kg nitrogen per ha per year) were investigated. The increased nitrogen level seemed to be the more important factor. Acidification was the next most discriminating factor: all species disappeared, except for the moss Pohlia nutans at high additions of acidity (Dirkse & Van Dobben, 1989; Dirkse et al., 1991).

In southern Sweden, Tyler et al. (1992) studied the effects of the application of ammonium nitrate (60–180 kg nitrogen per ha per year) over a 5-year period on stands of Fagus sylvatica. They observed a large reduction in biomass of the ground vegetation with the application of nitrogen, and the frequency of most herb layer species declined significantly. Soil measurements revealed that, in addition to eutrophication effects, the acidification of the soil solution was also important for the decline of the original ground vegetation. In an experiment on the effects of nitrogen fertilizer application on bryophytes, it appeared that Brachythecium oedipodium, B. reflexum and B. starkei increased significantly at levels up to 60 kg nitrogen per ha per year. At higher doses these species tended to decline, however. Hylocomium
and Pleurozium schreberi declined considerably at doses of 30 to 60 kg nitrogen per ha per year (Dirkse & Martaki, 1992).

c) Effects on macrofungi and mycorrhizas

During the last two decades many reports have described a decrease in species diversity and abundance of macrofungi. These changes can probably be attributed to indirect effects of air pollution, in particular to increases in the amount of available nitrogen (possibly in combination with acidification), and/or to decreased health of trees with concomitant reduction of transport to the roots (Arnolds, 1991).

When comparing sites over time, the number of fruiting bodies of macrofungi showed marked differences. Most studies in western Europe, however, have revealed that the number of ectomycorrhizal fungi species has declined (Arnolds, 1991). In the Netherlands the average number of ectomycorrhizal species per foray declined significantly from 71 in 1912-1954 to 38 in 1973-1982. Similar changes have been observed in Germany: 94 ectomycorrhizal species found in 1930-1979 in the Völklinger area (Saarland) have not been recorded recently. From the 236 species found in 1918-1942 in the Darmstadt area (Germany), only 137 were recorded in the early 1970s, a loss of 99 species, including many mycorrhizal fungi (Arnolds, 1991). In contrast to the decline in mycorrhizal fungi, the number of saprotrophic species remained practically unchanged, while the number of lignicolous species increased. This may be related to soil acidification with a increase in aluminium, since the proportion of forest areas in western Europe with a soil pH below 4.2 increased from less than 1% in 1960 to 15% in 1988 (Schneider & Bresser, 1988).

Arnolds (1988, 1991) concluded that acidification has very little effect on the diversity of ectomycorrhizal fungi, but rather triggers changes in species composition. He regarded the increased nitrogen flux to the forest floor as the most important factor in the decline of mycorrhizal fungi. Termorshuizen & Schaffers (1987) found a negative correlation between the total nitrogen input in mature Pinus sylvestris stands and the abundance of fruit bodies of ectomycorrhizal fungi. Similar results were obtained by Schlechte (1986) who compared two sites with Picea abies in the Göttingen area of Germany. An obvious negative relation was found between nitrogen input (23 versus 42 kg nitrogen per ha per year) and ectomycorrhizal species: 85 basidiomycetes including 21 ectomycorrhizas (25%) at the less polluted site compared with 55
Effects of Atmospheric Nitrogen Compounds on Plants

basidiomycetes including 3 ectomycorrhizas (5%) at the most polluted site. Environmental factors other than nitrogen did not differ significantly. The negative impact of nitrogen seems only to hold true for mature forests (Termorshuizen & Schaffers, 1987). Jansen & de Vries (1988) found a maximum in fruit-body production in > 20-year-old Pseudotsuga menziesii stands at about 25 kg nitrogen per ha per year. Meyer (1988) found a similar optimum when Picea abies was planted in soil mixed with different amounts of sawdust having a high carbon/nitrogen ratio.

Experiments with nitrogen fertilizer have produced similar results. In a fertilizer trial with simulated nitrogen deposition in a Fagus forest in southern Sweden (ambient deposition 15-20 kg nitrogen per ha per year), Ruhling & Tyler (1991) found, after applying NH₄NO₃ (60 and 180 kg nitrogen per ha per year), that within 3 to 4 years almost all mycorrhizal species ceased fruit-body production. In contrast, several decomposer species increased fruit-body production. Wood decomposers showed no obvious reaction to the treatment. No fruit-bodies were recovered when 300 kg nitrogen per ha was applied to Pinus sylvestris stands as liquid manure (Ritter & Tolle, 1978). The mycorrhizal frequency of the roots, however, was still 55% as compared to 87% in the controls. Application of 112 kg nitrogen (as NH₄NO₃) per ha to 11-year-old Pinus taeda stands revealed an 88% reduction in the number of fruit-bodies and a 14% decrease in the number of mycorrhizas per unit of soil volume (Menge & Grand, 1978). In the Lisselbo study the number of fruit-bodies decreased considerably at each nitrogen fertilizer dose (Wasterlund, 1982). Termorshuizen (1990) applied 0, 30 and 60 kg nitrogen (as ammonium sulfate or nitrate) per ha per year to young Pinus sylvestris stands. In general fruit-body production was more negatively influenced by the higher ammonium levels than nitrate levels. The mycorrhizal frequency and the number of mycorrhizas per unit of soil volume were not influenced. It was concluded by Termorshuizen (1990) that fruit-body production is much more sensitive to nitrogen enrichment that mycorrhizal formation. Branderud (1995) found after only 1.5 year a decrease in fruit-body production of mycorrhizal species at a nitrogen application of 35 kg nitrogen (as NH₄NO₃) per ha in a Picea abies stand at the Swedish Nitrex stand.

In contrast, some studies have shown an increase in the number of fruit-bodies of insensitive mycorrhizal fungi after nitrogen fertilizer application, e.g., Paxillus involutes (Hora, 1959), Laccaria bicolor (Ohenoja, 1988) and Lactarius rufus (Hora, 1959).
d) Effects on soil fauna of forests

Almost all studies of changes in faunal species composition due to nitrogen enrichment have been conducted in arable fields or agricultural grasslands using complete fertilization and thus cannot be used to substantiate critical loads for semi-natural forest ecosystems (Marshall, 1977). The relationship between acidity and soil fauna has also been studied in northern coniferous forests, but only very few studies have incorporated the effects of nitrogenous compounds (Gårdenfors, 1987). The abundance of Nematoda, Oligochaeta and microarthropods (especially Collembola) had increased in some studies, but decreased in others, after application of high doses of nitrogen fertilizers (> 150 kg nitrogen per ha per year) (Abrahamsen & Thompson, 1979; Huhta et al., 1983; Vilkamaa & Huhta, 1986). A reduction in the nitrogen deposition in a Pinus sylvestris stand (Nitrex site Ysselstein) to pre-industrial levels increased the species diversity of microarthropods due to a decreased dominance of some species (Boxman et al., 1995). However, it is not possible to use these few data to formulate a critical load for changes in forest soil fauna due to increased nitrogen deposition.

On the basis of the results presented in this overview, the critical load for changes in the ground vegetation of both coniferous and deciduous acidic forest may be 15 to 20 kg nitrogen per ha per year. The critical load for changes in the fruit-body production of ectomycorrhizal fungi is probably about 30 kg nitrogen per ha per year, while the critical load for changes in mycorrhizal frequency of tree roots is hard to estimate, but certainly considerably higher. There is insufficient data on the effects of enhanced nitrogen deposition on faunal components of forest ecosystems to allow critical loads to be set. Epiphytic or ground-living lichens with cyanobacteria as the photobiont probably form a sensitive part of forest ecosystems and have an estimated critical load of 10-15 kg nitrogen per ha per year. A summary of the critical loads for forests is given in chapter 8.

4.2.6 Effects on estuarine and marine ecosystems

Few topics in aquatic biology have received as much attention over the past decade as the debate over whether estuarine and coastal ecosystems are limited by nitrogen, phosphorus or some other factor (Hecky & Kilham, 1988). Numerous geochemical and experimental studies have suggested that nitrogen limitation is much more common in estuarine and coastal waters than in
Effects of Atmospheric Nitrogen Compounds on Plants

Freshwater systems. Taken as a whole, the productivity of estuarine waters in the USA correlates more closely with supply rates of nitrogen than with those of other nutrients (Nixon & Pilson, 1983).

Estimation of the contribution of nitrogen deposition to the eutrophication of estuarine and coastal waters is made difficult by the multiple direct anthropogenic sources (e.g., from agriculture and sewage) of nitrogen against which the importance of atmospheric sources must be weighed. Estuaries and coastal areas are common locations for cities and ports. The crux of any assessment of the importance of nitrogen deposition to estuarine eutrophication lies in establishing the relative importance of direct anthropogenic exposure (e.g., sewage and agricultural run-off) and indirect effects (e.g., atmospheric deposition).

The effects of nitrogen deposition in certain estuarine systems have been investigated. Complete nitrogen budgets, as well as information on nutrient limitation and seasonal nutrient dynamics, have been compiled for two large "estuaries", the Baltic Sea (Scandinavia) and the Chesapeake Bay (USA), and for the Mediterranean Sea. In the case of the Mediterranean, Loye-Pilot et al. (1990) suggest that 50% of the nitrogen load originates as deposition falling directly on the water surface. In the case of the Baltic and Chesapeake, deposition of atmospheric nitrogen has been suggested as a major contributor to eutrophication. Data for other coastal and estuarine systems are less complete, but similarities between these two systems and other estuarine systems suggest that their results may be more widely applicable. Discussion in this monograph is limited to these two case studies, with some speculation about how other estuaries may be related.

The Baltic Sea is perhaps the best-documented case study of the effects of nitrogen additions in causing estuarine eutrophication. Like many other coastal waters, the Baltic Sea has experienced a rapidly increasing anthropogenic nutrient load. It has been estimated that the supply of nitrogen has increased by a factor of 4, and phosphorus by a factor of 8, since the beginning of the 20th century (Larsson et al., 1985). The first observable changes attributable to eutrophication of the Baltic were declines in the concentration of dissolved oxygen in the 1960s (Rosenberg et al., 1990). Decreased dissolved oxygen concentrations result when decomposition in deeper waters is enhanced by the increased supply of sedimenting algal cells from the surface water layers to the sediment. In the case of the Baltic, the spring algal blooms
that now result from nutrient enrichment consist of large, rapidly sedimenting algal cells, which supply large amounts of organic matter to the sediment for decomposition (Enoksson et al., 1990). Since the 1960s, researchers in the Baltic have documented increases in algal productivity, increased incidence of nuisance algal blooms, and periodic failures and unpredictability in fish and Norway Lobster catches (Fleischer & Stibe, 1989; Rosenberg et al., 1990). It has now been shown by a number of methods that algal productivity in nearly all areas of the Baltic Sea is limited by nitrogen. Nitrogen-to-phosphorus ratios range from 6:1 to 60:1 (Rosenberg et al., 1990), but the higher ratios are only found in the remote and relatively unaffected area of the Bothnian Bay (between Sweden and Finland). Productivity in the spring (the season of highest algal biomass) is fuelled by nutrients supplied from deeper waters during spring overturn (Graneli et al., 1990); deep waters are low in nitrogen and high in phosphorus, resulting in nitrogen-to-phosphorus ratios near 5 (Rosenberg et al., 1990), suggesting potential nitrogen limitation when deep waters are mixed with surface waters. Low nitrogen-to-phosphorus ratios in deep water result from denitrification in the deep sediments (Shaffer & Ronner, 1984). Primary productivity measurements in the Kattegat (the portion of the Baltic between Denmark and Sweden) correlate closely with uptake of NO$_3^-$, but not of PO$_4^{3-}$ (Rydberg et al., 1990). Level II and III nutrient enrichment experiments conducted in coastal areas of the Baltic, as well as in the Kattegat, indicate nitrogen limitation at most seasons of the year (Graneli et al., 1990). Growth stimulation of algae has also been produced by addition of rain water to experimental enclosures, in amounts as small as 10% of the total volume (Graneli et al., 1990); rain water in the Baltic is rich in nitrogen but poor in phosphorus. In portions of the Baltic where freshwater inputs keep the salinity low, blooms of the nitrogen-fixing cyanobacterium *Aphanizomenon flos-aquae* are common (Graneli et al., 1990); cyanobacterial blooms are common features of nitrogen-limited freshwater lakes but are usually absent from marine waters.

Nitrogen budget estimates indicate that the Baltic Sea as a whole receives $7.6 \times 10^{10}$ eq of nitrogen per year, of which $2.8 \times 10^{10}$ eq per year (37%) comes directly from atmospheric deposition (Rosenberg et al., 1990). Fleischer & Stibe (1989) reported that the nitrogen flux from agricultural watersheds feeding the Baltic has been decreasing since about 1980 but that the nitrogen contribution from forested watersheds is increasing. They cite both increases in nitrogen deposition and the spread of
modern forestry practices as causes for the increase. It should be noted, however, that the Baltic also experiences a substantial phosphorus load from agricultural and urban lands, and that phosphorus inputs may help to maintain nitrogen-limited conditions (Granéli et al., 1990). If the Baltic had received consistent nitrogen additions (e.g., from the atmosphere or from agricultural run-off) in the absence of phosphorus additions, it might well have evolved into a phosphorus-limited system some time ago.

The physical structure of the Baltic Sea, with a shallow sill limiting exchange of water with the North Sea contributes to the eutrophication of the basin, by trapping nutrients in the basin once they reach the deeper waters. Because the larger algal cells that result from nutrient enrichment in the basin provide more nutrients to the deep water through sedimentation, and because only shallow waters have the ability to exchange with the North Sea, it is estimated that less than 10% of nutrients added to the Baltic are exported over the sill to the North Sea (Wulff et al., 1990). Throughout much of the year (i.e., especially during the dry months) productivity in the Baltic is maintained by nutrients recycled within the water column (Enoksson et al., 1990). The trapping of nutrients within the basin and recycling of nutrients from deeper water by circulation patterns suggest that eutrophication of the Baltic is a self-accelerating process (Enoksson et al., 1990) and has a long time-lag between reductions of inputs and improvements in water quality.

In the USA, a large effort has been made to establish the relative importance of sources of nitrogen to Chesapeake Bay (D’Elia et al., 1982; Smullen et al., 1982; Fisher et al., 1988; Tyler, 1988). Estimates of the contribution of nitrogen to Chesapeake Bay from each individual source are very uncertain; estimating the proportion of nitrogen deposition exported from forested watersheds is especially problematic but critical to the analysis, because about 80% of the Chesapeake Bay basin is forested. Nonetheless, three attempts at determining the proportion of the total nitrate load to the Bay attributable to nitrogen deposition all produce estimates in the range of 18 to 31%. Supplies of nitrogen from deposition exceed supplies from all other non-point sources to the Bay (e.g., agricultural run-off, pastureland run-off, urban run-off), and only point source inputs represent a greater input than deposition.
It is considered that the data from these studies are indicators of the impact of anthropogenic nitrogen. Nevertheless, they are insufficient to estimate critical loads for estuarine/marine systems. It may well be that critical loads for these systems differ for different climatic regions.

4.2.7 Appraisal and conclusions

Atmospheric deposition of nitrogen-containing and acidifying compounds have an impact on soil and groundwater quality and on the health and species composition of vegetation. Critical loads for these effects are given in Table 26. Critical loads have been derived using empirical data that relate loads directly to effects and steady-state soil models that calculate critical loads from critical chemical values for ion concentrations or ratios in foliage, soil solution and groundwater (De Vries, 1993). Information on the effects which occur when critical loads are exceeded is given in Table 27. The values given in Tables 26 and 27 apply to forest vegetation in a temperate climate. Whether they are representative of other climates is uncertain. An overview of the critical loads for atmospheric nitrogen deposition in a range of natural and semi-natural ecosystems is given in chapter 8.

Effects of nitrogen and acidifying deposition on soil and groundwater chemistry are most evident. Field studies showed that deposited nitrogen is partly retained in the forest soil. Even at high nitrogen deposition rates, as in the Netherlands, soil acidification (which is mainly manifested by leaching of aluminium and nitrate) is mainly caused by sulfur deposition. A relatively small contribution of nitrogen to acidification does not imply that sulfur has a larger impact on the health of forests, since the relationship between soil acidification and forest health is not very clear. The eutrophying impact of nitrogen is probably more important than the acidifying impact at present.

There is substantial evidence from field surveys in several countries of Europe that exceeding critical loads does not imply dieback of the forest trees in the short term (one or two decades). However, it does increase the risk of damage due to secondary stress factors and it affects the long-term sustainability of forests. These risks increase with the extent to which present loads exceed critical loads and with the duration.
Table 26. Critical loads for acidity and nitrogen for forest ecosystems in temperate climates (From: De Vries, 1993)

<table>
<thead>
<tr>
<th>Effects</th>
<th>Criteriaa</th>
<th>Critical loads (kg per ha per year)</th>
<th>Coniferous forests</th>
<th>Deciduous forests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(H for acidity; N for eutrophication)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>root damage;</td>
<td>Al &lt; 0.2 mol/m³</td>
<td>1.1b</td>
<td>1.4b</td>
<td></td>
</tr>
<tr>
<td>inhibition of uptake;</td>
<td>Al/Ca &lt; 1.0 mol/mol</td>
<td>1.4b</td>
<td>1.1b</td>
<td></td>
</tr>
<tr>
<td>Al depletion;</td>
<td>ΔK/(OH)⁺ = 0 mmol/m³</td>
<td>1.2b</td>
<td>1.3b</td>
<td></td>
</tr>
<tr>
<td>Al pollution</td>
<td>Al &lt; 0.02 mol/m³</td>
<td>0.3b</td>
<td>0.3b</td>
<td></td>
</tr>
<tr>
<td>Eutrophication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibition of uptake of K;</td>
<td>NH₄/K &lt; 5 mol/mol</td>
<td>17-70b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased susceptibility;</td>
<td>N &lt; 1.8%</td>
<td>21-42b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vegetation changes;</td>
<td>NO₃ &lt; 0.1 mol/m³</td>
<td>7-20b</td>
<td>11-20b</td>
<td></td>
</tr>
<tr>
<td>nitrate pollution</td>
<td>NO₃ &lt; 0.4-0.8 mol/m³</td>
<td>13-21b</td>
<td>24-41b</td>
<td></td>
</tr>
</tbody>
</table>

* Background information on the various criteria is given in De Vries (1993). Critical Al and NO₃⁻ concentrations and critical Al/Ca and NH₄/K ratios related to root damage, inhibition of nutrient uptake and vegetation changes refer to the soil solution. Critical Al and NO₃⁻ concentrations related to pollution refer to phreatic groundwater. Critical nitrogen contents related to an increased risk for frost damage and diseases refer to the foliage.

b Derived by a steady-state model. Al pollution refers to phreatic groundwater. For groundwater used for the preparation of drinking-water, a critical acid load of 1600 mol/ha per year was derived (De Vries, 1993).

c Derived by a steady-state model assuming 50% nitrification in the mineral topsoil (second value).

d Empirical data on the relation between nitrogen deposition and foliar nitrogen contents.

The first value is derived by a steady-state model (worst case) and the second value is based on empirical data.

f Derived by a steady-state model using critical NO₃⁻ concentrations of 0.4 and 0.8 mol/m³, respectively. NO₃⁻ pollution refers to phreatic groundwater. For deep groundwater, the critical load will be higher because of denitrification.
Table 27. Possible and observed effects when critical loads are exceeded

<table>
<thead>
<tr>
<th>Possible effects</th>
<th>Average critical load (kg per ha per year)*</th>
<th>Observed effects in the field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root damage</td>
<td>1.1-1.4 H</td>
<td>critical Al concentrations exceeded greatly</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Al</strong> concentrations exceeded greatly</td>
</tr>
<tr>
<td>Inhibition of uptake</td>
<td>1.1-1.4 H</td>
<td><strong>Al/Ca ratios</strong> exceeded greatly</td>
</tr>
<tr>
<td></td>
<td>17-70 N</td>
<td>critical <strong>NH4/K</strong> ratios exceeded slightly</td>
</tr>
<tr>
<td>Aluminium depletion</td>
<td>1.2-1.3 H</td>
<td>depletion of secondary <strong>Al</strong> compounds</td>
</tr>
<tr>
<td>Groundwater pollution</td>
<td>0.3-0.5 H</td>
<td>critical <strong>Al</strong> concentrations exceeded greatly</td>
</tr>
<tr>
<td></td>
<td>13-21 N</td>
<td>critical <strong>NO3</strong> concentrations exceeded substantially</td>
</tr>
<tr>
<td>Increased susceptibility</td>
<td>21-42 N</td>
<td>critical <strong>N</strong> contents exceeded substantially; nutrient imbalances; increased shoot/root ratios</td>
</tr>
<tr>
<td>Vegetation changes</td>
<td>7-20 N</td>
<td>strong increase in nitrophilous species</td>
</tr>
</tbody>
</table>

* H = acidity; N = total nitrogen
5. STUDIES OF THE EFFECTS OF NITROGEN OXIDES ON EXPERIMENTAL ANIMALS

5.1 Introduction

Most of the data reviewed in this chapter concerns the effects of NO\textsubscript{2}, since the bulk of the NO\textsubscript{2} literature is on NO\textsubscript{2}. The results of the few comparative NO\textsubscript{x} studies suggest that NO\textsubscript{2} is the most toxic species studied so far. Most of the reports describe the effects of NO\textsubscript{2} on the respiratory tract, but extrapulmonary effects are also briefly discussed. A broad range of NO\textsubscript{2} concentrations has been evaluated, but emphasis has been placed primarily on those studies with exposure concentrations of 9400 µg/m\textsuperscript{3} (5.0 ppm) or less, with the exception of studies on dosimetry and emphysema. Discussions of available literature on the effects of other nitrogen compounds, e.g., NO, HNO\textsubscript{3}, and mixtures containing NO\textsubscript{2}, also are included. WHO (1987), Berglund et al. (1993) and US EPA (1993) comprise other reviews of the animal toxicological literature concerning NO\textsubscript{2} effects.

5.2 Nitrogen dioxide

5.2.1 Dosimetry

It is generally agreed that effects of NO\textsubscript{2} observed in several laboratory animal species can be qualitatively extrapolated to humans. However, to extrapolate animal data quantitatively to humans, knowledge of both dosimetry and species sensitivity must be considered. Dosimetry refers to estimating the quantity of NO\textsubscript{2} absorbed by target sites within the respiratory tract. Even when two species receive an identical local tissue/cellular dose, cellular sensitivity to that dose is likely to show interspecies variability due to differences in defence and repair mechanisms and other physiological/metabolic parameters. Current knowledge of dosimetry is more advanced than that of species sensitivity, impeding quantitative animal-to-human extrapolation of effective NO\textsubscript{2} concentrations. Nevertheless, information on dosimetry alone can be crucial to interpretation of the data base. Both theoretical (modelling) and experimental dosimetry studies are discussed below.
5.2.1.1 Respiratory tract dosimetry

The uptake of NO\textsubscript{2} in the upper respiratory tract (above the larynx) has been experimentally studied in dogs, rats and rabbits. The upper airways of dogs and rabbits exposed to 7520 to 77 080 μg/m\textsuperscript{3} (4.0 to 41.0 ppm) NO\textsubscript{2} removed 42.1% of the NO\textsubscript{2} drawn through the nose (Yokoyama, 1968). The uptake of NO\textsubscript{2} by isolated upper respiratory tracts of naive and previously exposed rats (76 000 μg/m\textsuperscript{3}, 40.4 ppm NO\textsubscript{2}) was 28% and 25%, respectively (Cavanagh & Morris, 1987). Kleinman & Mautz (1987) exposed dogs to 1880 or 9400 μg/m\textsuperscript{3} (1.0 or 5.0 ppm) NO\textsubscript{2} and found that more NO\textsubscript{2} was absorbed in the upper respiratory tract with nasal breathing than with oral breathing. In addition, the percentage uptake of NO\textsubscript{2} by the upper respiratory tract decreased with increasing ventilation rates. As ventilation increased up to four times resting values, NO\textsubscript{2} uptake during nasal breathing decreased from approximately 85% to less than 80% and during oral breathing decreased from about 60% to approximately 45%. At rest, about 85% of the inhaled NO\textsubscript{2} entering the lungs was absorbed by the lower respiratory tract; this increased to 100% with high ventilation rates.

Miller et al. (1982) and Overton (1984) modelled NO\textsubscript{2} uptake in the lower respiratory tract using the same dosimetry model described by Miller et al. (1978) for ozone (O\textsubscript{3}), but with the diffusion coefficient and Henry's law constant appropriate to NO\textsubscript{2}; however, values of the latter constant and reaction chemistry were considered uncertain. For all species modelled (i.e., rat, guinea-pig, rabbit and humans), the results indicate that NO\textsubscript{2} is absorbed throughout the lower respiratory tract, but the major dose to tissue is delivered in the centriacinar region (i.e., junction between the conducting and respiratory airways), findings consistent with the site of morphological effects (see section 5.2.2.4).

Total respiratory tract uptake has been measured in healthy and diseased humans. In healthy humans exposed to an NO/NO\textsubscript{2} mixture containing 545 to 13 500 μg/m\textsuperscript{3} (0.29 to 7.2 ppm) NO\textsubscript{2} for brief (but unspecified) periods, 81 to 90% of the NO\textsubscript{2} was absorbed during normal respiration; this increased to 91 to 92% with maximal ventilation (Wagner, 1970). Bauer et al. (1986) exposed adult asthmatics to 564 μg/m\textsuperscript{3} (0.3 ppm) NO\textsubscript{2} via a mouthpiece for 30 min, including 10 min of exercise (30 litres/min) and measured inspired and expired NO\textsubscript{2} concentrations. At rest, the average uptake was 72%; during exercise, the average uptake was 87%, a statistically significant increase. Because of the large
increase in minute ventilation, the deposition was 3.1 µg/min at rest and 14.8 µg/min during exercise.

As discussed above, increased ventilation increases the quantity of NO₂ delivered to the respiratory tract and shifts the site of deposition. Typically, the percentage uptake of NO₂ in the upper respiratory tract decreases, with a consequent increase in uptake by the lower respiratory tract owing to the deeper penetration of the inspired gas with increased tidal volume. These experimental results are qualitatively similar to conclusions for the modelled effects of ventilation on O₃ dosimetry (Miller et al., 1985; Overton et al., 1987a,b).

5.2.1.2 Systemic dosimetry

Once deposited, NO₂ dissolves in lung fluids and various chemical reactions occur, giving rise to products that are found in the blood and other body fluids. Labelled ³⁵NO₂ (564 to 1710 µg/m³, 0.3 to 0.91 ppm) inhaled for 7 to 9 min by rhesus monkeys was distributed throughout the lungs (Goldstein et al., 1977b). These investigators also concluded that NO₂ probably reacts with water in the fluids of the respiratory tract to form nitrous and nitric acids. Saul & Archer (1983) provided support for this pathway using rats inhaling NO₂. This study subsequently led to the discovery of endogenous NO (Moncada et al., 1988, 1991).

The current database indicates that once NO₂ is absorbed in lung fluids, the subsequent reaction products are rapidly taken up and then translocated via the bloodstream. For example, Oda et al. (1981) reported a concentration-dependent increase in both NO₂ and NO₃ levels in the blood of mice during 1-h exposures to 9400 to 75 200 pg/m³ (5.0 to 40.0 ppm) NO₂. The blood levels of NO₂ and NO₃ declined rapidly after exposures ended, with decay half-times of a few minutes for NO₂ and about 1 h for NO₃.

5.2.2 Respiratory tract effects

5.2.2.1 Host defence mechanisms

Respiratory tract defences encompass many interrelated responses; however, for simplicity, they can be divided into physical and cellular defence mechanisms. Physical defence mechanisms include the mucociliary system of the conducting airways. Ciliary action moves particles and dissolved gases within
the mucous layer towards the pharynx, where the mucus is swallowed or expectorated. Both nasal and tracheobronchial regions are immunologically active (e.g., nasal-associated lymphoid tissue and bronchial-associated lymphoid tissue), but this function has not been studied following NO\textsubscript{2} exposure. Cellular defence mechanisms (phagocytic and immunological reactions) operate in the pulmonary region of the lung. Alveolar macrophages (AMs) are the first line of cellular defence. The AMs perform such activities as detoxifying and/or removing inhaled particles, maintaining sterility against inhaled microorganisms, interacting with lymphoid cells in a variety of immunological reactions, and removing damaged or dying cells from the alveoli through phagocytosis. Polymorphonuclear leukocytes (PMNs), another group of phagocytic cells, are present in relatively small numbers (i.e., a small percentage of cells obtained from bronchoalveolar lavage (BAL) fluid) from normal lungs, but in response to a variety of insults, there can be an influx of PMNs from blood into the lung tissues and onto the surface of the airways. Once recruited to the lung, PMNs then ingest and kill opsonized microbes and other foreign substances by mechanisms similar to those for AMs.

The responses of PMNs and AMs are frequently studied using BAL, the washing of the airways and alveolar spaces with saline. Cells and fluid obtained from this procedure can be used in a variety of ways to assess immune responses.

Humoral and cell-mediated immunity are also active in the respiratory tract. The humoral part of this system primarily involves the B cells that function in the synthesis and secretion of antibodies into the blood and body fluids. The cell-mediated component primarily involves T lymphocytes, which are involved in delayed hypersensitivity and defences against viral, fungal, bacterial and neoplastic disease.

a) Mucociliary clearance

Exposure to NO\textsubscript{2} can cause loss of cilia and ciliated epithelial cells, as discussed in section 5.2.2.4 on morphological changes. Such changes are reflected in the functional impairment of mucociliary clearance at high levels of NO\textsubscript{2} (≥ 9400 μg/m\textsuperscript{3}, 5.0 ppm) (Giordano & Morrow, 1972; Kita & Omichi, 1974). At lower exposures (2 h/day for 2, 7 and 14 days to 564 and 1880 μg/m\textsuperscript{3}, 0.3 and 1.0 ppm NO\textsubscript{2}), the mucociliary clearance of inhaled tracer particles deposited in the tracheobronchial tree of rabbits was not altered (Schlesinger et al., 1987).
Structural, biochemical, and functional changes in AMs observed in experimental animal studies to be caused by NO\textsubscript{2} exposure are summarized in Table 28. The adversity of these effects is not clearly understood at present, but they are taken as hallmarks of adverse reactions. Studies of AMs in humans are discussed in chapter 6.

Alveolar macrophages isolated from mice continuously exposed to 3760 \(\mu g/m^3\) (2.0 ppm) NO\textsubscript{2} or to 940 \(\mu g/m^3\) (0.5 ppm) NO\textsubscript{2} continuously with a 1-h peak to 3760 \(\mu g/m^3\) (2.0 ppm) for 5 days/week showed distinctive morphological changes after 21 weeks of exposure, compared to controls (Aranyi et al., 1976). Structural changes included the loss of surface processes, appearance of fenestrae, bleb formation and denuded surface areas. Continuous exposure to a lower NO\textsubscript{2} level did not result in any significant morphological changes. Numerous morphological studies have shown that NO\textsubscript{2} exposure increases the number of AMs (see section 5.2.2.4).

BAL methods have also been used to study AMs. Mochitate et al. (1986) reported a significant increase in the total number of AMs isolated from rats during 10 days of exposure to 7520 \(\mu g/m^3\) (4.0 ppm) NO\textsubscript{2}, but the number of PMNs did not increase. The AMs from exposed animals also exhibited increased metabolic activity, as measured by the activities of glucose-6-phosphate dehydrogenase, glutathione peroxidase and pyruvate kinase. The AMs also showed an increase in the rate of synthesis of protein and DNA. All responses peaked on day 4 and returned to control levels by the tenth day. Suzuki et al. (1986) made similar observations and, in addition, found that the viability of AMs was decreased on day 1 and remained depressed for the remainder of the exposure period. Increased numbers and metabolic activity of AMs would be expected to have a positive influence on host defences. However, AMs are rich in proteolytic enzymes, and increased numbers could result in some tissue destruction when the enzymes are released. Furthermore, as discussed below, although more AMs may be present, they often have a decreased phagocytic ability.

Schlesinger (1987a,b) found no significant changes in the number or the viability of AMs in BAL from rabbits exposed to 564 or 1880 \(\mu g/m^3\) (0.3 or 1.0 ppm) NO\textsubscript{2}, 2 h/day, for 13 days. Although there were no effects on the numbers of AMs that
Table 28. Effects of nitrogen dioxide (NO₂) on alveolar macrophages

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>564 ppm</td>
<td>0.3</td>
<td>2 h/day, 14 days</td>
<td>Rabbit</td>
<td>Increase in alveolar clearance.</td>
<td>Schlesinger &amp; Gearhart (1987)</td>
</tr>
<tr>
<td>1880 ppm</td>
<td>1.0</td>
<td>14 days</td>
<td>Rabbit</td>
<td>Decreased AM phagocytic capacity at 564 µg/m³; increase at 1880 µg/m³ after 2 days of exposure. No effect on cell number or viability; random mobility reduced at 564 µg/m³ only. No effects from 6 days of exposure on.</td>
<td>Schlesinger (1987a,b)</td>
</tr>
<tr>
<td>564 ppm</td>
<td>0.3</td>
<td>2 h/day, 1 or 14 days</td>
<td>Rabbit</td>
<td>Acceleration in alveolar clearance at ≤ 1880 µg/m³.</td>
<td>Vollmuth et al. (1996)</td>
</tr>
<tr>
<td>1880 ppm</td>
<td>1.0</td>
<td>14 days</td>
<td>Rabbit</td>
<td>No observable effects on AM morphology.</td>
<td>Aranyi et al. (1976)</td>
</tr>
<tr>
<td>564 ppm</td>
<td>3.0</td>
<td>Continuous base with 2 h/day peak (5 days/week), 24 weeks</td>
<td>Rabbit</td>
<td>Morphological changes, such as loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas.</td>
<td>Aranyi et al. (1976)</td>
</tr>
<tr>
<td>940 ppm or 158 base; 1880 peak</td>
<td>0.5 or 0.1 base; 1.0 peak</td>
<td>Continuous base</td>
<td>Mouse</td>
<td>No observable effects on AM morphology.</td>
<td>Aranyi et al. (1976)</td>
</tr>
<tr>
<td>3750 ppm or 2100 base; 3750 peak</td>
<td>2.0 or 0.5 base; 2.0 peak</td>
<td>Continuous base with 7 h/day peak (5 days/week), 21 weeks</td>
<td>Mouse</td>
<td>Morphological changes, such as loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas.</td>
<td>Aranyi et al. (1976)</td>
</tr>
</tbody>
</table>
Table 28 (contd).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Substance</th>
<th>Duration</th>
<th>Dispersion</th>
<th>Route</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1880</td>
<td>Goldstein at al. (1974)</td>
<td>1.0</td>
<td>17 h</td>
<td>Mouse</td>
<td>Bactericidal activity significantly decreased by 5 and 35% at 4320 and 12,400 μg/m³, respectively; no effect at 1880 μg/m³.</td>
<td></td>
</tr>
<tr>
<td>4320</td>
<td>Goldstein et al. (1974)</td>
<td>2.3</td>
<td>4320 and 12,400 μg/m³, respectively</td>
<td>1880</td>
<td>No effect at 1880 μg/m³.</td>
<td></td>
</tr>
<tr>
<td>12,400</td>
<td>Goldstein at al. (1974)</td>
<td>6.6</td>
<td>12,400 μg/m³</td>
<td>1880</td>
<td>No effect at 1880 μg/m³.</td>
<td></td>
</tr>
<tr>
<td>1880 base; 9400 peak</td>
<td>Gregory et al. (1983)</td>
<td>1.0 base; 5.0 peak</td>
<td>7 h/day, 5 days per week base with one 1.5-h peak/day, 15 weeks</td>
<td>Rat</td>
<td>Accumulation of AMs. Superimposed spikes produced changes that may persist with continued exposures.</td>
<td></td>
</tr>
<tr>
<td>4444-31,500</td>
<td>Amoruso et al. (1981)</td>
<td>1.3-17.0</td>
<td>Rat</td>
<td>Decreased production of superoxide anion radical.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>Greene &amp; Schneider (1978)</td>
<td>2.0</td>
<td>8 h/day, 5 days/week, 6 months</td>
<td>Baboon</td>
<td>Impaired AM responsiveness to migration inhibitory factor.</td>
<td></td>
</tr>
<tr>
<td>5640</td>
<td>Dowell et al. (1971)</td>
<td>3.0</td>
<td>3 h</td>
<td>Rabbit</td>
<td>Increased swelling of AMs.</td>
<td></td>
</tr>
<tr>
<td>6768</td>
<td>Goldstein et al. (1977a)</td>
<td>3.6</td>
<td>2 h</td>
<td>Rat</td>
<td>Enhanced AM agglutination with concanavalin A.</td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>Hootman et al. (1968)</td>
<td>4.0</td>
<td>8 h/day, 7, 14, or 21 days</td>
<td>Rat</td>
<td>Changes in AM morphology; no change in numbers of AMs or phagocytic capacity.</td>
<td></td>
</tr>
</tbody>
</table>
Table 28 (contd).

<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ug/m$^3$</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects$^b$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>10 days</td>
<td>Rat</td>
<td>Increase in number of AMs; no increase in PMNs; increased metabolic activity, protein and DNA synthesis; all responses peaked on day 4 and returned to normal on day 10.</td>
<td>Mochitate et al. (1986)</td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>Up to 10 days</td>
<td>Rat</td>
<td>Increase in number of AMs, reaching a peak on days 3 and 5; no increase in number of PMNs; decrease in AM viability throughout exposure period. Suppression of phagocytic activity on day 7 that returned to normal value at day 10. Decrease in superoxide radical production on days 3, 5 and 10.</td>
<td>Suzuki et al. (1986)</td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>7 days</td>
<td>Mouse</td>
<td>No effect on phagocytic activity.</td>
<td>Lefkowitz et al. (1986)</td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3 h</td>
<td>Rabbit</td>
<td>No change in AM resistance to pox virus.</td>
<td>Acton &amp; Myrvik (1972)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Modified from US EPA (1993)
$^b$ AM = alveolar macrophage; PMN = polymorphonuclear leukocyte
phagocytosed latex spheres, 2 days of exposure to 564 μg/m³ (0.3 ppm) decreased the phagocytic capacity (i.e., number of spheres per cell); the higher level of NO₂ increased phagocytosis. Longer exposures had no effect. The phagocytic activity of rat AMs was significantly depressed after 7 days of exposure to 7520 μg/m³ (4.0 ppm) but returned to the control value at 10 days of exposure (Suzuki et al., 1986). There may be a species difference in responsiveness because Lefkowitz et al. (1986) did not observe a depression in phagocytosis in mice exposed for 7 days to 9400 μg/m³ (5.0 ppm) NO₂. Suzuki et al. (1986) proposed that the inhibition of phagocytosis might be due to NO₂ effects on membrane lipid peroxidation. Studies by Dowell et al. (1971) and Goldstein et al. (1977a) add support to this hypothesis. Acute exposure to 5640–7520 μg/m³ (3.0–4.0 ppm) caused swelling of AMs (Dowell et al., 1971) and increased AM agglutination with concanavalin A (Goldstein et al., 1977a), suggesting damage to the membrane function.

Two independent studies have shown that NO₂ exposure decreases the ability of rat AMs to produce superoxide anion involved in antibacterial activity. Amoruso et al. (1981) presented evidence of such an effect at NO₂ concentrations ranging from 2440 to 32 000 μg/m³ (1.3 to 17.0 ppm). The duration of the NO₂ exposure was not given; all exposures were expressed in terms of parts per million × hours. A 50% decrease of superoxide anion production began after exposure to 54 700 μg/m³ × h (29.1 ppm × h) NO₂. Suzuki et al. (1986) reported a marked decrease in the ability of rat AMs to produce superoxide anion following a 10-day exposure to either 7520 or 15 000 μg/m³ (4.0 or 8.0 ppm) NO₂. At the highest concentration, the effect was significant each day, but at the lower concentration, the depression was significant only on exposure days 3, 5 and 10.

Alveolar macrophages obtained by BAL from baboons exposed to 3760 μg/m³ (2.0 ppm) NO₂ for 8 h/day, 5 days/week, for 6 months had impaired responsiveness to migration inhibitory factor produced by sensitized lymphocytes (Greene & Schneider, 1978). This substance affects the behaviour of AMs by inhibiting free migration, which, in turn, interferes with the functional capacity of these defence cells. In addition, the random mobility of AMs was significantly depressed in rabbits following a 2 h/day exposure for 13 days to 564 μg/m³ (0.3 ppm), but not to 1880 μg/m³ (1.0 ppm) (Schlesinger, 1987b).
Vollmuth et al. (1986) studied the clearance of strontium-85-tagged polystyrene latex spheres from the lungs of rabbits following a single 2-h exposure to 564, 1880, 5640 or 18 800 µg/m³ (0.3, 1.0, 3.0 or 10.0 ppm) NO₂. An acceleration in clearance occurred immediately after exposure to the two lowest NO₂ concentrations; a similar effect was found by Schlesinger & Gearhart (1987). At the higher levels of NO₂, an acceleration in clearance was not evident until midway through the 14-day post-exposure period. Repeated exposure for 14 days (2 h/day) to 1880 or 18 800 µg/m³ (1.0 or 10.0 ppm) NO₂ produced a response similar to a single exposure at the same concentration.

c) Humoral and cell-mediated immunity

Various humoral and cell-mediated effects are summarized in Table 29.

Exposing sheep to 9400 µg/m³ (5.0 ppm) NO₂, 1.5 h/day for 10 to 11 days showed that intermittent short-term exposure may temporarily alter the pulmonary immune responsiveness (Joel et al., 1982). One technique commonly used in determining the production of specific antibody-forming cells is to measure the number of plaque-forming cells (PFCs) in the blood or tissues of immunized animals. In this study, the authors assessed immunological response by monitoring the daily output of PFCs in the efferent lymph of caudal mediastinal lymph nodes of sheep immunized with horse erythrocytes (a T-cell dependent antigen). Although the number of animals used was small and the data were not analysed statistically, it would appear that, in the animals that were immunized 2 days (but not 4 days) after NO₂ exposure started, the output of PFC was below control values. Blastogenic responses of T cells from the efferent pulmonary lymph and venous blood also appeared to be decreased.

Hillam et al. (1983) examined the effects of a 24-h exposure to 9400, 18 800 and 48 900 µg/m³ (5.0, 10.0 and 26.0 ppm) NO₂ on cellular immunity in rats after intratracheal immunization with sheep erythrocytes (SRBCs). Cellular immunity was evaluated by antigen-specific lymphocyte stimulation assays of pooled lymphoid cell suspensions from either the thoracic lymph nodes or the spleen. Concentration-related elevation of cellular immunity in thoracic lymph nodes and spleen were reported after immunizing the lung with SRBCs.
Fujimaki et al. (1982) investigated the effect of a 4-week exposure to 752 and 3000 µg/m³ (0.4 and 1.6 ppm) NO₂ in mice (i.e., primary and secondary antibody response to SRBCs, using the splenic PFC response as the end-point). The primary PFC response was decreased by both NO₂ concentrations. Secondary antibody response was not affected at 752 µg/m³ (0.4 ppm), but was slightly enhanced at 3000 µg/m³ NO₂. Acute exposure (12 h) of mice to 9400 µg/m³ (5.0 ppm) NO₂ caused no such effects (Fujimaki & Shimizu, 1981; Fujimaki et al., 1981).

The effect of exposure pattern was examined by Maigetter et al. (1978) by exposing mice for up to 1 year to 940 µg NO₂/m³ (0.5 ppm) continuously or to three regimens having a continuous baseline of 188 µg/m³ (0.1 ppm) with 3-h peaks (5 days/week) of either 470, 940 or 1880 µg/m³ (0.25, 0.5 or 1.0 ppm). General mitogenic responses of splenic lymphocytes to phytohaemagglutinin (PHA) (a T cell dependent mitogen) and lipopolysaccharide (a B-cell dependent mitogen) decreased, but this was not related to the concentration or duration of exposure, with a single exception. The decrease in PHA-induced mitogenesis was linearly related to the increased duration of NO₂ exposure to 940 µg/m³ (0.5 ppm).

Shorter exposure (6 days) to 9400 µg/m³ (5.0 ppm) NO₂ did not affect mitogenesis of T cells (Lefkowitz et al., 1986). Although NO₂ did not affect haemagglutination antibody titres, it did reduce the number of splenic PFCs to SRBCs. The authors stated (data were not shown) that mice exposed to 2820 µg/m³ (1.5 ppm) NO₂ for 14 or 21 days showed a 33 and 50% decrease, respectively, in the number of PFCs.

Kosmider et al. (1973) exposed guinea-pigs to 1880 µg/m³ (1.0 ppm) NO₂ for 6 months and reported a significant reduction in all serum immunoglobulin (Ig) fractions and complement. Decreased levels of these substances may lead to an increase in the frequency, duration and severity of an infectious disease. Mice exposed to NO₂ had decreased serum levels of IgA and exhibited nonspecific increases in serum IgM, IgG and IgG₂ (Ehrlich et al., 1975).

These effects on lymphocyte function may reflect changes in lymphocyte populations. Richters & Damji (1988) found that the percentage of the total T lymphocyte population was reduced in the spleens of AKR/cum mice exposed for 7 weeks (7 h/day, 5 days/week) to 470 µg/m³ (0.25 ppm) NO₂. The percentages of
<table>
<thead>
<tr>
<th>NO₂ concentration (ppm)</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>188 base, 0.1 base, 0.25, 0.5, or 0 peak peak</td>
<td>Continuous base; 0.1 base; 0.25, 0.5, or 0 peak peak</td>
<td>Mouse</td>
<td>Suppression of splenic T and B cell responsiveness to mitogens variable and not related to concentration or duration, except for the 940 ppm continuous group, with a linear decrease in PHA-induced mitogenesis with exposure duration.</td>
</tr>
<tr>
<td>470 or 940</td>
<td>7 h/day, 5 days/week, 6 weeks to 36 weeks</td>
<td>Mouse</td>
<td>Reduced percentage of total T cell population and trend towards reduced percentages of certain T cell subpopulations. No reduction of mature T cells or natural killer cells.</td>
</tr>
<tr>
<td>658</td>
<td>7 h/day, 5 days/week, 12 weeks</td>
<td>Mouse</td>
<td>Trend towards suppression in total percentage of T cells. No effects on percentages of other T cell subpopulations.</td>
</tr>
</tbody>
</table>

Reference: Richters & Damji (1988)
Table 29 (contd).

<p>| 752 | 0.4 | 24 h/day | Mouse | Decrease in primary PFC response at ≥752 μg/m³. Increase in secondary PFC response at 3010 μg/m³. | Fujimaki et al. (1982) |
| 3010 | 1.6 | 4 weeks | No effect on splenic or circulatory B or T cell response to mitogens. After 3 weeks of exposure only, decrease in splenic natural killer cell activity. No histological changes in lymphoid tissues. | Selgrade et al. (1991) |
| 940 base; 2820 peak | 0.5 base; 1.5 peak | 22-h/day base (7 days/week); 6-h ramped peak (5 days/week) | 1, 3, 13, 52, 78 weeks | No effect on splenic or circulatory B or T cell response to mitogens. After 3 weeks of exposure only, decrease in splenic natural killer cell activity. No histological changes in lymphoid tissues. | Selgrade et al. (1991) |
| 940 base, 3760 peak | 0.5 base, 2.0 peak | Continuous base with 1 h/day peak, 3 months | Mouse | Vaccination with influenza A2/Taiwan virus after exposure. Decrease in serum neutralizing antibody; haemagglutination inhibition titres unchanged. Before virus challenge, NO₂ exposure decreased serum IgA and increased IgG₁, IgM, and IgG₂ after virus, serum IgA unchanged and IgM increased. | Ehrlich et al. (1973) |
| 3760 | 2.0 | | | | |
| 1880 | 1.0 | 493 days | Monkey | Monkeys challenged five times with monkey-adapted influenza virus during NO₂ exposure. Haemagglutination inhibition antibody titres not altered. Compared to controls, NO₂ caused an earlier and greater increase in serum neutralization antibody titres to the virus. | Fenters et al. (1973) |</p>
<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³  ppm Exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1880 1.0 6 months</td>
<td>Guinea-pig</td>
<td>Inhala/c challenge with K. pneumoniae after exposure. Decreased haemolytic activity of complement; decrease in all immunoelectrophoretic fractions.</td>
<td>Kosmider et al. (1973)</td>
</tr>
<tr>
<td>2820 1.5 24 h/day, 6, 14, or 21 days</td>
<td>Mouse</td>
<td>Reduction in number of splenic PFCs; lowering concentration to 2820 µg/m³ and extending the length to 14 or 21 days decreased PFCs by 33 and 50%, respectively; no effect on cell-mediated immune system or haemagglutination titres.</td>
<td>Lefkowitz et al. (1986)</td>
</tr>
<tr>
<td>9400 5.0 1.5 h/day, 10-11 days</td>
<td>Sheep</td>
<td>Reduction in PFCs from pulmonary lymph and in mitogenesis of T cells from pulmonary lymph and blood.</td>
<td>Joel et al. (1982)</td>
</tr>
<tr>
<td>9400 5.0 4 h/day, 5 days/week, 5.52 months</td>
<td>Guinea-pig</td>
<td>Serum antibodies against lung tissue increased with concentration and duration of exposure.</td>
<td>Balchum et al. (1965)</td>
</tr>
</tbody>
</table>
Table 29 (contd).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration</th>
<th>Species</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Continuous, 169 days, challenged 4x with mouse-adapted influenza virus</td>
<td>Monkey Initial depression in serum neutralization titres with return to normal by day 133; no effect on secondary response on haemagglutinin inhibition titre.</td>
<td>Fenters et al. (1971)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3-7 days</td>
<td>Mouse No effect on serum interferon levels.</td>
<td>Lefkowitz et al. (1963, 1964)</td>
</tr>
<tr>
<td>47 000</td>
<td>25.0</td>
<td>24 h</td>
<td>Rat Concentration-related elevation of cellular immunity in thoracic lymph nodes and spleen after immunizing the lung with sheep RBCs.</td>
<td>Hillam et al. (1983)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Continuous, 6 months</td>
<td>Monkey Depressed postvaccination serum neutralizing antibody formation.</td>
<td>Ehrlich &amp; Fenters (1973)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>12 h</td>
<td>Mouse No effect on primary and secondary splenic PFC response.</td>
<td>Fujimaki &amp; Shimizu (1981); Fujimaki et al. (1981)</td>
</tr>
</tbody>
</table>

* Source: Modified from US EPA (1993)

** PFC = plaque-forming cell; PHA = phytohaemagglutinin; Ig = immunoglobulin; RBCs = red blood cells
mature helper/inducer T and T cytotoxic/suppressor lymphocytes were also lower in the spleens of exposed animals. There were no changes in the percentages of natural killer cells or mature T cells. Upon a longer (36-week) exposure, Richters & Damji (1990) found that the numbers of T-helper/inducer (CD4⁺) lymphocytes (spleen) were reduced, but no effects were observed on T cytotoxic/suppressor cells. Spontaneously developing lymphomas in NO₂-exposed animals progressed more slowly than those in control animals. This was attributed to the NO₂-induced reduction in the T-helper/inducer lymphocytes. C57BL/6J mice exposed to 658 µg/m³ (0.35 ppm) for 7 h/day, 5 days/week for 12 weeks, also showed a suppression in the percentage of total matured T lymphocytes, but no effect on any specific subpopulation upon longer exposure (36 weeks) to 470 µg/m³ (0.25 ppm) (Richters & Damji, 1988). Selgrade et al. (1991) found that chronic exposure (up to 78 weeks) to an urban pattern of NO₂ (baseline of 940 µg/m³ (0.5 ppm) with a ramped 6-h peak to 2820 µg/m³ (1.5 ppm)) had no effect on splenic or circulating B or T cell mitogenic response. However, there was a transient decrease in splenic natural killer cell activity (at 3 weeks only).

Few studies have been undertaken to assess the effects of NO₂ on interferon production. Mice exposed to either 9400 or 47 000 µg/m³ (5.0 or 25.0 ppm) NO₂ for 3 to 7 days had serum levels of interferon similar to those of controls (Lefkowitz et al., 1983, 1984).

Induction of autoimmunity was suggested by the work of Balchum et al. (1965). Guinea-pigs exposed to 9400 µg/m³ (5.0 ppm) and 28 200 µg/m³ (15.0 ppm) NO₂ had an increase in the titre of serum antibodies against lung tissue, starting after 160 h of NO₂ exposure. These antibody titres continued to increase with NO₂ concentration and duration of exposure.

The impact of NO₂ on the humoral immune response of squirrel monkeys to intratracheally delivered influenza vaccine was studied by Fenters et al. (1971, 1973) and Ehrlich & Fenters (1973). In monkeys exposed for 493 days to 1880 µg/m³ (1.0 ppm) NO₂ and immunized with monkey-adapted virus (A/PR/8/34), the serum neutralizing antibody titres were significantly increased earlier and to a greater degree than those of controls (Fenters et al., 1973; Ehrlich & Fenters, 1973). In monkeys exposed to 9400 µg/m³ (5.0 ppm) NO₂ for a total of 169 days and immunized with mouse-adapted influenza virus (A/PR/8), serum neutralization titres were lower than controls initially; no significant difference was observed by 133 days of exposure (Fenters et al., 1971;
Studies of the Effects of Nitrogen Oxides on Experimental Animals

Ehrlich & Fenters, 1973). In all of these studies, the haemagglutination inhibition antibody titres were not affected. Differences between studies might be due to the difference in the virus used for immunization, along with exposure differences. Also, exposure to 1880 μg/m³ (1.0 ppm) NO₂ may have increased the establishment of infection and the survival of the monkey-adapted virus within the respiratory tract, resulting in an increase in antibody production.

Mice that were vaccinated with influenza virus (A-2/Taiwan/1/64) after 3 months of continuous exposure to 3760 μg/m³ (2.0 ppm) or to 940 μg/m³ (0.5 ppm) NO₂ with a 1-h daily (5 days/week) spike exposure to 3760 μg/m³ (2.0 ppm) had mean serum neutralizing antibody titres that were four-fold lower than those of clean air controls (Ehrlich et al., 1975). The haemagglutination inhibition antibody titres in these animals were unchanged. This agrees with the Fenters et al. (1973) findings in monkeys exposed to 1880 μg/m³ (1.0 ppm) for over 1 year.

d) Interaction with infectious agents

Various experimental approaches have been employed using animals in an effort to determine the overall functional efficiency of the host's pulmonary defences following NO₂ exposure. In the most commonly used infectivity model, animals are exposed to either NO₂ or filtered air. After NO₂ exposure, the treatment groups are combined and exposed briefly to an aerosol of a viable agent, such as Streptococcus sp., Klebsiella pneumoniae, Diplococcus pneumoniae or influenza virus. The animals are then returned to clean air for a holding period (usually 15 days), and the mortality in the NO₂-exposed and the control groups are compared. If host defences are compromised by the NO₂ exposure, mortality rates will be higher (Ehrlich, 1966; Henry et al., 1970; Coffin & Gardner, 1972; Ehrlich et al., 1979; Gardner, 1982).

Although the end-point is mortality, it is a sensitive indicator of the depression of the defence mechanisms used to control infection. Because these specific defence mechanisms are common to laboratory animals and humans, the increased susceptibility to infection can be qualitatively extrapolated to humans, even though mortality would not be an expected outcome in humans receiving appropriate medical treatment. However, different exposure levels of NO₂ and infectious agents may be required to produce changes in human host defences. Effects of NO₂ on pulmonary infectious disease in humans are discussed in chapters 6 and 7. Table 30 summarizes effects of exposure to NO₂ and infectious agents observed in animals.

209
Table 30. Interaction of nitrogen dioxide (NO\textsubscript{2}) with infectious agents

<table>
<thead>
<tr>
<th>NO\textsubscript{2} concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu g/m^3 )</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>100 base, 188 peak</td>
</tr>
<tr>
<td>940 + 1880 peak</td>
</tr>
<tr>
<td>2260 + 4700 peak</td>
</tr>
<tr>
<td>376 base, 1500 peak</td>
</tr>
<tr>
<td>564-940</td>
</tr>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>940 0.5</td>
</tr>
<tr>
<td>940 0.5</td>
</tr>
<tr>
<td>940-1880 0.5-1.0</td>
</tr>
<tr>
<td>18 800 10.0</td>
</tr>
<tr>
<td>940-52 600 0.5-28.0</td>
</tr>
<tr>
<td>$\text{NO}_2$ concentration ($\mu$g/m$^3$)</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>1880-4700</td>
</tr>
<tr>
<td>1880</td>
</tr>
<tr>
<td>2320</td>
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<td>4320</td>
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<td>5640</td>
</tr>
<tr>
<td>1880</td>
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<td>5640</td>
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Table 30 (contd).

<table>
<thead>
<tr>
<th>Base</th>
<th>Duration</th>
<th>Methodology</th>
<th>Species</th>
<th>Mortality</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2820</td>
<td>1.5</td>
<td>Continuous or intermittent (7 h/day), 7 days per week, 2 weeks</td>
<td>Mouse Streptococcus sp.</td>
<td>After 1 week, mortality with continuous exposure was greater than that for intermittent; after 2 weeks, no significant difference between continuous and intermittent exposure.</td>
<td>Gardner et al. (1979)</td>
</tr>
<tr>
<td>6560</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2820 base, 8460 peak</td>
<td>1.5 base, 4.5 peak</td>
<td>Continuous 60 h then peak for 1, 3.5 or 7 h, then continuous 18 h</td>
<td>Mouse Streptococcus sp.</td>
<td>Mortality increased with 3.5- and 7-h single spike when bacterial challenge was immediate, and 18 h after the spike</td>
<td>Gardner (1980); Gardner et al. (1992); Graham et al. (1997)</td>
</tr>
<tr>
<td>8460</td>
<td>4.5</td>
<td>1, 3.5, or 7 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2820</td>
<td>1.5</td>
<td>7 h/day, 4, 5, and 7 days</td>
<td>Mouse Streptococcus sp.</td>
<td>Elevated temperature (32 °C) increased mortality after 7 days.</td>
<td>Gardner et al. (1980)</td>
</tr>
<tr>
<td>NO₂ concentration</td>
<td>2820</td>
<td>1.5</td>
<td>2 h</td>
<td>Mouse</td>
<td>K. pneumoniae</td>
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<tr>
<td>-------------------</td>
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<tr>
<td>4750</td>
<td>2.5</td>
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<td>6580</td>
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<td>9400</td>
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<td>18 800</td>
<td>10.0</td>
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</tr>
<tr>
<td>28 200</td>
<td>15.0</td>
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</tr>
<tr>
<td>3570</td>
<td>1.9</td>
<td>4 h</td>
<td></td>
<td>Mouse</td>
<td>S. aureus</td>
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<td>7140</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td>prior to NO₂ exposure</td>
</tr>
<tr>
<td>13 160</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>17 300</td>
<td>9.2</td>
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</tr>
<tr>
<td>27 800</td>
<td>14.8</td>
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</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>3 h</td>
<td></td>
<td>Mouse</td>
<td>Streptococcus sp.</td>
</tr>
</tbody>
</table>

Table 30 (contd.)
<table>
<thead>
<tr>
<th>Concentration (mg/m³)</th>
<th>Duration</th>
<th>Species</th>
<th>Organism</th>
<th>Effect Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4700</td>
<td>2.5-30.0</td>
<td>Mouse</td>
<td>S. aureus, Pasteurella and Proteus</td>
<td>Concentration-related decrease in bactericidal activity, starting at 7500 µg/m³ with S. aureus when NO₂ exposure was after bacterial challenge; when NO₂ was before bacterial challenge, effect at 18 800 µg/m³. Higher concentration required to affect other organisms. (Jakab, 1987)</td>
</tr>
<tr>
<td>56 400</td>
<td>3.5</td>
<td>Mouse</td>
<td>K. pneumoniae</td>
<td>Increased mortality of all species (Ehrlich, 1975)</td>
</tr>
<tr>
<td>65 830</td>
<td>35.0</td>
<td>Hamster</td>
<td>Proteus</td>
<td>Increased virus susceptibility (Rose et al., 1988)</td>
</tr>
<tr>
<td>94 090</td>
<td>50.0</td>
<td>Squirrel monkey</td>
<td>K. pneumoniae or A/PR/8 influenza virus</td>
<td>Increased viral-induced mortality (1/3); increase in Klebsiella-induced mortality (2/7); no control deaths. (Henry et al., 1970)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Mouse</td>
<td>Cytomegalovirus</td>
<td>Increase in virus susceptibility (Rose et al., 1988)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Squirrel monkey</td>
<td>K. pneumoniae or A/PR/8 influenza virus</td>
<td>Increased viral-induced mortality (1/3); increase in Klebsiella-induced mortality (2/7); no control deaths. (Henry et al., 1970)</td>
</tr>
<tr>
<td>19 000</td>
<td>10.0</td>
<td>Continuous, 1 month</td>
<td>Proteus</td>
<td>Increased virus-induced mortality (5/6) within 2-3 days after infection; no control deaths. Increase in Klebsiella-induced mortality (1/4); no control deaths.</td>
</tr>
</tbody>
</table>
Table 30 (contd).

<table>
<thead>
<tr>
<th>NO_2 concentration</th>
<th>Species</th>
<th>Infective agent</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μg/m³</strong></td>
<td><strong>ppm</strong></td>
<td><strong>Exposure</strong></td>
<td><strong>Species</strong></td>
<td><strong>Infective agent</strong></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>4 h</td>
<td>Mouse</td>
<td>Mycoplasma pulmonis</td>
</tr>
<tr>
<td>19 000</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>2 months</td>
<td>Squirrel monkey</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>65 800</td>
<td>35.0</td>
<td>1 month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94 000</td>
<td>50.0</td>
<td>2 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)
Studies of the Effects of Nitrogen Oxides on Experimental Animals

An enhancement in mortality following exposure to NO$_2$ in combination with a pathogenic microorganism could be due to several factors. Goldstein et al. (1973) showed decreases in pulmonary bactericidal activity following NO$_2$ exposure. In their first experiments, mice breathed aerosols of *Staphylococcus aureus* (*S. aureus*) labelled with radioactive phosphorus and were then exposed to NO$_2$ for 4 h. Physical removal of the bacteria was not affected by any of the NO$_2$ concentrations used up to 27 800 µg/m$^3$ (14.8 ppm). Concentrations ≥ 13 200 µg/m$^3$ (7.0 ppm) NO$_2$ lowered the bactericidal activity by ≥ 7%. Lower concentrations (3570 and 7140 µg/m$^3$ (1.9 and 3.8 ppm)) had no significant effect. In another experiment (Goldstein et al., 1974), mice breathed 1800, 4320 and 12 400 (1.0, 2.3 and 6.6 ppm) NO$_2$ for 17 h and then were exposed to an aerosol of *S. aureus*. Four hours later, the animals were examined for the number of organisms present in the lungs. No difference in the number of bacteria inhaled was found in the NO$_2$-exposed animals. Concentrations of 4320 and 12 400 µg/m$^3$ (2.3 and 6.6 ppm) NO$_2$ decreased pulmonary bactericidal activity by 6 and 35%, respectively, compared to controls. Exposure to 1880 µg/m$^3$ (1.0 ppm) NO$_2$ had no significant effect. Goldstein et al. (1974) hypothesized that the decreased bactericidal activity was due to defects in AM function. Jakab (1987) confirmed these findings and found that the concentration of NO$_2$ required to suppress pulmonary bactericidal activity in mice depended on the specific organism. For example, exposure to ≥ 7520 µg/m$^3$ (≥ 4.0 ppm) NO$_2$ for 4 h after bacterial challenge depressed bactericidal activity against *S. aureus*, but it required a concentration of 18 800 to 37 600 µg/m$^3$ (10.0 to 20.0 ppm) before the lung's ability to kill deposited *Pasteurella* and *Proteus* was impaired. Parker et al. (1989) made similar observations in mice exposed for 4 h to 9400 or 18 800 µg/m$^3$ (5.0 or 10.0 ppm) NO$_2$ and infected with *Mycoplasma pulmonis*. The higher concentration of NO$_2$ increased mortality. Both concentrations: (1) reduced lung bactericidal activity and increased bacterial growth, without affecting deposition or physical clearance; and (2) increased the incidence of lung lesions as well as their severity. Davis et al. (1991) found no effects of lower NO$_2$ concentrations on bactericidal activity using the same model system.

Differences in species susceptibility to NO$_2$ or to a pathogen may play a role in the enhancement of mortality seen in experimental animals. An enhancement in mortality was noted in mice, hamsters and monkeys acutely exposed to NO$_2$ for 2 h followed by a challenge of *K. pneumonia* (Ehrlich, 1975). However, differences in susceptibility were noted between the
species. Ehrlich found increased mortality occurred in monkeys only at 94 000 μg/m³ (50.0 ppm), whereas, lower NO₂ levels increased mortality in mice (6580 μg/m³, 3.5 ppm) and hamsters (65 800 μg/m³, 35.0 ppm). The mouse model was the most sensitive to NO₂ exposure, as shown by enhanced mortality from K. pneumoniae following exposure to 6580 μg/m³ (3.5 ppm) but not to 2820–4700 μg/m³ (1.5–2.5 ppm) NO₂ for 2 h (Purvis & Ehrlich, 1963; Ehrlich, 1975). With prolonged (2 month) exposure, Henry et al. (1969) found that lower levels of NO₂ (9400 μg/m³, 5.0 ppm) increased susceptibility to bacterial infections in monkeys than the 50.0 ppm concentration found to be effective by Ehrlich (1975) with acute (2 h) exposure. The sensitivity is also affected by the test organism. For example, when Streptococcus sp. was the infectious agent, a 3-h exposure to 3760 μg/m³ (2.0 ppm) NO₂ caused an increased in mortality in mice (Ehrlich et al., 1977).

Sherwood et al. (1981) illustrated that exposure to 1880 μg/m³ (1.0 ppm) NO₂ for 48 h increased the propensity of virulent group-C streptococci, but not S. aureus, to proliferate within mouse lungs and cause earlier mortality.

Additional factors can influence the interaction of NO₂ and infectious agents. Mice placed on continuously moving exercise wheels during exposure to 3640 μg/m³ (3.0 ppm) NO₂, but not 1880 μg/m³ (1.0 ppm), for 3 h showed enhanced mortality over non-exercised NO₂-exposed mice using the streptococcal infectivity model (Huling et al., 1980). The presence of other environmental factors, such as O₃ (Ehrlich et al., 1977; Gardner, 1980; Gardner et al., 1982; Graham et al., 1987) or elevated temperatures (Gardner et al., 1982), also exacerbated the effects of NO₂.

The influence of a wide variety of exposure regimens has been evaluated using the infectivity model. For example, Gardner et al. (1977b) examined the effect of varying durations of continuous exposure on the mortality of mice exposed to six concentrations of NO₂ (940 to 52 600 μg/m³ (0.5 to 28.0 ppm)) for durations ranging from 15 min to 1 year. Streptococcus sp. was used for all concentrations, except 940 μg/m³, in which case K. pneumoniae was used. Mortality increased linearly with increasing duration of exposure to a given concentration of NO₂. Mortality also increased with increasing concentration of NO₂ as indicated by the steeper slopes with higher concentrations. When the product of concentration and time (C x T) was held constant, the relationship between concentration and time produced significantly different mortality responses. At a constant C x T of approximately
21 ppm-h, a 14-h exposure to 2820 μg/m³ (1.5 ppm) NO₂ increased mortality by 12.5%, whereas a 1.5-h exposure to 27 300 μg/m³ (14.0 ppm) NO₂ enhanced mortality by 58.5%. These findings demonstrate that concentration is more important than time in determining the degree of injury induced by NO₂ in this model, and they were confirmed at additional C x T values (Gardner et al., 1977a,b, 1982; Coffin et al., 1977).

Gardner et al. (1979) also compared the effect of continuous versus intermittent exposure to NO₂ followed by bacterial challenge with Streptococcus sp. Mice were exposed either continuously or intermittently (7 h/day, 7 days/week) to 2820 or 6580 μg/m³ (1.5 or 3.5 ppm) NO₂. The continuous exposure of mice to 2820 μg/m³ NO₂ increased mortality after 24 h of exposure. During the first week of exposure, the mortality was significantly higher in mice exposed continuously to NO₂ than in those exposed intermittently. By the 14th day of exposure, the difference between intermittent and continuous exposure became indistinguishable. At the higher concentration, there was essentially no difference between continuous and intermittent regimens. This suggests that fluctuating levels of NO₂ may ultimately be as toxic as sustained high levels (Gardner et al., 1979).

Mice were exposed continuously or intermittently (6 or 18 h/day) to 940 μg/m³ (0.5 ppm) NO₂ for up to 12 months (Ehrlich & Henry, 1968). None of the exposure regimens affected resistance to K. pneumoniae infection during the first month. Those exposed continuously exhibited decreased resistance to the infectious agent, as demonstrated by a significant enhancement in mortality at 3, 6, 9 and 12 months. In another experiment, a significant enhancement did not occur at 3 months, but was observed after 6 months of exposure. After 6 months, mice exposed intermittently (6 or 18 h/day) to NO₂ showed significant increases in mortality over controls (18%). Only the continuously exposed animals showed significant mortality (23%) over controls following 12 months of exposure. After 12 months of exposure, mice in the three experimental groups showed a reduced capacity to clear viable bacteria from their lungs. This was first observed after 6 months in the continuously exposed group and after 9 months in the intermittently exposed groups. These changes, however, were not statistically tested for significance. Although it is not possible to compare directly the results of the studies using Streptococcus sp. to those using K. pneumoniae, the data suggest that, as the concentration of NO₂ is decreased, a longer
exposure time is necessary for the intermittent exposure regimen to produce a level of effect equivalent to that of a continuous exposure. McGrath & Oyervides (1985) did not confirm these findings in mice exposed to 940, 1880 and 2820 μg/m³ (0.5, 1.0 and 1.5 ppm) NO₂ for 3 months. The inconsistency may be attributed to the fact that the McGrath & Oyervides (1985) study had 95% mortality in the control groups, making it virtually impossible to detect a further enhancement in mortality due to NO₂.

Gardner (1980), Gardner et al. (1982) and Graham et al. (1987) reported extensive investigations on the response to airborne infections in mice breathing NO₂ spike exposures superimposed on a lower continuous background level of NO₂, which simulated the pattern (although not the NO₂ concentrations) of exposure in the urban environment in the USA. Mice were exposed to spikes of 8460 μg/m³ (4.5 ppm) for 1, 3.5 or 7 h and then were challenged with *Streptococcus* sp. either immediately or 18 h after exposure. Mortality was proportional to the duration of the spike when the mice were challenged with bacteria immediately after exposure, but mice had recovered from the exposure by 18 h. Similar findings were reported by Purvis & Ehrlich (1963) using *K. pneumoniae*. When a spike of 8460 μg/m³ (4.5 ppm) was superimposed on a continuous background of 2820 μg/m³ (1.5 ppm) for 62 h preceding and 18 h following the spike, mortality was significantly enhanced by a spike lasting 3.5 or 7 h when the infectious agent was administered 18 h after the spike (Gardner, 1980; Gardner et al., 1982; Graham et al., 1987). Possible explanations for these differences due to the presence or absence of a background exposure are that mice continuously exposed are not capable of recovery or that new AMs or PMNs recruited to the site of infection are impaired by the continuous exposure to NO₂.

The effect of multiple spikes was examined by exposing mice for 2 weeks to two daily 1-h spikes (morning and afternoon, 5 days/week) of 8460 μg/m³ (4.5 ppm) superimposed on a continuous background of 2820 μg/m³ (1.5 ppm) NO₂. Mice were challenged with the infectious agent either immediately before or after the morning spike. When the infectious agent was given before the morning spike, the increase in mortality did not closely approach that of a continuous exposure to 2820 μg/m³ (1.5 ppm) NO₂. However, in mice challenged after the morning spike, by 2 weeks of exposure, the increased mortality over controls approached that equivalent to continuous exposure to 2820 μg/m³ (1.5 ppm) NO₂. Thus, the magnitude of the effect of the base-plus-spike group, which had a higher C × T than the continuous
groups, did not exceed the effect of the continuous group. These findings demonstrate that the pattern of exposure determines the response and that the response is not predictable based on a simple C X T relationship.

Further investigations into the effects of chronic exposure to NO₂ spikes on murine antibacterial lung defences have been conducted using a spike-to-baseline ratio of 4:1, which is not uncommon in the urban environment in the USA (Miller et al., 1987). For 1 year, mice were exposed 23 h/day, 7 days/week, to a baseline of 376 μg/m³ (0.2 ppm) or to this baseline level on which was superimposed a 1-h spike of 1500 μg/m³ (0.8 ppm) NO₂, twice a day, 5 days/week. The animals exposed to the baseline level did not exhibit any significant effects; however, the streptococcal-induced mortality of the mice exposed to the baseline plus spike regimen was significantly greater than that of either the NO₂-background-exposed mice or the control mice. Human epidemiological studies in chapter 7 indicate increased risk of respiratory infection. Data from experimental animals support the epidemiological responses in humans.

Antiviral defences are also compromised by NO₂. Squirrel monkeys exposed to 9400 or 18 800 μg/m³ (5.0 or 10.0 ppm) NO₂ for 2 or 1 month, respectively, had an increased susceptibility to a laboratory-induced viral influenza infection (Henry et al., 1970). All six animals exposed to the highest concentration died within 2 to 3 days of infection with the influenza virus; at the lower concentration, one out of three monkeys died.

Mice exposed continuously for 3 months to 564–940 μg/m³ (0.3–0.5 ppm) NO₂ followed by a challenge with A/PR/8 influenza virus exhibited significant pulmonary pathological responses (Motomiya et al., 1973). A greater incidence of adenomatous proliferation of bronchial epithelial cells resulted from the combined exposures of virus plus NO₂ than with either the viral or NO₂ exposures alone. Continuous NO₂ exposure for an additional 3 months did not enhance the effect of NO₂ or the subsequent virus challenge.

Ito (1971) challenged mice with influenza A/PR/8 virus after continuous exposure to 940 to 1880 μg/m³ (0.5 to 1.0 ppm) NO₂ for 39 days and to 18 800 μg/m³ (10.0 ppm) NO₂, 2 h daily for 1, 3 and 5 days. Acute and intermittent exposure to 18 800 μg/m³ (10.0 ppm) NO₂ as well as continuous exposure to 940 to 1880 μg/m³ (0.5 to 1.0 ppm) NO₂ increased the susceptibility of mice to influenza virus as demonstrated by increased mortality.
The lower respiratory tract of mice became significantly more susceptible to murine cytomegalovirus infection after 6-h exposures for 6 days to 9400 µg/m³ (5.0 ppm) NO₂ (Rose et al., 1988). No effects occurred at levels ≤ 4700 µg/m³ (2.5 ppm). Exposure to 9400 µg/m³ (5.0 ppm) NO₂ did not significantly alter the course of a parainfluenza (murine sendai virus) infection in mice as measured by the infectious pulmonary virus titres in the lungs. However, this concentration of NO₂, when combined with the virus exposure, did increase the severity of the pulmonary disease process (viral pneumonitis) (Jakab, 1988).

5.2.2 Lung biochemistry

Studies of lung biochemistry in animals exposed to NO₂ have focused on either the putative mechanisms of toxic action of NO₂ or on detection of indicators of tissue and cell damage. One theory of the mechanism underlying NO₂ toxicity is that NO₂ initiates lipid peroxidation in unsaturated fatty acids in membranes of target cells, thereby causing cell injury or death (Menzel, 1976). Another theory is that NO₂ oxidizes water-soluble, low molecular weight reducing substances and proteins, resulting in a metabolic dysfunction that manifests itself in toxicity (Freeman & Mudd, 1981). It is likely that NO₂ acts by both means. Several potential biochemical mechanisms related to detoxification of NO₂ or to responses to NO₂ intoxication have been proposed and summarized below according to impacts on lipids, proteins, and antioxidant metabolism and antioxidants. The following discussion focuses on inhalation studies because they are more interpretable for risk assessment purposes; in vitro exposure studies have been reviewed elsewhere (US EPA, 1993).

a) Lipid peroxidation

Animal toxicology studies evaluating effects of NO₂ on lipid peroxidation are summarized in Table 31.

Lipid peroxidation induced by NO₂ exposure has been detected at exposure levels as low as 75 µg/m³ (0.04 ppm). Lipid peroxidation, measured as ethane exhalation, was detected after 9 months of exposure of rats to 75-750 µg/m³ (0.04–0.4 ppm) (Sagai et al., 1984). Lipid peroxidation has also been evaluated by measuring the content of lipid peroxides or substances reactive to thiobarbituric acid in alveolar lavage fluid and lung tissue after exposure to similar NO₂ concentrations (Ichinose & Sagai, 1982; Ichinose et al., 1983). Acute or subacute exposure to higher
Table 31. Effects of nitrogen dioxide (NO$_2$) on lung lipid metabolism

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu g/m^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.04</td>
<td>Continuous, 9, 18 or 27 months</td>
<td>Rat</td>
<td>Increased TBA products at 7520 $\mu g/m^3$ after 9 months and at $\geq$ 752 $\mu g/m^3$ after 18 months; increased ethane exhalation at all levels. No changes in total lipid, phospholipid, total cholesterol or triglyceride contents.</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>9 and 18 months</td>
<td>Rat</td>
<td>Increased ethane exhalation after 9 and 18 months.</td>
</tr>
<tr>
<td>225</td>
<td>0.12</td>
<td>Continuous, 6, 2 weeks</td>
<td>Rat</td>
<td>Changes in TBA-reactive substances, exhaled ethane and enzyme activities in lung homogenates, dependent on concentration and duration of exposure.</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>1-16 weeks</td>
<td>Rat</td>
<td>Changes in TBA-reactive substances, exhaled ethane and enzyme activities in lung homogenates, dependent on concentration and duration of exposure.</td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td>1-16 weeks</td>
<td>Rat</td>
<td>Changes in TBA-reactive substances, exhaled ethane and enzyme activities in lung homogenates, dependent on concentration and duration of exposure.</td>
</tr>
<tr>
<td>75</td>
<td>0.04</td>
<td>9, 18, 27 months</td>
<td>Rat</td>
<td>Changes in TBA-reactive substances, exhaled ethane and enzyme activities in lung homogenates, dependent on concentration and duration of exposure.</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>27 months</td>
<td>Rat</td>
<td>Changes in TBA-reactive substances, exhaled ethane and enzyme activities in lung homogenates, dependent on concentration and duration of exposure.</td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Sagai et al. (1984)
Ichinose et al. (1983)
Table 31 (contd).

<table>
<thead>
<tr>
<th>NO&lt;sub&gt;2&lt;/sub&gt; concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>752</td>
<td>0.4</td>
<td>4 months</td>
<td>Rat</td>
</tr>
<tr>
<td>2560</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>72 h</td>
<td>Guinea-pig</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5640</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3 h</td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>1 week</td>
<td></td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>Continuous, 2 weeks</td>
<td>Decrease in lecithin synthesis after 1 week; less marked depression after 2 weeks.</td>
</tr>
<tr>
<td>Vitamin E supplement</td>
<td>Animal</td>
<td>Duration</td>
<td>Dose</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>Reduced lipid peroxidation</td>
<td>Rat</td>
<td>4 h/day, 5 days</td>
<td>1.0</td>
</tr>
<tr>
<td>Increased lung wet weight</td>
<td>Rat</td>
<td>Continuous, 5 months</td>
<td>5450</td>
</tr>
<tr>
<td>Decreased saturated fatty acid content of lung tissue</td>
<td>Rat</td>
<td>6 days</td>
<td>2.9</td>
</tr>
<tr>
<td>Increased surface tension of lung lavage fluid</td>
<td>Rat</td>
<td>Continuous, 9 months</td>
<td>1880</td>
</tr>
<tr>
<td>Decreased lung compliance</td>
<td>Rat</td>
<td>1880</td>
<td>1.0</td>
</tr>
<tr>
<td>Elevated thromboxane B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Rabbit</td>
<td>1800 Mg/rm³</td>
<td>3.0</td>
</tr>
<tr>
<td>Depressed thromboxane B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Rabbit</td>
<td>1800 Mg/rm³</td>
<td>3.0</td>
</tr>
<tr>
<td>Decrease in linoleic and linolenic acid content of BAL fluid</td>
<td>Rat</td>
<td>Continuous, 17 days</td>
<td>5450</td>
</tr>
<tr>
<td>Increased TBA reagents with vitamin E deficiency</td>
<td>Rat</td>
<td>Continuous, 5 days</td>
<td>5640</td>
</tr>
</tbody>
</table>

*Modified from US EPA (1993)*

TBA = Thiobarbituric acid; BAL = Bronchoalveolar lavage
concentrations of NO\textsubscript{2} has also been shown to cause a rapid increase in lung peroxide levels in several species.

Lipid peroxidation results in an alteration in phospholipid composition. Exposure of either mice or guinea-pigs to an NO\textsubscript{2} level of 750 \mu g/m\textsuperscript{3} (0.4 ppm) for a week resulted in a decreased concentration of phosphatidyl ethanolamine and a relative increase in the phosphatidyl choline concentration (Sagai et al., 1987).

Several investigators have also demonstrated NO\textsubscript{2}-induced lipid peroxidation in \textit{in vitro} systems. The cell type most commonly used is the endothelial cell from either pig arteries or aorta. Studies using these cell types have recently attempted to relate the effect on lipid metabolism to functional parameters such as membrane fluidity and enzyme activation or inactivation.

Membrane fluidity changes are related to lipid peroxidation. NO\textsubscript{2}-induced changes in membrane fluidity have been demonstrated in alveolar macrophages and endothelial cells in culture. Endothelial cells exposed to a NO\textsubscript{2} level of 9400 \mu g/m\textsuperscript{3} (5 ppm), for instance, exhibit decreased membrane fluidity after 3 h. Thus, NO\textsubscript{2} changes the physical state of the membrane lipids, perhaps through initiating lipid peroxidation, and hence impairs membrane functions (Patel et al., 1988).

Lipid peroxidation can also activate phospholipase activities. Activation of phospholipase A\textsubscript{2} in cultured endothelial cells by NO\textsubscript{2} has been demonstrated. This activation, which is specific for phospholipase A\textsubscript{2}, occurs at an NO\textsubscript{2} concentration of 9400 \mu g/m\textsuperscript{3} (5 ppm) after 40 h of exposure and is speculated to depend on a specific NO\textsubscript{2}-induced increase in phosphatidyl serine in the plasma membranes (Sekharam et al., 1991).

One function of phospholipases is the release of arachidonic acid. The effect of NO\textsubscript{2} on the release and metabolism of arachidonic acid has been studied both \textit{in vivo} and \textit{in vitro}. Both an increase and a decrease in the metabolism of arachidonic acid has been observed in several species. \textit{In vivo} exposure of rats to 18 800 \mu g/m\textsuperscript{3} (10 ppm) for 2 h resulted in decreased levels of prostaglandins E\textsubscript{2} and F\textsubscript{2α}, as well as thromboxane B\textsubscript{2}, in lavage fluid. On the other hand, at an exposure level of 1880 \mu g/m\textsuperscript{3} (1 ppm), the concentrations of thromboxane B\textsubscript{2} were increased (Schlesinger et al., 1990).
**b) Effects on lung proteins and enzymes**

Nitrogen dioxide can cause lung inflammation (associated with concomitant infiltration of serum protein, enzymes and inflammatory cells) and hyperplasia of Type 2 cells. Thus, some changes in lung enzyme activity and protein content may reflect inflammation and/or changes in cell types, rather than direct effects of NO₂ on lung cell enzymes. Some direct effects of NO₂ on enzymes are possible because NO₂ can oxidize various reducible amino acids or side chains of proteins in aqueous solution (Freeman & Mudd, 1981). These effects are summarized in Table 32.

Nitrogen dioxide can increase the protein content of BAL in vitamin-C-deficient guinea-pigs (Sherwin & Carlson, 1973; Selgrade et al., 1981; Hatch et al., 1986; Slade et al., 1989). Selgrade et al. (1981) found effects at NO₂ levels as low as 1880 µg/m³ (1.0 ppm) after a 72-h exposure, but a 1-week exposure to 752 µg/m³ (0.4 ppm) did not increase protein levels. The results of the 1-week exposure apparently conflict with those of Sherwin & Carlson (1973), who found increased protein content of BAL from vitamin-C-deficient guinea-pigs exposed to 752 µg/m³ (0.4 ppm) NO₂ for 1 week. Differences in exposure techniques, protein measurement methods, and/or degree of vitamin C deficiencies may explain the difference between the two studies. Hatch et al. (1986) found that the NO₂-induced increase in BAL protein in vitamin-C-deficient guinea-pigs was accompanied by an increase in lung content of non-protein sulfhydryls and ascorbic acid and a decrease in vitamin E content. The increased susceptibility to NO₂ was observed when lung vitamin C was reduced (by diet) to levels below 50% of normal. A depletion of lung non-protein sulfhydryls also enhances susceptibility to a high level (18 800 µg/m³, 10.0 ppm) of NO₂ (Slade et al., 1989).

The effects of NO₂ on structural proteins of the lungs has been of major interest because elastic recoil is lost after exposure (section 5.2.2.3). Last et al. (1983) examined collagen synthesis rates by lung minces from animals exposed to NO₂. In rats continuously exposed to 9400 to 47 000 µg/m³ (5.0 to 25.0 ppm) NO₂ for 7 days, there was a linear concentration-related increase in collagen synthesis rate. In a subsequent paper, Last & Warren (1987) confirmed that 9400 µg/m³ (5.0 ppm) increased collagen synthesis. Such biochemical changes are typically interpreted as reflecting increases in total lung collagen, which, if sufficient, could result in pulmonary fibrosis after longer periods of exposure. However, such correlations have not been made directly after NO₂ exposure.
Table 32. Effects of nitrogen dioxide (NO₂) on lung proteins and enzymes

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>µg/m³</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.04</td>
<td></td>
<td>Continuous, Rat</td>
<td>NPSHs increased at the 2 higher NO₂ levels after 9 or 18 months; GSH peroxidase activity decreased at 752 µg/m³ after 18 months and at 7520 µg/m³ after 9 or 18 months; GSH reductase activity increased after a 9-month exposure to 7520 µg/m³; G-6-PD was increased after a 9- or 18-month exposure to 7520 µg/m³; no effects on 6-phosphogluconate dehydrogenase, superoxide dismutase, or disulfide reductase; some GSH S-transferases had decreased activities after an 18-month exposure to 752 or 7520 µg/m³.</td>
<td>Segal et al. (1984)</td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>9</td>
<td>and 18 months</td>
<td>Rat</td>
<td>No effect at 752 µg/m³; increase in BAL protein in vitamin-C-depleted but not normal animals at ≥ 1880 µg/m³.</td>
<td>Selgrade et al. (1961)</td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td></td>
<td>Rat</td>
<td>No effect at 752 µg/m³; increase in BAL protein in vitamin-C-depleted guinea-pigs 15-h post-exposure.</td>
<td>Selgrade et al. (1961)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>72 h</td>
<td>Guinea-pig</td>
<td>Guinea-pig</td>
<td>No effect at 752 µg/m³; increase in BAL protein in vitamin-C-depleted guinea-pigs 15-h post-exposure.</td>
<td>Selgrade et al. (1961)</td>
</tr>
<tr>
<td>5640</td>
<td>1.0</td>
<td>9400</td>
<td>3.0</td>
<td>Guinea-pig</td>
<td>Increased BAL protein in vitamin-C-depleted guinea-pigs 15-h post-exposure.</td>
<td>Selgrade et al. (1961)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3 h</td>
<td>Guinea-pig</td>
<td>Guinea-pig</td>
<td>Increased BAL protein in vitamin-C-depleted guinea-pigs 15-h post-exposure.</td>
<td>Selgrade et al. (1961)</td>
</tr>
<tr>
<td>Experiment</td>
<td>Time</td>
<td>Species</td>
<td>Description</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>---------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Guinea-pig</td>
<td>Continuous, no effect on BAL protein in vitamin-C-depleted guinea-pigs.</td>
<td>Sherwin &amp; Carlson (1973)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Guinea-pig</td>
<td>Increase in BAL protein content of guinea-pigs with an unquantified vitamin C deficiency.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>752, 2260, 7520</td>
<td>1 to 14 weeks</td>
<td>Rat</td>
<td>Complex concentration and duration dependence of effects. Example: at 752 µg/m³, cytochrome P-450 levels decreased at 2 weeks, returned to control level by 5 weeks. At 2260 µg/m³, cytochrome P-450 levels decreased initially, increased at 5 weeks, and decreased at 10 weeks. Effects on succinate-cytochrome c reductase also.</td>
<td>Takahashi et al. (1966)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752, 2260, 7520</td>
<td>4 months</td>
<td>Rat</td>
<td>Duration-dependent pattern for increase in activities of antioxidant enzymes; increase, peaking at week 4, and then decreasing; concentration-dependent effects.</td>
<td>Ichinose &amp; Sagai (1982)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752, 2260, 7520</td>
<td>2 weeks</td>
<td>Guinea-pig</td>
<td>No effect on TBA reactants, antioxidants or antioxidant enzyme activities.</td>
<td>Ichinose &amp; Sagai (1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Rat</td>
<td>Decrease in cytochrome P-450 level at ≥ 2260 µg/m³.</td>
<td>Mochitate et al. (1964)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 32 (contd)

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>846</td>
<td>0.45</td>
<td>7 h/day</td>
<td>Mouse</td>
<td>No changes in lung serotonin levels.</td>
<td>Sherwin et al. (1986)</td>
</tr>
<tr>
<td>864</td>
<td>0.47</td>
<td>Continuous, 10, 12, 14 days</td>
<td>Mouse</td>
<td>Increased content of serum proteins in homogenized whole lung tissue.</td>
<td>Sherwin &amp; Layfield (1974)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous, 17 months</td>
<td>Mouse</td>
<td>Decrease in lung GSH peroxidase activity at 1880 µg/m³ in vitamin-E-deficient mice. Increased activity in vitamin-E-supplemented mice at ≤ 940 µg/m³.</td>
<td>Ayaz &amp; Csallany (1978)</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>Continuous</td>
<td>Rat</td>
<td>Activities of GSH reductase and G-6-PD increased at 11 700 µg/m³ per m² proportional to duration of exposure; no effect on GSH peroxidase. No effects at ≤ 4320 µg/m³.</td>
<td>Chow et al. (1974)</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>4 days</td>
<td>Rat</td>
<td>Changes in BAL fluid and lung tissue levels of enzymes early in exposure, resolved by 15 weeks.</td>
<td>Gregory et al. (1983)</td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>3 days</td>
<td>Rat</td>
<td>Decreased superoxide dismutase activity.</td>
<td>Azoulay-Dupuis et al. (1983)</td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td></td>
<td>Guinea-pig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (μg/m³)</td>
<td>Duration</td>
<td>Species</td>
<td>Effect</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
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<td>--------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>Rat</td>
<td>Continuous, increased activities of several glycolytic enzymes. At ≤ 7520 μg/m³, pyruvate kinase increased on days 4 and 7; recovery occurred by day 14. G-6-PD increased at all levels; at 3760 μg/m³, 14 days of exposure needed.</td>
<td>Mochitate et al. (1985)</td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>Rat</td>
<td>7, 10, 14 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td>Rat</td>
<td>Increased lung protein content; increase in microsomal succinate cytochrome c reductase activity.</td>
<td>Mochitate et al. (1964)</td>
<td></td>
</tr>
<tr>
<td>5640</td>
<td>3.0</td>
<td>Rat</td>
<td>Various changes in lung homogenate protein and DNA content and enzyme activities; changes more severe in vitamin-E-deficient rats.</td>
<td>Elsayed &amp; Mustafa (1982)</td>
<td></td>
</tr>
<tr>
<td>5640</td>
<td>3.0</td>
<td>Rat</td>
<td>1-7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Rat</td>
<td>No effects on antioxidant metabolism or oxygen consumption enzymes at ≤ 9400 μg/m³.</td>
<td>Mustafa et al. (1979)</td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>Rat</td>
<td>7, 14 and 21 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td>Rat</td>
<td>Increased gamma-glutamyl transferase on days 14 and 21; no consistent effect on alkaline phosphatase, lactate dehydrogenase or total protein.</td>
<td>Hoofman et al. (1966)</td>
<td></td>
</tr>
<tr>
<td>47 000</td>
<td>25.0</td>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9020</td>
<td>4.6</td>
<td>Guinea-pig</td>
<td>Increased BAL protein content in vitamin-C-deficient guinea-pigs.</td>
<td>Hatch et al. (1986)</td>
<td></td>
</tr>
<tr>
<td>8450</td>
<td>4.5</td>
<td>Guinea-pig</td>
<td>3 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 h</td>
<td></td>
<td>Increased lung wet weight, alterations in lung antioxidant levels in vitamin-C-deficient guinea-pigs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-------------------</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/m³</td>
<td>ppm</td>
<td>Exposure</td>
<td>Species</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>9020</td>
<td>4.8</td>
<td>7 days</td>
<td>Mouse</td>
<td>No significant changes in lung homogenate parameters.</td>
<td>Mustafa et al. (1984)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>14-72 h</td>
<td>Mouse</td>
<td>Increase in lung protein (14 to 56 h) by radioactive label incorporation.</td>
<td>Csallany (1975)</td>
</tr>
<tr>
<td>9400-47 000</td>
<td>5.0-25.0</td>
<td>Continuous, 7 days</td>
<td>Rat</td>
<td>Concentration-related increase in rate of collagen synthesis; 125% increase at 9400 µg/m³.</td>
<td>Last et al. (1983)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3 h</td>
<td>Rabbit</td>
<td>Benzo[a]pyrene hydroxylase activity of tracheal mucosa not affected.</td>
<td>Palmer et al. (1972)</td>
</tr>
<tr>
<td>37 600</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94 000</td>
<td>50.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

** NPSHs = Non-protein sulfhydryls; GSH = Glutathione; G-6-PD = Glucose-6-phosphate dehydrogenase; BAL = Bronchoalveolar lavage
Alterations in lung xenobiotic metabolism follow a complex duration of exposure pattern in rats exposed to 752, 2260 and 7520 μg/m³ (0.4, 1.2 and 4.0 ppm) NO₂ (Takahashi et al., 1986). At the lowest NO₂ concentration tested, cytochrome P-450 levels decreased initially (at 2 weeks) and then returned to control levels by 5 weeks, where they remained throughout exposure. At 2260 μg/m³ (1.2 ppm), cytochrome P-450 levels decreased initially, then increased after 5 weeks of exposure and decreased again by 10 weeks. A similar pattern of response occurred at the highest concentration. Only 7520 μg/m³ (4.0 ppm) NO₂ affected other microsomal electron-transport systems. The activity of succinate-cytochrome c reductase was decreased by 14 weeks of exposure to 752 μg/m³ (0.4 ppm), but at the higher NO₂ levels, the activity was decreased sooner. In contrast, Mochitate et al. (1984) also found a decrease in levels of cytochrome P-450 at ≥ 2260 μg/m³ (1.2 ppm) in rats exposed for 7 days.

Glycolytic pathways are also increased by NO₂ exposure, apparently due to a concurrent increase in Type 2 cells (Mochitate et al., 1985). The most sensitive enzyme was pyruvate kinase, exhibiting an increased activity after a 14-day exposure to 3760 μg/m³ (2.0 ppm) NO₂. At higher NO₂ concentrations (e.g., 7520 μg/m³, 4.0 ppm), pyruvate kinase activity increased sooner (4 and 7 days) and then decreased to control levels by 14 days.

c) Antioxidant defence systems

Since NO₂ is an oxidant and lipid peroxidation is believed to be a major molecular event responsible for the toxic effects of NO₂, much attention has been focused on the effect of the antioxidant defence system in the epithelial lining fluid and in pulmonary cells. Investigations with subacute and chronic NO₂ exposure levels of 75 to 62040 μg/m³ (0.04-33 ppm) have been performed both in vivo and in vitro and focused on effects on low molecular weight antioxidants such as glutathione, vitamin E and vitamin C, as well as on some enzymes involved in the synthesis and catabolism of glutathione. Experiments made in vitro using human plasma have shown a rapid depletion of vitamin C and glutathione and a loss of vitamin E. This result was achieved with a concentration of 26320 μg/m³ (14 ppm) (Halliwell et al., 1992).

Menzel (1970) proposed that antioxidants might protect the lung from NO₂ damage by inhibiting lipid peroxidation. Data related to this hypothesis have been reported (Thomas et al., 1968; Menzel et al., 1972; Fletcher & Tappel, 1973; Csallany, 1975; Ayaz
Several laboratories have observed changes in the activity of enzymes in the lungs of NO₂-exposed animals that regulate levels of glutathione (GSH), the major water-soluble reductant in the lung. Chow et al. (1974) exposed rats to 1880, 4320 or 11 700 µg/m³ (1.0, 2.3 or 6.2 ppm) NO₂ continuously for 4 days to examine the effect on activities of GSH reductase, glucose-6-phosphate dehydrogenase and GSH peroxidase in the soluble fraction of exposed rat lungs. Linear regression analysis of the correlation between the NO₂ concentration and enzymatic activity showed a significant positive correlation coefficient of 0.63 for GSH reductase and of 0.84 for glucose-6-phosphate dehydrogenase. No correlation was found between the GSH peroxidase activity and the NO₂ concentration. The activities of GSH reductase and glucose-6-phosphate dehydrogenase were significantly increased during exposure to 11 700 µg/m³ (6.2 ppm) NO₂. GSH peroxidase activity was not affected. The possible role of oedema and cellular inflammation in these findings was not examined. These researchers concluded that after a slightly longer exposure (14 days), 3760 µg/m³ (2.0 ppm) NO₂ increased the activity of glucose-6-phosphate dehydrogenase in rats (Mochitate et al., 1985). There is evidence from recent studies that glutathione and vitamins C and E are all involved in normal protection of the lung from NO₂ (Rietjens et al., 1986; Hatch et al., 1986; Slade et al., 1989).

Sagai et al. (1984) studied the effects of prolonged (9 and 18 months) exposure to 75, 752 and 7520 µg/m³ (0.04, 0.4 and 4.0 ppm) NO₂ on rats. After both exposure durations, non-protein sulphydryl levels were increased at ≥ 752 µg/m³; exposure to 7520 µg/m³ (4.0 ppm) decreased the activity of GSH peroxidase and increased glucose-6-phosphate dehydrogenase activity. Glutathione peroxidase activity was also decreased in rats exposed to 752 µg/m³ NO₂ for 18 months. Three GSH S-transferases were also studied, two of which (aryl S-transferase and aralkyl S-transferase) exhibited decreased activities after 18 months of exposure to ≥ 752 µg/m³ NO₂. No effects were observed on the activities of 6-phosphogluconate dehydrogenase, superoxide dismutase or disulfide reductase. When effects were observed, they followed a concentration and exposure-duration response function. The decreases in antioxidant metabolism were inversely related to the apparent formation of lipid peroxides (see lipid peroxidation subsection). Shorter exposures (4 months) to NO₂ between 752 and 7520 µg/m³ (0.4 and 4.0 ppm) also caused concentration- and duration-dependent effects on antioxidant enzyme activities (Ichinose & Sagai, 1982). For example,
glucose-6-phosphate dehydrogenase increased, reaching a peak at 1 month, and then decreased towards the control value. Briefer (2-week) exposures to 752 μg/m³ (0.4 ppm) NO₂ caused no such effects in rats or guinea-pigs (Ichinose & Sagai, 1989).

Ayaz & Csallany (1978) exposed vitamin-E-deficient and vitamin-E-supplemented mice continuously for 17 months to 940 or 1880 μg/m³ (0.5 or 1.0 ppm) NO₂ and assayed them for GSH peroxidase activity. Exposure to 1880 μg/m³ (1.0 ppm) NO₂ decreased enzyme activity in the vitamin-E-deficient mice. However, in vitamin-E-supplemented mice, GSH peroxidase activity increased at 940 μg/m³ (0.5 ppm) NO₂.

5.2.2.3 Pulmonary function

Animal studies of NO₂ effects on pulmonary function are summarized in Table 33. NO₂ concentrations in many urban areas of the USA and elsewhere consist of spikes superimposed on a relatively constant background level. Miller et al. (1987) evaluated this urban pattern of NO₂ exposure in mice using continuous 7 days/week, 23 h/day exposures to 376 μg/m³ (0.2 ppm) NO₂ with twice daily (5 days/week) 1-h spike exposures to 1500 μg/m³ (0.8 ppm) NO₂ for 32 and 52 weeks. Mice exposed to clean air and to the constant background concentration of 376 μg/m³ (0.2 ppm) served as controls. Vital capacity tended to be lower (p = 0.054) in mice exposed to NO₂ with diurnal spikes than in mice exposed to air. Lung distensibility, measured as respiratory system compliance, also tended to be lower in mice exposed to diurnal spikes of NO₂ compared with constant NO₂ exposure or air exposure. These changes suggest that up to 52 weeks of low-level NO₂ exposure with diurnal spikes may produce a subtle decrease in lung distensibility, although part of this loss in compliance may be a reflection of the reduced vital capacity. Vital capacity appeared to remain suppressed for at least 30 days after exposure. Lung morphology in these mice was evaluated only by light microscopy (a relatively insensitive method) and showed no exposure-related lesions. The decrease in lung distensibility suggested by this study is consistent with the thickening of collagen fibrils in monkeys (Bils, 1976) and the increase in lung collagen synthesis rates of rats (Last et al., 1983) after exposure to higher levels of NO₂.

Tepper et al. (1993) exposed 60-day-old rats to 940 μg/m³ (0.5 ppm) NO₂, 22 h/day, 7 days/week, with a 2-h spike of 2820 μg/m³ (1.5 ppm) NO₂, 5 days/week for up to 78 weeks. There
Table 33. Effects of nitrogen dioxide (NO$_2$) on pulmonary function

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>376</td>
<td>0.2</td>
<td>23 h/day base (7 days/week), 1-h peaks twice/day, 1500 peak 0.8 base, 32 and 52 weeks</td>
<td>Mouse</td>
<td>Decreased vital capacity following base + spike NO$_2$ exposures compared with control and base NO$_2$ exposures. Tendency toward decreased respiratory system compliance following spike NO$_2$ exposures compared and control and base NO$_2$ exposures.</td>
<td>Miller et al. (1987)</td>
</tr>
<tr>
<td>940 base, 2820 peak</td>
<td>0.5 base, 1.5 peak</td>
<td>23 h/day (7 days/week) base, 1-h peaks twice/day, 3-week, but not 6-week, exposure to the two higher exposure levels. Decreased body weight and lung compliance in adult rats following 6-week exposure to 3760 µg/m$^3$ + spike. Adults recovered 3 weeks after exposure.</td>
<td>Rat (1-day and 7-weeks old)</td>
<td>Increased lung volume and compliance in neonates following 3-week, but not 6-week, exposure to the two higher exposure levels. Decreased body weight and lung compliance in adult rats following 6-week exposure to 3760 µg/m$^3$ + spike. Adults recovered 3 weeks after exposure.</td>
<td>Stevens et al. (1999)</td>
</tr>
</tbody>
</table>
Table 33 (contd).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Duration</th>
<th>Species</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 base, 2820 peak</td>
<td>22 h/day (7 days per week), 2-h peak (5 days/week); 1, 3, 12, 52 and 78 weeks</td>
<td>Rat</td>
<td>Decreased ∆FEF₂₅ and frequency of breathing following 78-week NO₂ exposure.</td>
<td>Tepper et al. (1993)</td>
</tr>
<tr>
<td>3750</td>
<td>8 h/day, 5 days/week, 8 weeks</td>
<td>Hamster</td>
<td>No change in vital capacity or lung compliance following NO₂ exposures in both normal and elastase-treated animals.</td>
<td>Lafuma et al. (1987)</td>
</tr>
<tr>
<td>10 200</td>
<td>3 h/day for 7, 14 or 30 days</td>
<td>Rat</td>
<td>Tendency toward increased lung volume at low inflation pressures.</td>
<td>Yokoyama et al. (1983)</td>
</tr>
</tbody>
</table>


PaO₂ = Arterial oxygen tension; ∆FEF₂₅ = Change in forced expiratory flow at 25% of forced vital capacity; PaCO₂ = Arterial carbon dioxide tension.
Nitrogen Oxides were no effects on pulmonary function between 1 and 52 weeks of exposure. Following 78 weeks of exposure, flow at 25% forced vital capacity was decreased, perhaps indicating airway obstruction. A significant decrease in the frequency of breathing was also observed at 78 weeks that was paralleled by a trend toward increased expiratory resistance and expiratory time. Taken together, these results suggest that few, if any, significant effects were seen that suggest incipient lung degeneration.

The age sensitivity to exposure to diurnal spikes of NO₂ was studied by Stevens et al. (1988), who exposed 1-day- and 7-week-old rats to continuous baselines of 940, 1880 and 3760 µg/m³ (0.5, 1.0 and 2.0 ppm) NO₂ with twice daily 1-h spikes at 3 times these baseline concentrations for 1, 3 and 7 weeks. In neonatal rats, vital capacity and respiratory system compliance increased following 3 weeks, but not 6 weeks, of exposure to the 1880 and 3760 µg/m³ NO₂ baselines with spikes. In young adult rats, respiratory system compliance decreased following 6 weeks of exposure, and body weight decreased following 3 and 6 weeks of exposure to the 3760 µg/m³ baseline with spike. In the young adult rats, pulmonary function changes returned to normal values 3 weeks after exposure ceased. A correlated morphometric study (Chang et al., 1986) is summarized in section 5.2.2.4.

Lafuma et al. (1987) exposed 12-week-old hamsters with and without laboratory-induced (elastase) emphysema to 3760 µg/m³ (2.0 ppm) NO₂, 8 h/day, 5 days/week for 8 weeks. Vital capacity and pulmonary compliance were not affected by NO₂ exposure.

5.2.2.4 Morphological studies

Inhalation of NO₂ produces morphological alterations in the respiratory tract, as summarized in Tables 34 and 35. This discussion is generally limited to those studies using NO₂ levels ≤ 9400 µg/m³ (5.0 ppm), but results of studies of emphysema conducted at higher concentrations are also discussed. Examination of the tables shows variability in responses at similar exposure levels in different studies. This may be due to differences in animal species or strain, age, diet, microbiological status of the animals, or aspects of experimental protocol. The latter includes the methodology used to evaluate the morphological response. For example, simple light microscopic examination may reveal no effect, whereas other techniques, such as quantitative morphological (morphometric) procedures with electron microscopy, can detect more subtle structural changes.

238
There is a large degree of interspecies variability in responsiveness to NO₂. This is clearly evident from those few studies where different species were exposed under identical conditions (Wagner et al., 1965; Furiosi et al., 1973; Azoulay-Dupuis et al., 1983). Variability in response may be due to differences in effective dose of NO₂ reaching target sites, but other species differences are likely to play a role. Guinea-pigs, hamsters, and monkeys all appear to be more severely affected morphologically by equivalent exposure to NO₂ than are rats, the most commonly used experimental animal. However, in most cases, similar types of histological lesions are produced when similar effective concentrations are used.

a) Sites affected and time course of effects

The anatomic region most sensitive to NO₂ and within which injury is first noted is the centriacinar region. This region includes the terminal conducting airways (terminal bronchioles), respiratory bronchioles, and adjacent alveolar ducts and alveoli. Within this region, those cells that are most sensitive to NO₂-induced injury are the ciliated cells of the bronchiolar epithelium and the Type I cells of the alveolar epithelium, which are then replaced with non-ciliated bronchiolar (Clara) cells and Type 2 cells, respectively. In addition to these dynamic changes, permanent alterations resembling emphysema-like disease may result from chronic exposure.

The temporal progression of early events due to NO₂ exposure has been described best in the rat (e.g., Freeman et al., 1966, 1968c, 1972; Stephens et al., 1971a, 1972; Evans et al., 1972, 1973a,b, 1974, 1975, 1976, 1977; Cabral-Anderson et al., 1977; Rombout et al., 1986) and guinea-pig (Sherwin et al., 1973). The earliest alterations resulting from exposure to concentrations of ≥ 3760 μg/m³ (2.0 ppm) are seen within 24 to 72 h of exposure and include increased AM aggregation, desquamation of Type I cells and ciliated bronchiolar cells, and accumulation of fibrin in small airways. However, repair of injured tissue and replacement of destroyed cells can begin within 24 to 48 h of continuous exposure. Hyperplasia of nonciliated bronchiolar (Clara) cells occurs in the bronchioles, whereas in the alveoli, the damaged Type 1 cells are replaced with Type 2 cells. These new cells are relatively resistant to effects of continued NO₂ exposure.

The time course of alveolar lesions over a chronic exposure was examined by Kubota et al. (1987) in small groups of rats exposed
<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$207$</td>
<td>0.11 ppm</td>
<td>Continuous, Rat</td>
<td>Various morphometric changes, depending on age and exposure level. Multiphasic pattern (e.g., decrease in air-blood barrier thickness from 1 to 12 months of age, and increase in 21-month-old rats).</td>
<td>Kyono &amp; Kawai (1982)</td>
</tr>
<tr>
<td>865</td>
<td>0.46 ppm</td>
<td>1 month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5260</td>
<td>2.8 ppm</td>
<td>Continuous, Rat</td>
<td>Loss of cytoplasmic granules in mast cells, and rupture of mast cells.</td>
<td>Hayashi et al. (1987)</td>
</tr>
<tr>
<td>16500</td>
<td>8.8 ppm</td>
<td>Various morphometric changes, depending on age and exposure level. Multiphasic pattern (e.g., decrease in air-blood barrier thickness from 1 to 12 months of age, and increase in 21-month-old rats).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>639</td>
<td>0.34 ppm</td>
<td>6 h/day, 5 days</td>
<td>Loss of cytoplasmic granules in mast cells.</td>
<td>Thomas et al. (1967)</td>
</tr>
<tr>
<td>940</td>
<td>0.5 ppm</td>
<td>Continuous, up to 6 days</td>
<td>Increased number of mast cells in trachea as exposure duration increased.</td>
<td>Hayashi et al. (1987)</td>
</tr>
<tr>
<td>Concentration (pg/m³)</td>
<td>Duration</td>
<td>Effect</td>
<td>Changes</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------</td>
<td>--------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>940 base, 2820 peak</td>
<td>0.5 base, 1.5 peak</td>
<td>23 h/day (7 days per week) base, 1-h peaks twice/day (5 days/week)</td>
<td>Ret (1 day and 6 weeks old)</td>
<td></td>
</tr>
<tr>
<td>3760 base, 11290 peak</td>
<td>2.0 base, 6.0 peak</td>
<td>5 weeks</td>
<td>In proximal alveolar region: base (940 μg/m³) + peak caused Type 2 cells to become spread over more surface area in neonates and adults; Type 2 cell hypertrophy and increase in number of AMs in adults; Type 2 cells thinner in neonates. Base (3760 μg/m³) + peak (only adults studied) caused similar changes plus an increase in numbers of Type 1 cells, which were smaller than normal Type 1 cells. In terminal bronchiolar region: base (940 μg/m³) + peak caused no effects on percentage distribution of ciliated cells and Clara cells in neonates or adults, but neonates (only) had an increase in ciliated cell surface area and mean luminal surface area of Clara cells. Base (3760 μg/m³) + peak (only adults studied) had fewer ciliated cells with a reduced surface area and alterations in the shape of Clara cells.</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.53</td>
<td>Continuous (24 h/day)</td>
<td>At ≤ 2500 μg/m³, no pathology. At 5000 μg/m³, focal thickening of centriacinar septa by 2 days, progressive loss of cilia and abnormal cilia in trachea and main bronchi at ≥ 4 days; hypertrophy of bronchiolar epithelium at ≥ 8 days. At days 16 and 28, all epithelial cells hypertrophied.</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>1.33</td>
<td>Ret</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>2.66</td>
<td>28 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 34 (contd).**

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.53</td>
<td>24 h/day, 90 days</td>
<td>Guinea-pig, rabbit, dog, monkey, rat</td>
<td>No pathology</td>
<td>Steadman et al. (1966)</td>
</tr>
<tr>
<td>1320-1500</td>
<td>0.7-0.8</td>
<td>Continuous, 1 month</td>
<td>Mouse</td>
<td>Mucous hypersecretion; focal degeneration and desquamation of mucous membrane; terminal bronchiolar epithelial hyperplasia; some alveolar enlargement; shortening of cilia.</td>
<td>Nakajima et al. (1980)</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>Continuous, 1 month</td>
<td>Mouse</td>
<td>Terminal bronchiolar epithelial hyperplasia; some alveolar enlargement.</td>
<td>Nakajima et al. (1980)</td>
</tr>
<tr>
<td>2820</td>
<td>1.5</td>
<td>Continuous, 1 month</td>
<td>Mouse</td>
<td>Terminal bronchiolar epithelial hyperplasia; some alveolar enlargement.</td>
<td>Nakajima et al. (1980)</td>
</tr>
<tr>
<td>1860</td>
<td>1.0</td>
<td>1 h</td>
<td>Rat</td>
<td>Degranulation and decreased number of mast cells.</td>
<td>Thomas et al. (1997)</td>
</tr>
<tr>
<td>3750</td>
<td>2.0</td>
<td>3 days</td>
<td>Rat</td>
<td>No historical changes</td>
<td>Azoulay-Dupuis et al. (1983)</td>
</tr>
<tr>
<td>Exposure</td>
<td>Duration</td>
<td>Species</td>
<td>Lesions</td>
<td>Authors and Year</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>---------</td>
<td>-------------------------------------------------------------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>3760 2.0</td>
<td>3 days</td>
<td>Guinea-pig</td>
<td>Thickening of alveolar walls; oedema; increase in AM numbers; loss of bronchiolar cilia; inflammation.</td>
<td>Azoulay-Dupuis et al. (1983)</td>
<td></td>
</tr>
<tr>
<td>3760 2.0</td>
<td>8 h/day, 5 days/week, 8 weeks</td>
<td>Hamster</td>
<td>Moderate alveolar enlargement, primarily at bronchiolar-alveolar duct junction; increase in mean linear intercept; decrease internal surface area of lung; no lesions in bronchial, bronchiolar, alveolar duct, or alveolar epithelium; no change in macrophage number.</td>
<td>Lafuma et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>3760 2.0</td>
<td>Continuous, 7-21 days</td>
<td>Guinea-pig</td>
<td>Type 2 cell hypertrophy at 7 or 21 days.</td>
<td>Sherwin et al. (1973)</td>
<td></td>
</tr>
<tr>
<td>3760 2.0</td>
<td>Continuous, 1-3 weeks</td>
<td>Guinea-pig</td>
<td>Increase in number of LDH-positive cells with time of exposure. Correlated to increase in Type 2 cells (LDH positive).</td>
<td>Sherwin et al. (1973)</td>
<td></td>
</tr>
<tr>
<td>3760 2.0</td>
<td>Continuous, 6 weeks</td>
<td>Rat</td>
<td>Minimal effect: some cilia loss in terminal bronchioles; some distended or disrupted alveolar walls.</td>
<td>Azoulay et al. (1978)</td>
<td></td>
</tr>
<tr>
<td>9400 5.0</td>
<td>Continuous, 90 days</td>
<td>Cynomolgus monkey</td>
<td>Bronchiolar epithelium hyperplasia; some focal pulmonary oedema.</td>
<td>Busey et al. (1974)</td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

AMs = Alveolar macrophages; LDH = Lactate dehydrogenase
Table 35. Effects of chronic exposure to nitrogen dioxide (NO$_2$) on lung morphology.

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.04</td>
<td>Continuous, 9-27 months</td>
<td>Rat</td>
<td>At 75 mg/m$^3$: no significant change, but some tendency towards increase in arithmetic mean thickness of air-blood barrier. At 752 mg/m$^3$: slight increase in arithmetic mean thickness of air-blood barrier by 18 months, becoming significant by 27 months; some interstitial oedema and slight change in bronchiolar and alveolar epithelium by 27 months. At 7520 mg/m$^3$: hypertrophy and hyperplasia of bronchiolar epithelium and increase in arithmetic mean thickness of air-blood barrier by 9 months, which became significant at 18 months and decreased slightly by 27 months; Clara cell hyperplasia. By 27 months: interstitial fibrosis and hypertrophy of Type 1 and Type 2 cells.</td>
<td>Kubota et al. (1987)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188 base; 1880 peak</td>
<td>0.1 base; 1.0 peak</td>
<td>Continuous baseline; 2-h daily peak; 6 months</td>
<td>Mouse</td>
<td>Dilated airspaces and alveolar wall destruction (small sample size).</td>
<td>Port et al. (1977)</td>
</tr>
<tr>
<td>Concentration</td>
<td>Exposure Time</td>
<td>Species</td>
<td>Duration</td>
<td>Pathology Description</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
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<td></td>
</tr>
<tr>
<td>940 mg/m³</td>
<td>0.5</td>
<td>Continuous, Rat</td>
<td>7 months</td>
<td>At 940 mg/m³, swelling of terminal bronchial cilia and hyperplasia of Type 2 cells.</td>
<td></td>
</tr>
<tr>
<td>1880 mg/m³</td>
<td>1.0</td>
<td>7 months</td>
<td></td>
<td>At 1880 mg/m³, cilia loss in terminal bronchioles; hyperplasia of Type 2 cells; and interstitial oedema.</td>
<td></td>
</tr>
<tr>
<td>7520 mg/m³</td>
<td>4.0</td>
<td>7 months</td>
<td></td>
<td>At 7520 mg/m³, cilia loss in terminal bronchioles; hyperplasia of Type 2 cells, interstitial oedema; decrease in number of lamellar bodies in Type 2 cells; lysosomes with osmiophilic lamellar structure in ciliated cells of terminal bronchioles.</td>
<td></td>
</tr>
<tr>
<td>940 mg/m³</td>
<td>0.5</td>
<td>Continuous, up to 19 months</td>
<td></td>
<td>Type 2 cell hypertrophy and interstitial oedema by 4 months; increased thickness of alveolar septa by 6 months; fibrous pleural thickening by 19 months.</td>
<td></td>
</tr>
<tr>
<td>1880 mg/m³</td>
<td>1.0</td>
<td>Continuous, 6-24 h/day, 3-12 months</td>
<td>Mouse</td>
<td>Pneumonitis and alveolar size increase; loss of cilia in respiratory bronchioles and bronchiolar inflammation with 24 h/day.</td>
<td></td>
</tr>
<tr>
<td>940 mg/m³</td>
<td>0.5</td>
<td>Continuous, lifetime (up to 33 months)</td>
<td>Rat</td>
<td>Minimal changes: slight enlargement of alveoli and alveolar ducts; some rounding of bronchiolar epithelial cells; increase in elastic fibers around alveolar ducts.</td>
<td></td>
</tr>
<tr>
<td>1500 mg/m³</td>
<td>0.8</td>
<td>Continuous, lifetime (up to 33 months)</td>
<td>Rat</td>
<td>No pathology</td>
<td></td>
</tr>
<tr>
<td>1880 mg/m³</td>
<td>1.0</td>
<td>Continuous, 16 months</td>
<td>Squirrel monkey</td>
<td>No pathology</td>
<td></td>
</tr>
<tr>
<td>NO₂ concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Species</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>6 h/day, 5 days/week, up to 16 months</td>
<td>Dog</td>
<td>At 1880 µg/m³ - 6 months: no pathology; 12 months: dilated alveoli and alveolar ducts; 18 months: dilated alveoli, oedema, thickening alveolar septa due to inflammation.</td>
<td>Wagner et al. (1965)</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td></td>
<td>Guineas-pig</td>
<td>Mild thickening of alveolar septa due to inflammation; some alveolar dilatation.</td>
<td>Wagner et al. (1965)</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>7 h/day, 5 days/week, 15 weeks</td>
<td>Rat</td>
<td>No pathology</td>
<td>Gregory et al. (1983)</td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>Continuous, 2 years</td>
<td>Rat</td>
<td>Loss of cilia in terminal bronchioles; abnormal ciliogenesis; crystalloid inclusions in broncholar epithelial cells; increased thickness of collagen fibrils and basement membrane in terminal bronchioles.</td>
<td>Stephens et al. (1971a,b)</td>
</tr>
<tr>
<td>Concentration (mg/m³)</td>
<td>Duration</td>
<td>Species</td>
<td>Effects</td>
<td>References</td>
<td></td>
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<tr>
<td>-----------------------</td>
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<td>-----------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0, continuous, up to 12 months</td>
<td>Rat</td>
<td>Hypertrophy of ciliated cells and cilia loss by 72 h; decreased number of ciliated cells by 7 days; normal ciliated cells from 21 days-12 months.</td>
<td>Stephens et al. (1972)</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0, continuous, up to 360 days</td>
<td>Rat</td>
<td>No change in turnover of terminal bronchiolar epithelial cells; increase in turnover of Type 2 cells in peripheral alveoli by 1 day, but normal by 7 days.</td>
<td>Evans et al. (1972)</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0, continuous, 14 months</td>
<td>Monkey (Macaca fascicularis)</td>
<td>Bronchiolar epithelial hypertrophy, especially adjacent to alveolar ducts; change to cuboidal cells in proximal bronchiolar epithelium.</td>
<td>Furiosi et al. (1973)</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0, continuous, 14 months</td>
<td>Rat</td>
<td>Minimal effect: some terminal bronchiolar epithelial hypertrophy.</td>
<td>Furiosi et al. (1973)</td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0, continuous, lifetime (up to 763 days); 1500 mg/m³ for 1st 69 days, then 3760 mg/m³</td>
<td>Rat</td>
<td>Alveolar distension, especially near alveolar duct level; increased variability in alveolar size; loss of cilia and hypertrophy in terminal bronchiolar cells; no inflammation.</td>
<td>Freeman et al. (1966b)</td>
<td></td>
</tr>
</tbody>
</table>
Table 35 (contd).

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>µg/m³</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td>Continuous,</td>
<td>Rat</td>
<td>Bronchial epithelial hyperplasia</td>
<td>Haydon et al. (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td></td>
<td>6 h/day,</td>
<td>Mouse</td>
<td>No pathology</td>
<td>Wagner et al. (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 days/week,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td></td>
<td>4-7.5 h/day,</td>
<td>Guinea-pig</td>
<td>Some dilatation of terminal bronchioles; tracheal inflammation; pneumonitis.</td>
<td>Balchum et al. (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 days/week,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.5 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td></td>
<td>7 h/day,</td>
<td>Rat</td>
<td>Focal hyperinflation and areas of subpleural accumulation of macrophages.</td>
<td>Gregory et al. (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 days/week,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 weeks</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)
Studies of the Effects of Nitrogen Oxides on Experimental Animals

to 7520 µg/m³ (4.0 ppm) NO₂, 24 h/day for up to 27 months. One phase, which lasted for 9 to 18 months of exposure, consisted of a decrease in number and an increase in cell volume of Type I epithelium, an increase in the relative ratio of Type 2 to Type 1 cells, and an increase in the number and volume of Type 2 cells. A second phase, between 18 to 27 months of exposure, showed some recovery of the alveolar epithelium, but the total volume of interstitial tissue decreased and collagen fibres in the interstitium increased. Thus, some lesions resolved with continued exposure, whereas others progressed. At 752 µg/m³ (0.4 ppm), Kubota et al. (1987) found that the lesion typically was milder and its initiation delayed, compared to the higher concentration. In general, most NO₂-induced lesions were resolved following a recovery period. This period may be as short as 30 days for exposures at ≤ 9400 µg/m³ (5.0 ppm). With continuous exposure, early morphological damage may also be resolved. For example, in rats exposed continuously for 7 months to 940 µg/m³ (0.5 ppm) NO₂, resolution of epithelial lesions occurred by 4 to 6 months of exposure (Yamamoto & Takahashi, 1984).

b) Effects of nitrogen dioxide as a function of exposure pattern

Several morphological studies were designed to evaluate ambient NO₂ patterns consisting of a low baseline level with transient spikes of NO₂. However, in some cases, there was no group at the baseline exposure only, preventing evaluation of the contribution of peaks to the responses. Gregory et al. (1983) exposed rats (14 to 16 weeks old) for 7 h/day, 5 days/week for up to 15 weeks to atmospheres consisting of the following concentrations of NO₂: (1) 1880 µg/m³ (1.0 ppm), (2) 9400 µg/m³ (5.0 ppm), or (3) 1880 µg/m³ (1.0 ppm) with two 1.5-h spikes of 9400 µg/m³ (5.0 ppm) per day. After 15 weeks of exposure, histopathology was minimal, with focal hyperinflation and areas of subpleural accumulation of macrophages found in some of the animals exposed either to the baseline of 9400 µg/m³ (5.0 ppm) or to 1880 µg/m³ (1.0 ppm) with the 9400 µg/m³ (5.0 ppm) spikes.

Port et al. (1977) observed dilated respiratory bronchioles and alveolar ducts in mice exposed to 188 µg/m³ (0.1 ppm) NO₂ with daily 2-h peaks to 1880 µg/m³ (1.0 ppm), for 6 months. Miller et al. (1987) found no morphological effects in mice exposed for 1 year, although host defence and functional changes were noted (see sections 5.2.2.1 and 5.2.2.3).
Crapo et al. (1984) and Chang et al. (1986) used quantitative morphometric analyses to examine the proximal alveolar and terminal bronchiolar regions of rats exposed for 6 weeks to a baseline concentration of 940 or 3760 µg/m³ (0.5 or 2.0 ppm) NO₂, 23 h/day for 7 days/week, onto which were superimposed two daily 30 min spikes of 3 times the baseline concentration for 5 days/week. At the lower exposure level, the volumes of the Type 2 epithelium, interstitial matrix, and AMs increased, whereas the volume of the fibroblasts decreased. The surface area of Type 2 cells increased. Most of these changes also occurred at the higher exposure level, and in some cases the change was greater than that at the lower level (i.e., increase in Type 1 and Type 2 epithelial volume). At both levels of exposure, the volume of Type 2 cells and interstitial fibroblasts increased, with no significant changes in their numbers, and the number of AMs decreased. The number of Type 1 cells decreased and their average surface area increased in the highest exposure group. Generally, there was a spreading and hypertrophy of Type 2 cells. A correlation between decreased compliance (Stevens et al., 1988) and thickening of the alveolar interstitium was found (see section 5.2.2.3 for details of the pulmonary function portion of the study). Examination of the terminal bronchiolar region revealed no effects at the lower exposure level. At the higher level, there was a 19% decrease in ciliated cells per unit area of the epithelial basement membrane and a reduction in the mean ciliated surface area. The size of the dome protrusions of non-ciliated bronchiolar (Clara) cells was decreased, giving the bronchial epithelium a flattened appearance, but there was no change in the number of cells.

c) Factors affecting susceptibility to morphological changes

Age-related responsiveness to an urban pattern of NO₂ was evaluated by Chang et al. (1986, 1988) using 1-day- or 6-week-old rats exposed for 6 weeks to a baseline of 940 µg/m³ (0.5 ppm) NO₂ for 23 h/day, 7 days/week, with two 1-h spikes (given in the morning and afternoon) of 2820 µg/m³ (1.5 ppm) 5 days/week. Electron microscopic morphometric procedures were used. In the proximal alveolar region, only the older animals showed an increase in the surface density of the alveolar basement membrane. The increase in the mean cellular volume of Type 2 cells was greater in the young adult animals, although the neonates also exhibited this effect. Although there was no qualitative evidence of morphological injury in the terminal bronchioles of the neonatal rats, there was a 19% increase in the average ciliated cell surface and a 13% increase of the mean luminal surface area of
non-ciliated bronchiolar (Clara) cells that was not evident in the young adult rats. Generally, the neonatal rats were as sensitive or more susceptible than young adults, depending upon the end-point. However, the terminal bronchioles of the neonatal rats were more susceptible than those of young adults (Chang et al., 1988). For example, the lower exposure altered ciliated cells and non-ciliated bronchiolar (Clara) cells in the neonates but not the young adults. Other indices were unaffected. Pulmonary function was also altered in similarly exposed rats (Stevens et al., 1988) (see section 5.2.2.3). Interpretation of the neonatal effects is difficult. Assuming that rats prior to weaning are more resistant to NO₂ (Stephens et al., 1978) (see below), effects observed after a 6-week exposure from birth may have resulted from the last 3 weeks of exposure, as the first 3 weeks may constitute a more resistant period. In contrast, effects observed in young adults probably reflect the impact of the entire 6-week exposure.

In one of the more extensive studies, Kyono & Kawai (1982) exposed rats at 1, 3, 12, and 21 months of age continuously for 1 month to 207 μg/m³, 865 μg/m³, 5260 μg/m³ or 16 500 μg/m³ (0.11, 0.46, 2.8 or 8.8 ppm) NO₂. Various morphometric parameters were assessed, including arithmetic mean thickness of the air-blood barrier and the volume density of various alveolar wall components. Quantitative estimations deliberately excluded the site of main damage (i.e., the peripheral alveolar wall was examined). Analysis of individual results was complex, but depending upon the animal's age and the specified end-point, exposure levels as low as 207 μg/m³ (0.11 ppm) changed specific morphometric parameters. There was a trend towards a concentration-dependent increase in air-blood barrier thickness in all age groups, with evidence of age-related differences in response. At any concentration, the response of this end-point decreased in rats from 1 to 12 months old, but increased again in 21-month-old animals. Type 1 and 2 cells showed various degrees of response, depending on both age at onset of exposure and exposure concentration. The response of each lung component did not always show a simple concentration-dependent increase or decrease, but suggested a multiphasic reaction pattern.

The above studies with rats may not have used the most susceptible animal model, as demonstrated by Azoulay-Dupuis et al. (1983), who exposed both rats and guinea-pigs aged 5 to ≥ 60 days old to 3760 (2.0 ppm) for 3 days. Rats at all ages and guinea-pigs < 45 days old were not affected. The 45-day-old guinea-pigs showed thickening of alveolar walls, alveolar oedema,
and inflammation, whereas animals older than 45 days showed similar, but more frequent, alterations that seemed to increase with age. Adults also had focal loss of cilia in bronchioli.

In general, it appears that neonates, prior to weaning, are relatively resistant to NO₂, and that responsiveness then increases (Stephens et al., 1978). Furthermore, the responsiveness of mature animals appears to decline somewhat with age, until an increase in responsiveness occurs at some point in senescence. However, the morphological response to NO₂ in animals of different ages involves similarities in the cell types affected and in the nature of the damage incurred. Age-related differences occur in the extent of damage and in the time required for repair, the latter taking longer in older animals. The reasons for age differences in susceptibility are not known, but may involve differences in doses to the target cells and variable sensitivity of target cells during different growth phases.

The database regarding the effects of levels of NO₂ < 9400 µg/m³ (5.0 ppm) on animals with pre-existing respiratory disease is very limited and only includes animals with laboratory-induced emphysema or infections. Lafuma et al. (1987) exposed both normal and elastase-induced emphysematous hamsters (2 months old) to 3260 µg/m³ (2.0 ppm) NO₂ for 8 h/day, 5 days/week, for 8 weeks. Morphometric analyses indicated that emphysematous lesions were exacerbated by NO₂ (i.e., NO₂ increased pulmonary volume and decreased internal alveolar surface area). The investigators suggested that these results may imply a role for NO₂ in enhancing pre-existing emphysema. Acute infectious (influenza) lung disease enhanced the morphological effects of NO₂ in squirrel monkeys exposed continuously to 1880 µg/m³ (1.0 ppm) NO₂ for 16 months (Fenters et al., 1973).

d) Emphysema following nitrogen dioxide exposure

Numerous investigators have observed morphological lesions that led them to the diagnosis of NO₂-induced emphysema. However, to evaluate these reports independently, it is necessary to apply the current definition of emphysema, especially because the definition changed after several of the reports were published. Such an evaluation is described in detail by the US EPA (1993), based upon the most recent definition of emphysema from the report of the US National Heart, Lung and Blood Institute (NHLBI), Division of Lung Diseases Workshop (National Institutes of Health, 1985). According to this document, in human lungs:
"Emphysema is defined as a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis". Destruction in emphysema is further defined as "non-uniformity in the pattern of respiratory airspace enlargement so that the orderly appearance of the acinus and its components is disturbed and may be lost". The report further indicates: "Destruction...may be recognized by subgross examination of an inflation-fixed lung slice...". However, emphysema in animal models was defined differently. An animal model of emphysema is defined as "an abnormal state of the lungs in which there is enlargement of the airspaces distal to the terminal bronchiole. Airspace enlargement should be determined qualitatively in appropriate specimens and quantitatively by stereologic methods". Thus, in animal models of emphysema, airspace wall destruction need not be present. "Appropriate specimens" presumably refers to lungs fixed in the inflated state.

When reports of emphysema following NO₂ exposures of animals are to be extrapolated to potential hazards for humans, the definition of human emphysema, rather than that for emphysema in experimental animals, should be used. The presence of airspace wall destruction, critical to the definition of human emphysema, can only be determined independently in published reports by careful review of the authors' description of the lesions or by examining the micrographs that the author selected for publication. Because descriptions in some reports are inadequate for independent evaluation, more evidence may exist for emphysema than is summarized here. All reports reviewed are summarized in Table 36, but only those showing emphysema of the type seen in human lungs are discussed in the text that follows.

Haydon et al. (1967) reported emphysema in rabbits exposed continuously (presumably 24 h/day) for 3 to 4 months to 15000 or 22 600 μg/m³ (8.0 or 12.0 ppm) NO₂. They reported enlarged lungs that failed to collapse when the thorax was opened. The lungs were fixed in an expanded state via the trachea. In 100-μm thick sections from formaldehyde-fixed dried lungs they reported "dilated" airspaces with "distorted architecture." In those and other tissue preparations, they reported that the airspaces appeared "grossly enlarged and irregular, which appears to be due to disrupted alveoli... and the absence of adjacent alveolar collapse." Thus, in appropriately fixed lungs, they reported evidence of enlarged airspaces with destructive changes in alveolar walls. Although no stereology was performed, this appears to be emphysema of the type seen in human lungs.

253
Table 36. Effects of nitrogen dioxide (NO$_2$) on the development of emphysema

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Emphysema$^a$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>188 with 2-h peaks to 1880</td>
<td>0.1 with peaks to 1.0</td>
<td>Daily, 6 months</td>
<td>Mouse</td>
<td>±</td>
<td>Port et al. (1977)</td>
</tr>
<tr>
<td>263 plus 2050 µg/m$^3$ NO</td>
<td>0.14</td>
<td>16 h/day, 68 months</td>
<td>Beagle dog</td>
<td>−</td>
<td>Hyde et al. (1978)</td>
</tr>
<tr>
<td>1200 plus 310 µg/m$^3$ NO</td>
<td>0.64</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>6, 18 or 24 h/day, 1-12 months</td>
<td>Mouse</td>
<td>−</td>
<td>Blair et al. (1969)</td>
</tr>
<tr>
<td>1500</td>
<td>0.8</td>
<td>51-813 days</td>
<td>Rat</td>
<td>−</td>
<td>Haydon et al. (1965)</td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1880 (with and without viral challenge)</td>
<td>1.0</td>
<td>16 months</td>
<td>Squirrel monkey</td>
<td>±</td>
<td>Ehrlich &amp; Fenters (1973)</td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>Continuous, 112-763 days</td>
<td>Rat</td>
<td>−</td>
<td>Freeman et al. (1968c)</td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>8 h/day, 5 days/week for 8 weeks</td>
<td>Hamster</td>
<td>−</td>
<td>Lafuma et al. (1987)</td>
</tr>
<tr>
<td>Concentration</td>
<td>Duration</td>
<td>Species</td>
<td>Notes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
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<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>3 months</td>
<td>Squirrel monkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td></td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>Up to 18 months</td>
<td>Dog, rabbit, guinea-pig, rat, hamster, mouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 000</td>
<td>8.0</td>
<td>3-4 months</td>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 560</td>
<td>12.0</td>
<td>24 h/day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 200</td>
<td>15.0</td>
<td>2-5 months</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 200</td>
<td>15.0</td>
<td>Continuously for 35 days then intermittently for at least 2 years</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 800</td>
<td>18.0</td>
<td>24 h/day for 1-6 days or 4 weeks</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 600 reduced to either 28 200 or 18 800</td>
<td>20.0 reduced to 15.0 or 10.0</td>
<td>Up to 33 months</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47 000</td>
<td>25.0</td>
<td>32-65 days</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 400</td>
<td>30.0</td>
<td>22 h/day, 12 months</td>
<td>Hamster</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 400</td>
<td>30.0</td>
<td>Continuous, up to 140 days</td>
<td>Rat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ehrlich & Fenters (1973)
Wagner et al. (1965)
Haydon et al. (1967)
Stephens et al. (1975)
Port et al. (1977)
Freeman et al. (1968a)
Freeman et al. (1972)
Freeman & Haydon (1964)
Kleinerman et al. (1985)
Glasgow et al. (1987)
Table 36 (contd).

<table>
<thead>
<tr>
<th>µg/m³</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Emphysema</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 400</td>
<td>30.0</td>
<td>Continuous, up to 8 weeks</td>
<td>Rat</td>
<td>-</td>
<td>Blank et al. (1978)</td>
</tr>
<tr>
<td>56 400 to 65 800</td>
<td>30.0-35.0</td>
<td>23 h/day for 7 days</td>
<td>Hamster</td>
<td>-</td>
<td>Lam et al. (1983)</td>
</tr>
<tr>
<td>65 800</td>
<td>35.0</td>
<td>6 h/day for 25 days</td>
<td>Rat</td>
<td>-</td>
<td>Stavert et al. (1986)</td>
</tr>
<tr>
<td>75 200</td>
<td>40.0</td>
<td>6 or 8 weeks</td>
<td>Mouse</td>
<td>-</td>
<td>Buckley &amp; Locati (1969)</td>
</tr>
<tr>
<td>94 000 to 169 200 for 4 weeks, reduced to 56 400 to 94 000</td>
<td>50-90 reduced to 30-50</td>
<td>2 h/day, 5 days/week, 12 months</td>
<td>Hamster, guinea-pig</td>
<td>±</td>
<td>Gross et al. (1968)</td>
</tr>
<tr>
<td>84 600 to 103 400</td>
<td>45-55</td>
<td>22-23 h/day, 10 weeks</td>
<td>Hamster</td>
<td>-</td>
<td>Kleinerman &amp; Cowdrey (1968)</td>
</tr>
</tbody>
</table>

- Modified from US EPA (1993)
- * = emphysema; - = no emphysema; ± = equivocal

Emphysema is defined according to the 1985 US National Heart, Lung, and Blood Institute Workshop criteria for human emphysema. Although many of the papers reviewed (US EPA, 1993) reported finding emphysema, some of these studies were reported according to previous, different criteria; some reports did not fully describe the methods used; and/or the results obtained were not in sufficient detail to allow independent confirmation of the presence of emphysema. Thus, a "-" (i.e. no emphysema) should only be interpreted as lack of proof of emphysema, because it is conceivable that if the study were repeated with current methods and the current criteria applied, it might be judged to be positive.
Freeman et al. (1972) exposed rats to 37,600 μg/m³ (20.0 ppm) NO₂, which was reduced during the exposure to 28,200 μg/m³ (15.0 ppm) or to 18,800 μg/m³ (10.0 ppm), for varying periods up to 33 months. Following removal at necropsy, the lungs were fixed via the trachea at 25 cm of fixative pressure. Morphometry of lung and alveolar size was performed in a suitable, although unconventional, manner. The morphometry indicated enlargement of alveoli and reduction in alveolar surface area. The authors also both reported alveolar destruction and illustrated alveolar destruction in their figures. They correctly concluded that they had demonstrated emphysema in their NO₂-exposed rats. However, it is not entirely clear whether both experimental groups or only the group exposed to 28,200 μg/m³ (15.0 ppm) had emphysema.

Hyde et al. (1978) studied beagle dogs that had been exposed 16 h daily for 68 months to either filtered air or to 1,200 μg/m³ (0.64 ppm) NO₂ with 310 μg/m³ (0.25 ppm) NO or to 263 μg/m³ (0.14 ppm) NO₂ with 2,050 μg/m³ (1.67 ppm) NO. The dogs then breathed clean air during a 32- to 36-month post-exposure period. The right lungs were fixed via the trachea at 30-cm fixative pressure in a distended state. Semiautomated image analysis was used for morphometry of air spaces. The dogs exposed to 1,200 μg/m³ NO₂ with 310 μg/m³ NO had significantly larger lungs with enlarged air spaces and evidence of destruction of alveolar walls. These effects were not observed in dogs exposed to 270 μg/m³ NO₂ with 2,050 μg/m³ NO, implying a significant role of the NO₂ in the production of the lesions. The lesions in dogs exposed to the higher NO₂ concentration meet the criteria of the 1985 NHLBI workshop for emphysema of the type seen in human lungs.

5.2.3 Genotoxicity, potential carcinogenic or co-carcinogenic effects

NO₂ forms nitrous and nitric acids in aqueous solutions, which are in equilibrium with the nitrite (NO₂⁻) and nitrate (NO₃⁻) ions that constitute the main metabolites of NO₂. Nitrous acid/NO₂⁻ is mutagenic in vitro, causing deamination of bases in DNA. The formation of N-nitroso compounds from secondary amines and amides is another mechanism for indirect mutagenic activity (Zimmermann, 1977).

In vitro studies with NO₂ have demonstrated mutations in bacteria (Salmonella strain TA100) (Isomura et al., 1984; Victorin & Stahlberg, 1988) but not in a mammalian cell culture (Isomura et al., 1984). Other experiments using cell cultures were positive
concerning chromatid-type chromosome aberrations, sister chromatid exchanges (SCE) and DNA single strand breaks (Tsuda et al., 1981; Shiraishi & Bandow, 1985; Gorsdorf et al., 1990).

NO\textsubscript{2} did not induce recessive lethal mutations or somatic mutations in Drosophila (Inoue et al., 1981; Victorin et al., 1990) and was negative in in vivo studies with mice concerning chromosome aberrations in peripheral lymphocytes or spermatoocytes (Gooch et al., 1977) and micronuclei in bone marrow cells in mice (Victorin et al., 1990).

Two studies have dealt with genotoxic effects in the relevant target organ, i.e. the lung, and both were positive at high concentrations. In the first one, Isomura et al. (1984) demonstrated the induction of mutations and chromosome aberrations in lung cells of rats exposed to 27 ppm (50 000 µg/m\textsuperscript{3}) for 3 h. In the other (Walles et al., 1995), DNA single strand breaks were induced in lung cells of mice exposed to 54 000 µg/m\textsuperscript{3} (30 ppm) for 16 h or 94 000 µg/m\textsuperscript{3} (50 ppm) for 5 h.

Several studies have evaluated the issue of carcinogenesis and co-carcinogenesis, but results are often unclear or conflicting (Table 37). However, there do not appear to be any published reports on studies using classical carcinogenesis whole-animal bioassays. An excellent critical review and discussion of some of the important theoretical issues in interpreting these types of studies has been published (Witschi, 1988). Although lung epithelial hyperplasia (section 5.2.2.4) and enhancement of endogenous retrovirus expression (Roy-Burman et al., 1982) have been thought by some to suggest increased carcinogenic potential, such findings are not conclusive (see US EPA, 1993).

Wagner et al. (1965) suggested that NO\textsubscript{2} may accelerate the production of tumours in CAF/Jax mice (a strain that has spontaneously high pulmonary tumour rates) after continuous exposure to 9400 µg/m\textsuperscript{3} (5.0 ppm) NO\textsubscript{2}. After 12 months of exposure, 7 out of 10 mice in the exposed group had tumours, compared to 4 of 10 in the controls. No differences in tumour production were observed after 14 and 16 months of exposure. A statistical evaluation of the data was not presented. The frequency and incidence of spontaneously occurring pulmonary adenomas was increased in strain A/J mice (with spontaneously high tumour rates) after exposure to 18 800 µg/m\textsuperscript{3} (10.0 ppm) NO\textsubscript{2} for 6 h/day, 5 days/week, for 6 months (Adkins et al., 1986). These small, but statistically significant, increases were only detectable when the
Table 37. Effects of nitrogen dioxide ($NO_2$) on carcinogenesis or co-carcinogenesis*

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects $^b$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>188-18 800 0.1-10.0</td>
<td>0.5-4 h</td>
<td>Mouse</td>
<td>Mice exposed to DMA had whole-body concentration-related increase in DMN.</td>
<td>Iqbal et al. (1981)</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>0.25</td>
<td>7 h/day, 5 days/week, up to 26 weeks</td>
<td>Mouse</td>
<td>NO$_2$ slowed progression of spontaneous T cell lymphomas in AKR/cum mice, increased survival, and decreased number of splenic CD4$^+$ T cells.</td>
<td>Richters &amp; Darnji (1990)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>7-8 h/day, 5 days/week, 12 weeks</td>
<td>Mouse</td>
<td>Increased lung tumors and mortality in mice injected with melanoma cells after NO$_2$ exposure.</td>
<td>Richters &amp; Kuraitis (1981, 1963); Richters et al. (1985)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous, 30 days</td>
<td>Mouse</td>
<td>Hyperplastic foci identical to that observed after exposure to known carcinogens.</td>
<td>Nakajima et al. (1972)</td>
</tr>
<tr>
<td>1500</td>
<td>0.8</td>
<td>6 h/day, 5 days/week, 16 weeks</td>
<td>Mouse</td>
<td>Enhanced retrovirus expression in two strains of mice.</td>
<td>Roy-Burman et al. (1982)</td>
</tr>
</tbody>
</table>
Table 37 (contd).

<table>
<thead>
<tr>
<th>NO\textsubscript{2} concentration</th>
<th>Exposure</th>
<th>Species</th>
<th>Effects\textsuperscript{b}</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{mg/m}^3)</td>
<td>ppm</td>
<td>6 h/day, 5 days/week, 6 weeks</td>
<td>Mouse</td>
<td>No effect at 1880 or 9400 (\text{mg/m}^3). At 18 800 (\text{mg/m}^3), spontaneous adenomas in strain A/J mice increased only when compared to pooled control group.</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>6 days/week</td>
<td>Mouse</td>
<td>(\text{NO}_2) plus (\text{NO}_2) did not produce tumors. Design and statistical analyses not appropriate; exposure methods not described.</td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>5 days/week</td>
<td>Rat</td>
<td>DMA plus (\text{NO}_2) had no effect on tumor production.</td>
</tr>
<tr>
<td>18 800</td>
<td>10.0</td>
<td>2 h/day, 5 days/week, 50 weeks</td>
<td>Mouse</td>
<td>Mice given 4-nitroquinoline-1-oxide during (\text{NO}_2) exposure; (\text{NO}_2) had no effect on tumor production.</td>
</tr>
</tbody>
</table>

\textsuperscript{b} Effects listed are those of \(\text{NO}_2\) unless indicated otherwise.

Reference: 2000-18 800, 18 800, 5.0-10.0, 18 800.
Table 37 (contd).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Exposure Time</th>
<th>Species</th>
<th>Effect Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 200-94 000</td>
<td>15.0-50.0</td>
<td>1-4 h</td>
<td>Mouse</td>
<td>Mice gavaged with morpholine had concentration-dependent increase in whole-body content of NMOR. (Kobayashi et al. 1980)</td>
</tr>
<tr>
<td>31 020-38 500</td>
<td>16.5-20.5</td>
<td>5-6 h/day, 4 days; plus 3 h on 5th day</td>
<td>Mouse</td>
<td>In vivo production of NMOR when 1 g/kg of morpholine was administered each day prior to exposure. (Van Steen et al. 1983)</td>
</tr>
<tr>
<td>84 500</td>
<td>45.0</td>
<td>2 h</td>
<td>Mouse</td>
<td>Mice gavaged with morpholine had an in vivo increase in NMOR production. (Norkus et al. 1984)</td>
</tr>
<tr>
<td>199 000</td>
<td>106.0</td>
<td>0.5-4 h</td>
<td>Rat</td>
<td>Rats given morpholine in their diets or by gavage had no NMOR detected in their bodies. In mice morpholine, by gavage, yielded no significant in vivo NMOR production. (Mirvish et al. 1981)</td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

** DMA = Dimethylamine; DMN = Dimethylnitrosamine; NMOR = N-nitrosomorpholine
control response from nine groups (n = 400) were pooled. Exposure to 1880 and 9400 µg/m³ (1.0 and 5.0 ppm) NO₂ had no effect. In contrast, Richters & Damji (1990) found that an intermittent exposure to 470 µg/m³ (0.25 ppm) NO₂ for up to 26 weeks decreased the progression of a spontaneous T cell lymphoma in AKR/cum mice and increased survival rates. The investigators attribute this effect to an NO₂-induced decrease in the proliferation of T cell subpopulation (especially T-helper/inducer lymphocytes) that produce growth factors for the lymphoma.

Whether NO₂ facilitates metastases has been the subject of several experiments by Richters & Kuraitis (1981, 1983), Richters & Richters (1983) and Richters et al. (1985). Mice were exposed to several concentrations and durations of NO₂ and were injected intravenously with a cultured-derived melanoma cell line (B16) after exposure; subsequent tumours in the lung were counted. Although some of the experiments showed an increased number of lung tumours, statistical methods were inappropriate. Furthermore, the experimental technique used in these studies probably did not evaluate metastases formation, as the term is generally understood, but more correctly, colonization of the lung by tumour cells.

Ide & Otsu (1973) did not find that chronic exposure to high concentrations of NO₂ (somewhere between 9400 and 18800 µg/m³, 5.0 and 10.0 ppm) enhanced tumour production in conventional mice receiving five weekly injections of 0.25 mg 4-nitroquinoline-1-oxide (a lung-tumour-specific carcinogen). Benemansky et al. (1981) used a known carcinogen, nitroso-dimethylamine or its precursor dimethylamine (DMA) to test for interactions with a chronic exposure to NO₂. However, appropriate statistical techniques and control groups were not employed and the methods of exposure and monitoring of NO₂ were not reported, thus precluding accurate evaluation. In another study, rats were injected with N-bis (2-hydroxy-propyl)nitrosamine (BHPN) and continuously exposed to 75, 750 or 7500 µg/m³ (0.04, 0.4 or 4.0 ppm) NO₂ for 17 months. Although the data indicated five times as many lung adenomas or adenocarcinomas in the rats injected with BHPN and exposed to 7500 µg/m³ NO₂ (5/40 compared to 1/10), the results failed to achieve statistical significance (Ichinose et al., 1991).

Because of evidence that NO₂ could produce NO₂⁻ and NO₃⁻ in the blood and the fact that NO₂⁻ is known to react with amines to produce animal carcinogens (nitrosamines), the possibility that
NO\textsubscript{2} could produce cancer via nitrosamine formation has been investigated. Iqbal et al. (1980) was the first to demonstrate a linear time-dependent and concentration-dependent relationship between the amount of \textit{N}-nitrosomorpholine (NMOR) (an animal carcinogen) found in whole-mouse homogenates after the mice were gavaged with 2 mg of morpholine (an exogenous amine that is rapidly nitrosated) and exposure to 28 200 to 94 000 \mu g/m\textsuperscript{3} (15.0 to 50.0 ppm) NO\textsubscript{2} for between 1 and 4 h. In a follow-up study, Iqbal et al. (1981) used DMA, an amine that is slowly nitrosated to dimethylnitrosamine (DMN). They reported a concentration-related increase in biosynthesis of DMN at NO\textsubscript{2} concentrations as low as 188 \mu g/m\textsuperscript{3} (0.1 ppm); however, the rate was significantly greater at concentrations above 18 800 \mu g/m\textsuperscript{3} (10.0 ppm) NO\textsubscript{2}.

Increased length of exposure also increased DMN formation between 0.5 and 2 h, but synthesis of DMN was less after 3 or 4 h of exposure than after 0.5 h.

Mirvish et al. (1981) conducted analogous research and concluded that the results of Iqbal et al. (1980) were technically flawed, but found that \textit{in vivo} exposure to NO\textsubscript{2} could produce a nitrosating agent (NSA) that would nitrosate morpholine only when morpholine was added \textit{in vitro}. Further experiments showed that the NSA was localized in the skin (Mirvish et al., 1983) and that mouse skin cholesterol was a likely NSA (Mirvish et al., 1986). It has also been reported that only very lipid-soluble amines, which can penetrate the skin, would be available to the NSA. Compounds such as morpholine, which are not lipid-soluble, could only react with NO\textsubscript{2} when it was painted directly on the skin (Mirvish et al., 1988). Iqbal (1984), responding to the Mirvish et al. (1981) criticisms, verified their earlier studies (Iqbal et al., 1980). \textit{In vivo} nitrosation was also demonstrated by Norkus et al. (1984) after morpholine administration and a 2-h exposure to 84 600 \mu g/m\textsuperscript{3} (45 ppm) NO\textsubscript{2}.

Another study (Van Stee et al., 1983) reported that mice gavaged with 1 g of morpholine/kg body weight per day and then exposed (5-6 h daily for 5 days) to 31 000 to 38 500 \mu g/m\textsuperscript{3} (16.5 to 20.5 ppm) NO\textsubscript{2} revealed that NMOR could be produced \textit{in vivo}. The single site containing the greatest amount of NMOR was the gastrointestinal tract.

Shoaf et al. (1989) studied the uptake and nitrosation of primary amines by NO\textsubscript{2} in isolated ventilated rat lungs. The rate of nitrosation was very low because the nitrosation of primary amines is a general acid/base catalysed reaction that would be at
a minimum at pH 7. The authors could not replicate the previous nitrosation studies. At a maximum, only 0.0001% of an amine would be nitrosated. Such a rate is at or below the detection limit for nitrosamine. The studies reporting nitrosation may be seriously in error. Nitrosation may be a very minimal reaction and of little consequence.

Victorin (1994) reviewed the genotoxicity of nitrogen oxides and concluded that there is no clear evidence of a carcinogenic potential of NO₂. Victorin (1994) also directed attention to the possibility that NO compounds in photochemical smog may contribute secondarily to formation of other genotoxic compounds. For example, it was noted that strongly mutagenic nitro-PAH compounds are easily formed and mutagenic reaction products may be formed from alkenes through photochemical reactions.

Overall, the above critical evaluation indicates that there is no evidence establishing that tumours can be directly induced by NO₂ exposure alone. Also, the available evidence for NO₂ promoting or enhancing the production or growth of tumours caused by other agents is quite limited and conflicting. It must therefore be concluded that the evidence for carcinogenicity of nitrogen oxides is at present inadequate, but the issue should be addressed by further research.

5.2.4 Extrapulmonary effects

Exposure to NO₂ produces a wide array of health effects beyond the confines of the lung. Thus, NO₂ and/or some of its reactive products penetrate the lung or nasal epithelial and endothelial layers to enter the blood and produce alterations in blood and various other organs (Shoaf et al., 1989). Effects on the systemic immune system are discussed under section 5.2.2.1. Information regarding the effects of NO₂ on animal behaviour and brain enzymes is limited to a few studies that cannot be readily interpreted in terms of human risks and will not be discussed. The summary of other systemic effects is quite brief because the database suggests that effects on the respiratory tract are of more concern. A more detailed discussion of extrapulmonary responses can be found in US EPA (1993).

Results of research on the number of erythrocytes and leukocytes, haemoglobin concentration, and contents of erythrocyte membranes are inconsistent. In the only such study conducted below 9400 μg/m³ (5.0 ppm) NO₂, Nakajima & Kusumoto (1968)
found that the amount of methaemoglobin was not increased when mice were exposed to 1500 μg/m³ (0.8 ppm) NO₂ for 5 days. This topic was of interest because some (but not all) in vitro studies and high concentration in vivo NO₂ studies found methaemoglobin effects (US EPA, 1993).

Several studies have examined hepatic function either directly or indirectly after NO₂ exposure. Changes in serum chemistry (e.g., plasma cholinesterase, Drozdz et al., 1976; Menzel et al., 1977) suggest that NO₂ exposure may affect the liver. Xenobiotic metabolism appears to be affected by NO₂. A 3-h exposure to NO₂ concentrations as low as 470 μg/m³ (0.25 ppm) increased pentobarbital-induced sleeping times in female, but not male, mice (Miller et al., 1980; Graham et al., 1982). Higher exposures (9400 μg/m³, 5.0 ppm; 3 h) did not affect the level of hepatic cytochrome P-450 or the activities of several mixed-function oxidases in mice (Graham et al., 1982). Other authors found mixed effects (i.e. increase or decrease depending on exposures) on liver cytochrome P-450 levels in rats (Takano & Miyazaki, 1984; Takahashi et al., 1986). Significant decreases in cytochrome P-450 from rat liver microsomes were also found after 7 days of exposure to 752 or 7520 μg/m³ (0.4 or 4.0 ppm) NO₂, but not after exposure to 2260 μg/m³ (1.2 ppm) NO₂ (Mochitate et al., 1984). NADPH-cytochrome C reductase was reduced with 5 days of exposure to 7520 and 18 800 μg/m³ (4.0 and 10.0 ppm) NO₂. Drozdz et al. (1976) found decreased total liver protein and sialic acid, but increased protein-bound hexoses in guinea-pigs exposed to 2000 μg/m³ (1.05 ppm) NO₂, 8 h/day for 180 days. Liver alanine and aspartate aminotransferase activity was increased in the mitochondrial fraction but decreased in the cytoplasmic fraction of the liver. Electron micrographs of the liver showed intracellular oedema and inflammatory and parenchymal degenerative changes.

Takahashi et al. (1986) found that continuous exposure to 2260 and 7520 μg/m³ (1.2 and 4.0 ppm) NO₂ increased the amount of cytochrome P-450 and cytochrome b₅ in the kidney after 8 weeks of exposure. Continued exposure for 12 weeks resulted in less substantial increases in the amount and activity of the microsomal electron-transport enzymes. This is in contrast to the decreased activity in the liver.

Increases in urinary protein and specific gravity of the urine were reported by Sherwin & Layfield (1974) in guinea-pigs exposed continuously to 940 μg/m³ (0.5 ppm) NO₂ for 14 days.
Proteinuria was detected in another group of animals when the exposure was reduced to 752 μg/m³ (0.4 ppm) NO₂ for 4 h/day. Disc electrophoresis of the urinary proteins demonstrated the presence of albumin and alpha-, beta-, and gamma-globulins. The presence of high molecular weight proteins in urine is characteristic of the nephrotic syndrome. Differences in water consumption or in the histology of the kidney were not found.

Few studies have examined the effects of NO₂ on reproduction and development or the heritable mutagenic potential of NO₂. Exposure to 1800 μg/m³ (1.0 ppm) NO₂ for 7 h/day (5 days/week for 21 days) resulted in no alterations in spermatogenesis, germinal cells or interstitial cells of the testes of six rats (Kripke & Sherwin, 1984). Similarly, breeding studies by Shalamberidze & Tsereteli (1971) found that long-term NO₂ exposure had no effect on fertility. However, there was a statistically significant decrease in litter size and neonatal weight when male and female rats exposed to 2440 μg/m³ (1.3 ppm) NO₂, 12 h/day for 3 months were bred. In utero death due to NO₂ exposure resulted in smaller litter sizes, but no direct teratogenic effects were observed in the offspring. In fact, after several weeks, NO₂-exposed litters approached weights similar to those of controls.

Inhalation exposure of pregnant Wistar rats to NO₂ concentrations of 1000 and 10 000 μg/m³ for 6 h/day throughout gestation (21 days) was found to have maternal toxic effects and to induce developmental disturbances in the progeny (Tabacova et al., 1984; Balabaeva & Tabacova, 1985; Tabacova & Balabaeva, 1988). The maternal weight gain during gestation was significantly reduced at 10 000 μg/m³ (5.3 ppm). Pathomorphological changes, manifested at the higher exposure level, were found in maternal organs, e.g., desquamative bronchitis and bronchiolitis in the lung, mild parenchymal dystrophy and reduction of glycogen in the liver, and blood stasis and inflammatory reaction in the placenta. At gross examination, the placentae of the dams exposed to 10 000 μg/m³ were smaller in size than those of control rats. A marked increase of lipid peroxides was found in maternal lungs and particularly in the placenta at both exposure levels by the end of gestation (Balabaeva & Tabacova, 1985). Disturbances in the prenatal development of the progeny were registered, such as two- to four-fold increase in late post-implantation lethality at 1000 and 10 000 μg/m³ (0.5 and 5.3 ppm), respectively, as well as reduced fetal weight at term and stunted growth at 10 000 μg/m³ (Tabacova et al., 1984). These effects were significantly related to the content of lipid peroxides in the placenta, which was
suggestive of a pathogenetic role of placental damage (Tabacova & Balabaeva, 1988). Teratogenic effects were not observed, but dose-dependent morphological signs of embryotoxicity and retarded intrauterine development, such as generalized oedema, subcutaneous haematoma, retarded ossification and skeletal aberrations, were found at both exposure levels.

In the only study that has examined postnatal development, a significant delay in eye opening and incisor eruption was observed in the progeny of maternally exposed Wistar rats (Tabacova et al., 1985). The dams were exposed to 50, 100, 1000 or 10 000 µg/m³ (0.03, 0.05, 0.53 or 5.3 ppm) NO₂ for 6 h/day, 7 days/week throughout gestation, and the offspring were studied for 2-month post-exposure. Significant deficits in the onset of normal neuromotor development and reduced open field activity were detected in the offspring of dams exposed to 1000 and 10 000 µg/m³ NO₂.

5.3 Effects of mixtures containing nitrogen dioxide

Humans are exposed to pollutant mixtures in the ambient air, and, because pollutant interactions do occur, it is difficult to predict the effects of NO₂ in a mixture based upon the effects of NO₂ alone. Epidemiological studies (chapter 7), by their very nature, evaluate ambient air mixtures, but the presence of confounding variables makes it difficult to demonstrate a cause-effect relationship. In contrast, controlled animal and human clinical studies can often demonstrate the cause of a response, but are typically limited to binary or tertiary mixtures, which do not truly reflect ambient air exposures. When combinations of air pollutants are studied, there are a number of possible outcomes on human or animal responses. The result of exposure to two or more pollutants may be simply the sum of the responses to individual pollutants; this is referred to as additive. Another possibility is that the resultant response may be greater than the sum of the individual responses, suggesting some type of interaction or augmentation of the response; this is referred to as synergism. Finally, responses may be less than additive; this is often called antagonism. Generally, such human clinical studies, which focused on pulmonary function, have not found that acute exposures to NO₂ have any impact on the response to other co-occurring pollutants (e.g., O₃) or that additive effects occur. Animal toxicological studies, with a wider array of designs and end-points, have shown an array of interactions, including no interaction, additivity and synergism. Because no clear
understanding of NO2 interactions has yet emerged from this database, only a brief overview is provided here. A more substantive review can be found in US EPA (1993). Other animal studies sought to understand the effects of ambient air mixtures containing NO2 or vehicular combustion exhausts containing NOx. Generally, these studies provide useful information on the mixtures, but lack NO2-only groups, making it impossible to discern the influence of NO2. Therefore, this class of research is not described here, but is reviewed elsewhere (US EPA, 1993).

The vast majority of interaction studies have involved NO2 and O3. For lung morphology end-points, NO2 had no interaction with O3 (Freeman et al., 1974) or with sulfur dioxide (SO2) (Azouley et al., 1980) after a subchronic exposure. Some biochemical responses to NO2 plus O3 display no positive interaction or synergism. For example, Mustafa et al. (1984) found synergism for some endpoints (e.g., increased activities of O2 consumption and antioxidant enzymes), but no interaction for others (e.g., DNA or protein content) in rats exposed for 7 days. Ichinose & Sagai (1989) observed a species-dependence in regard to the interaction of O3 (752 µg/m3, 0.4 ppm) and NO2 (752 µg/m3, 0.4 ppm) after 2 weeks of exposure. Guinea-pigs, but not rats, had a synergistic increase in lung lipid peroxides. Rats, but not guinea-pigs, had synergistic increases in antioxidant factors (e.g., non-protein thiols, vitamin C, glucose-6-phosphate dehydrogenase, GSH peroxidase). Schlesinger et al. (1990) observed a synergistic increase in prostaglandin E2 and F2 in the lung lavage of acutely exposed rabbits; the response appeared to have been driven by O3. However, with 7 or 14 days of repeated 2-h exposures, only prostaglandin E2 was decreased and appeared to have been driven by NO2; there was no synergism (Schlesinger et al., 1991).

The infectivity model has been frequently used to study NO2-O3 mixtures. In this model, mice are exposed to O3 and NO2 alone or in mixtures for various durations. The mice are then challenged with an aerosol of viable bacteria. An increase in mortality indicates detrimental effects on lung host-defence mechanisms. Ehrlich et al. (1977) found additivity after acute exposure to mixtures of NO2 and O3. They reported synergism after subchronic exposures. Exposure scenarios involving NO2 and O3 have also been performed using a continuous baseline exposure to one concentration or mixture, with superimposed short-term peaks to a higher level. This body of work (Ehrlich et al., 1979; Gardner, 1980; Gardner et al., 1982; Graham et al., 1987) shows that differences in the pattern and concentrations of the exposure
are responsible for the increased susceptibility to pulmonary infection, without indicating clearly the mechanism controlling the interaction.

Some aerosols may potentiate response to NO\textsubscript{2} by producing local changes in the lungs that enhance the toxic action of co-inhaled NO\textsubscript{2}. The impacts of NO\textsubscript{2} and H\textsubscript{2}SO\textsubscript{4} on lung host defences have been examined by Schlesinger & Gearhart (1987) and Schlesinger (1987a). In the former study, rabbits were exposed for 2 h/day for 14 days to either 564 µg/m\textsuperscript{3} (0.3 ppm) or 1880 µg/m\textsuperscript{3} (1.0 ppm) NO\textsubscript{2}, or 500 µg/m\textsuperscript{3} H\textsubscript{2}SO\textsubscript{4} alone, or to mixtures of the low and high NO\textsubscript{2} concentrations with H\textsubscript{2}SO\textsubscript{4}. Exposure to either concentration of NO\textsubscript{2} accelerated alveolar clearance, whereas H\textsubscript{2}SO\textsubscript{4} alone retarded clearance. Exposure to either concentration of NO\textsubscript{2} with H\textsubscript{2}SO\textsubscript{4} resulted in retardation of clearance in a similar manner to that seen with H\textsubscript{2}SO\textsubscript{4} alone.

Schlesinger (1987a) used a similar exposure design, but different end-points. Exposure to 1800 µg/m\textsuperscript{3} (1.0 ppm) NO\textsubscript{2} with acid resulted in an increase in the numbers of PMNs in lavage fluid at all time points (not seen with either pollutant alone), and an increase in phagocytic capacity of AMs after two or six exposures. In contrast, exposure to 564 µg/m\textsuperscript{3} (0.3 ppm) NO\textsubscript{2} with acid resulted in depressed phagocytic capacity and mobility. The NO\textsubscript{2}/H\textsubscript{2}SO\textsubscript{4} mixture was generally either additive or synergistic, depending on the specific cellular end-point being examined.

Last et al. (1983) and Last & Warren (1987) found that exposure to high levels of NO\textsubscript{2} (≤ 9400 µg/m\textsuperscript{3}, 5.0 ppm) with very high concentrations of H\textsubscript{2}SO\textsubscript{4} (1 mg/m\textsuperscript{3}) caused a synergistic increase in collagen synthesis rate and protein content of the lavage fluid of rats.

Dogs were exposed for 68 months (16 h/day) to raw or photo-chemically reactive vehicle exhaust which included mixtures of NO\textsubscript{x} — one with a high NO\textsubscript{2} level and a low NO level (1200 µg/m\textsuperscript{3}, 0.64 ppm, NO\textsubscript{3} 310 µg/m\textsuperscript{3}, 0.25 ppm, NO\textsubscript{1}, and one with a low NO\textsubscript{2} level and a high NO level (270 µg/m\textsuperscript{3}, 0.14 ppm, NO\textsubscript{2} 2050 µg/m\textsuperscript{3}, 1.67 ppm, NO\textsubscript{2}) (Stara et al., 1980). Following the end of exposure, the animals were maintained for about 3 years in normal indoor air. Numerous pulmonary function, haematological, and histological end-points were examined at various times during and after exposure. The lack of an NO\textsubscript{2}-only or NO-only group precludes determination of the nature of the interaction. Even so, the main findings are of interest. Pulmonary function changes
appeared to progress after exposure ceased. Dogs in the high NO₂ group had morphological changes considered to be analogous to human centrilobular emphysema (see section 2.2.2.4). Because these morphological measurements were made after a 2.5- to 3-year holding period in clean air, it cannot be determined with certainty whether these disease processes abated or progressed during this time. This study suggests progression of damage after exposure ends.

5.4 Effects of other nitrogen oxide compounds

5.4.1 Nitric oxide

The toxicological database for NO is small, relative to NO₂. It is often difficult to obtain pure NO in air without some contamination with NO₂. An excellent review on the effects of NO on animals and humans has been prepared by Gustafsson (1993) for the Swedish Environmental Protection Agency. The following sections are based on the information in this review.

5.4.1.1 Endogenous formation of NO

Endogenous NO synthesis occurs by NO formation from physiological substrate (the amino acid L-arginine) in cells of many of the organ systems, such as nerve tissue, blood vessels and the immune system. NO has been found to be produced by at least three different oxygen-utilizing NO synthases, for purposes such as signalling in the nervous system, mediating vasodilation in both systemic and pulmonary circulation, and mediating cytotoxicity and host defence reactions in the immune system (Garthwaite, 1991; Barinaga, 1991; Moncada et al., 1991; McCall & Valance, 1992; Snyder & Bredt, 1992; Moncada, 1992). The impact of these findings for an understanding of the toxicological effects of NO is still difficult to assess.

The actions of endogenous NO can be divided into two main groups. The first group involves low concentrations of NO (nano- to picomolar) formed by constitutive enzymes in nerve and endothelial cells. Nitric oxide has local discrete actions exerted via activation of an enzyme, guanylate cyclase, in the target cell (Ignarro, 1989). The second group involves high concentrations of NO (micro- to nanomolar) formed by enzymes that can increase in amount through the induction of these enzymes upon exposure to bacterial toxins or to growth-regulating factors (cytokinins). The inducible NO formation occurs especially in macrophages and
Studies of the Effects of Nitrogen Oxides on Experimental Animals

neutrophil leukocytes and is important for the killing of bacteria and parasites, and possibly also for cytostasis in antitumour reactions (Hibbs et al., 1988; Ignarro, 1989; Moncada et al., 1991; Moncada, 1992).

For effects of inhaled NO it is important to consider that endogenous NO regulates pulmonary vascular resistance; it is found in small amounts in exhaled air and has been suggested to be necessary for normal oxygenation of the blood (Persson et al., 1990; Gustafsson et al., 1991).

5.4.1.2 Absorption of NO

Yoshida et al. (1981) found that < 10% of the NO "inhaled" by isolated perfused lungs of rabbits was absorbed. In normally breathing humans, 85 to 92% of NO was absorbed at concentrations ranging from 400 to 6100 µg/m³ (0.33 to 5.0 ppm) (Wagner, 1970; Yoshida & Kasama, 1987); values for NO₂ were 81 to 90% (Wagner, 1970). Absorption of NO with exercise was 91 to 93% in humans (Wagner, 1970). Yoshida et al. (1980) found the percentage of absorption of NO in rats acutely exposed to 169 300 µg/m³ (138 ppm), 331 300 µg/m³ (270 ppm) and 1 079 800 µg/m³ (880 ppm) to be 90%, 60% and 20%, respectively. The progressive decrease in absorption was ascribed to an exposure-induced decrease in ventilation. In dogs exposed to vehicle exhaust mixtures, 73% of the constituent NO was removed by the nasopharyngeal region; this compared to 90% removal for NO₂ (Vaughan et al., 1969). Thus, respiratory tract absorption of NO has some similarities to that for NO₂, in spite of solubility differences. The lower solubility of NO may, however, result in greater amounts reaching the pulmonary region, where it may then diffuse into blood and react with haemoglobin (Yoshida & Kasama, 1987). In vivo exposures seem to indicate that NO has a faster rate of diffusion through tissue than NO₂ (Chiodi & Mohler, 1985).

5.4.1.3 Effects of NO on pulmonary function, morphology and host lung defence function

No change in respiratory function was found in guinea-pigs exposed to NO at 19 600 µg/m³ (16 ppm) or 61 300 µg/m³ (50 ppm) for 4 h (Murphy et al., 1964). Increased airway responsiveness to acetylcholine was observed in guinea-pigs exposed to 6130 µg/m³ (5 ppm) NO for 30 min, twice a week for 7 weeks. In sheep, significant reversal of vasoconstriction to an
infused thromboxane analogue was seen with acute exposure to 6130 µg/m³ NO (Fratacci et al., 1991). At the same exposure level, hypoxic vasoconstriction was significantly diminished and was nearly abolished at 49 000 µg/m³ (40 ppm) NO in inhaled air (Frostell et al., 1991).

Reversal of methacholine-induced bronchoconstriction by NO has been reported in guinea-pigs at 6130 µg/m³ (5 ppm) (Dupuy et al., 1992), while in rabbits full reversal of methacholine bronchoconstriction was seen at 98 100 µg/m³ (80 ppm) (Högman et al., 1993). Relaxation of bronchial smooth muscle can be exerted in vitro by mechanisms dependent on an intact airway epithelium. An endogenous muscle-relaxing factor released by the epithelium has been suggested, but it is not clear whether it is endogenous NO (Barnes, 1993).

The few studies that have examined histological response to non-lethal levels of NO are outlined in Table 38. With chronic exposure, the morphological changes seen are similar to those with NO₂ (see section 5.2.2.4 on morphological effects of NO₂), except that NO levels needed to produce them are higher. Additionally, Hugod (1979) noted that the absence of NO-induced alterations in the alveolar epithelium suggested that the observed responses occurred after absorption of NO; that is, they were not caused by direct action of deposited NO. Perhaps higher exposure concentrations of NO are needed for direct toxic action (e.g., results of Holt et al., 1979). Some of the effects seen by Oda et al. (1976) with 12 270 µg/m³ (10.0 ppm) NO may have been due to the presence of 1880 to 2820 µg/m³ (1.0 to 1.5 ppm) NO₂ in the exposure atmosphere.

It is important to note that in all existing studies of NO toxicity in the lungs, histological evaluation of the lungs was rudimentary and no quantitative measurements were carried out to test for airspace enlargement or destruction.

A recent study (Mercer et al., 1995) suggests that NO may be more potent than NO₂ in introducing certain changes in lung morphology. More specifically, male rats were exposed to either NO or NO₂ at 0.5 ppm with twice daily 1-h spikes of 1.5 ppm for 9 weeks. The number of pores of Kohn and detached alveolar septa were evaluated by electron microscopy, using stereological procedures for the study of lung structure that involved morphometric analyses of electron micrographs. The average number of
<table>
<thead>
<tr>
<th>NO₂ concentration (ppm)</th>
<th>NO₂ concentration (mg/m³)</th>
<th>Animal Species</th>
<th>Reference</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 240 0.08 ppm</td>
<td>2 240 0.08 ppm</td>
<td>Rat</td>
<td>Azoulay et al. (1977)</td>
<td>Slight emphysema-like alterations of alveoli</td>
</tr>
<tr>
<td>2.4 2950 0.01-0.04 ppm</td>
<td>2.4 2950 0.01-0.04 ppm</td>
<td>Mouse</td>
<td>Oda et al. (1982)</td>
<td>No difference from control</td>
</tr>
<tr>
<td>5 6150 0.1 ppm</td>
<td>5 6150 0.1 ppm</td>
<td>Rabbit</td>
<td>Hugod (1979)</td>
<td>Oedema; thickening of alveolo-capillary membrane due to fluid in interstitial space; fluid-filled vacuoles seen in arteriolar endothelial cells and at junctions of endothelial cells; no changes in alveolar epithelium; no inflammation</td>
</tr>
<tr>
<td>6 6150 0.1 ppm</td>
<td>6 6150 0.1 ppm</td>
<td>Mouse</td>
<td>Holt et al. (1979)</td>
<td>Enlarged air spaces in lung periphery; paraseptal emphysema; some haemorrhage; some congestion in alveolar septa; increased concentration of goblet cells in bronchi</td>
</tr>
<tr>
<td>10 12300 1.1-1.5 ppm</td>
<td>10 12300 1.1-1.5 ppm</td>
<td>Mouse</td>
<td>Call et al. (1978)</td>
<td>Bronchiolar epithelial hyperplasia; hypertrophy; increased ratio of lung to body weight</td>
</tr>
</tbody>
</table>

Modified from US EPA (1993)
pores per lung for the NO group exceeded by ~2.5 times the mean number for the NO₂ groups, which was more than 10 times that for controls. Analogously, the average number of detached septa per lung was significantly higher for the NO group (X = 117) than the NO₂ group (X = 20) or the controls (X = 4). There was also a statistically significant 30% reduction in interstitial cells in the NO group, but no significant differences in the other parenchymal cell types between the controls and the NO- or NO₂-exposed groups. Lastly, the thickness of the interstitial space was reduced for the NO group (X = 0.24 μm versus 0.32 μm for controls) but not for the NO₂ group (X = 0.29 μm), and epithelial cell thickness did not differ between the groups.

The effects of NO on host defence function of the lungs has been examined in two studies. Holt et al. (1979) found immunological alterations in mice exposed to 12,270 μg/m³ (10 ppm) NO for 2 h/day (5 days/week for 30 weeks). However, interpretation is complicated by the duration dependence of some of the responses (e.g., an enhancement of the humoral immune response to SRBCs was seen at 10 weeks, but this was not evident at the end of the exposure series). The effects of NO on bacterial defences were examined by Azoulay et al. (1981). Male and female mice were exposed continuously to 3760 μg/m³ (2.0 ppm) NO for 6 h to 4 weeks to assess the effect on resistance to infection induced by a bacterial aerosol administered after each NO exposure. There were no statistically significant effects for either sex at any of the time points studied.

5.4.1.4 Metabolic effects

Mice exposed to NO concentrations of 12,300 to 25,800 μg/m³ (10 to 21 ppm) for 3 h daily for 7 days showed no change in the levels of reduced glutathione in their lungs (Watanabe et al., 1980). In vitro data indicate that NO stimulates guanylate cyclase and therefore leads to smooth muscle relaxation and vasodilation and functional effects on the nervous system (Katsuki et al., 1977; Ignarro, 1989; Garthwaite, 1991; Moncada et al., 1991). These effects are probably responsible for vasodilation in the pulmonary circulation and an acute bronchodilator effect of inhaled NO. However, it is unclear whether other effects might be exerted from ambient NO via this pathway. Due to the rapid inactivation of NO in haemoglobin, internal organs other than the lungs are unlikely to be affected directly by cyclic GMP-mediated vasodilator influence from ambient concentrations of NO.
Methaemoglobin formation, via the formation of nitrosylhaemoglobin (Oda et al., 1975, 1979, 1980a,b; Case et al., 1979; Nakajima et al., 1980) and subsequent oxidation with oxygen, is well known (Kon et al., 1977; Chiiodi & Mohler, 1983). During NO exposure of mice to 24 500 to 98 100 µg/m³ (20-80 ppm), the levels of methaemoglobin were found to increase exponentially with the NO concentration (Oda et al., 1980b). After the cessation of NO exposure, methaemoglobin decreased rapidly, with a halftime of only a few minutes. In humans the ability to reduce methaemoglobin varies genetically and is lower in infants. Of the NO reaction products with haemoglobin, methaemoglobin attains higher levels than nitrosylhaemoglobin (Maeda et al., 1987). Exposure of mice to 2940 µg/m³ (2.4 ppm) NO for 23-29 months resulted in nitrosylhaemoglobin levels at 0.01%, while the maximal methaemoglobin level was 0.3% (Oda et al., 1980b). At 12 300 µg/m³ for 6.5 months the nitrosylhaemoglobin level was 0.13% and the level of methaemoglobin was 0.2% (Oda et al., 1976). Rats exposed to 2450 µg/m³ (2 ppm) continuously for six weeks showed no detectable methaemoglobin (Azoulay et al., 1977).

5.4.1.5 Haematological changes

Mice exposed to NO at 11 070 µg/m³ (9 ppm) for 16 h had decreased iron transferrin (Case et al., 1979), and when exposed to 12 300 µg/m³ (10 ppm) for 6.5 months had increased leucocyte count and proportion of polymorphonuclear cells (Oda et al., 1976). Red blood cell morphology, spleen weight and bilirubin were also affected. A slight increase in haemolysis was seen in mice exposed to 2940 µg/m³ (2.4 ppm) of NO (Oda et al., 1980a).

5.4.1.6 Biochemical mechanisms for nitric oxide effects: reaction with iron and effects on enzymes and nucleic acids

NO has an affinity for haem-bound iron which is two times higher than that of carbon monoxide. This affinity leads to the formation of methaemoglobin and the stimulation of guanylate cyclase. Furthermore, NO reacts with thiol-associated iron in enzymes and eventually displaces the iron. This is a possible mechanism for the cytotoxic effects of NO (Hibbs et al., 1988; Weinberg, 1992). In vitro, the NO donor sodium nitroprusside has been shown to mobilize iron from ferritin (Reif & Simmons, 1990). NO might possibly modulate arachidonic acid metabolism via interference with iron (Kanner et al., 1991a,b).
NO inhibits aconitase, an enzyme in the Krebs cycle, and also complex 1 and 2 of the respiratory chain (Hibbs et al., 1988; Persson et al., 1990; Stadler et al., 1991). Permanent modification of haemoglobin has been found; possibly via deamination (Moriguchi et al., 1992). NO can also deaminate DNA, evoke DNA chain breaks, and inhibit DNA polymerase and ribonucleotide reductase (Wink et al., 1991; Lepoivre et al., 1991; Kwon et al., 1991; Nguy et al., 1992). NO might be antimitogenic and inhibit T cell proliferation in rat spleen cells (Fu & Blankenhorn, 1992), and NO donors inhibit DNA synthesis, cell proliferation, and mitogenesis in vascular tissue (Garg & Hassid, 1989; Nakaki et al., 1990). ADP (adenosine diphosphate) ribosylation is stimulated by NO-generating agents (Nakaki et al., 1990).

Substantial in vitro evidence has recently been published describing other effects of NO in tissues. These include: inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) via ADP ribosylation (Alheid et al., 1987; Dimmeler et al., 1992); macrophage mediated-nitric oxide dependent mechanisms which include inhibition of the electron transport chain (Nathan, 1992); inhibition of DNA synthesis (Hibbs et al., 1988); inhibition of protein synthesis (Curran et al., 1991) and decrease in cytosolic free calcium by a cGMP-independent mechanism (Garg & Hassid, 1991).

5.4.2 Nitric acid

There have been only a few toxicological studies of HNO₃, which exists in ambient air generally as a highly water-soluble vapour. A few investigators have examined the histological response to instilled HNO₃ (usually 1%), a procedure used in developing models of bronchiolitis obliterans in various animals, namely dogs, rabbits and rats (Totten & Moran, 1961; Greenberg et al., 1971; Gardiner & Schanker, 1976; Mink et al., 1984). However, the relevance of such instillation studies is questionable, except to provide information for the design of inhalation studies.

Only two studies have been designed specifically to examine the pulmonary response to pure HNO₃ vapour. Abraham et al. (1982) exposed both normal sheep and allergic sheep (i.e., having airway responses similar to those occurring in humans with allergic airway disease) for 4 h to 4120 µg/m³ (1.6 ppm) HNO₃ vapour. The exposure, using a “head-only” chamber, decreased specific pulmonary flow resistance in both groups of sheep; this indicated the absence of any bronchoconstriction. Allergic, but not normal,
sheep showed increased airway reactivity to carbachol, both immediately and 24 h after HNO₃ exposure. In another study, rats exposed for 4 h to 1000 µg/m² (0.38 ppm) HNO₃ vapour or for 4 h/day for 4 days to 250 µg/m³ (0.1 ppm) HNO₃ showed a decrease in stimulated or unstimulated respiratory burst activity of alveolar macrophages (AMs) obtained by lavage, as well as an increase in elastase inhibitory capacity of BAL (Nadziejko et al., 1992).

5.4.3 Nitrates

Only one inhalation study conducted at levels ≤ 1 mg/m³ NO₂ has been reported. Busch et al. (1986) exposed rats and guinea-pigs with either normal lungs or elastase-induced emphysema to ammonium nitrate aerosols at 1 mg/m³ for 6 h/day, 5 days/week for 4 weeks. Using both light and electron microscopy, the investigators concluded that there were no significant effects of exposure on lung structure.

5.5 Summary of studies of the effects of nitrogen compounds on experimental animals

Responses to NO₂ exposure have been observed in several laboratory animal species, resulting in the conclusion that these effects could occur in humans. In addition, mathematical dosimetry models suggest that the greatest dose of NO₂ is delivered to the same region in both animal and human lungs (i.e., the centriacinar region which is the junction of the conducting airway with the gas exchange area). Thus, the responses of laboratory animals can be qualitatively extrapolated to humans.

NO₂ exposure causes lung structural alterations. Exposure to 3760 µg/m³ (2.0 ppm) for 3 days has resulted in centriacinar damage, including damaged cilia and alveolar wall oedema. Prolonged exposures produce changes in the cells lining the centriacinar region, and the tissue in this region (i.e., alveolar interstitium) becomes thicker. These effects were seen in rats exposed to 940 µg/m³ (0.5 ppm) baseline with brief peaks of 2800 µg/m³ (1.5 ppm) for 6 weeks or exposures to 940 µg/m³ (0.5 ppm) NO₂ for 4 to 6 months.

Several animal studies clearly demonstrate that chronic exposure to concentrations of NO₂ ≥ 9400 µg/m³ (≥ 5.0 ppm) can cause emphysema of the type seen in human lungs. Increased lung
distensibility was reported in mice exposed to 375 µg/m³ (0.2 ppm) with peaks of 1500 µg/m³ (0.8 ppm) after 1 year of exposure.

NO₂ increases susceptibility to bacterial and viral pulmonary infections in animals. Reduced phagocytic activity and reduced mobility were observed in AMs from rabbits exposed for 13 days to 500 µg/m³ (0.3 ppm). The lowest observed concentration that increases lung susceptibility to bacterial infections after acute exposure is 3750 µg/m³ (2.0 ppm) NO₂ (a 3-h exposure study in mice). Acute (17 h) exposures to ≥4250 µg/m³ (≥2.3 ppm) NO₂ also decrease pulmonary bactericidal activity in mice. After long-term exposures (e.g., 3 to 6 months) to 940 µg/m³ (0.5 ppm) NO₂, mice have decreased resistance to lung bacterial infections. Exposure of mice for 1 year to 375 µg/m³ (0.2 ppm/week) with 1480 µg/m³ (0.8 ppm) spike followed by infection with streptococcus resulted in increased mortality. NO₂ also increases lung susceptibility to viral infections in mice. Subchronic (7-week) exposures to concentrations as low as 470 µg/m³ (0.25 ppm) NO₂ can alter the systemic immune system in mice.

NO₂ exposure has been shown to cause a clear dose-related decrease in pulmonary antibacterial defences. Decreases in pulmonary antibacterial defences occurred at concentrations ranging from 7520 µg/m³ (4 ppm) for Staphylococcus aureus to 37500 µg/m³ (20 ppm) for Proteus mirabilis. Dose-response increases in bacterial-induced mortality in mice was demonstrated with continuous exposure to 940 µg/m³ (0.5 ppm) after 3 months.

When the relationship of NO₂ exposure concentration and duration was studied, concentration had more influence than duration on the outcome. This conclusion is primarily based on investigations of lung antibacterial defences of mice, which also indicate that the exposure pattern (e.g., baseline level with daily peaks of NO₂ or exposure 24 h/day versus 6 to 7 h/day) has an impact on the study results.

Structural changes in the lung become more severe as exposure progresses from weeks to months at a given NO₂ concentration. Longer exposures resulted in effects at lower concentrations.

NO₂ showed positive effects in some studies with Salmonella strain TA100 and caused DNA strand breaks in a mammalian cell culture. NO seems to be less active. High concentrations of NO₂ have induced mutations in lung cells in vivo, but not in other
organs. There are no classical chronic bioassays for carcinogenicity. Studies concerning enhancement of spontaneous tumours, co-carcinogenic effects, or facilitation of the metastases of tumours to the lung are inadequate to form conclusions. Possible secondary effects concern the \textit{in vivo} formation of nitrite and nitrosamines and atmospherically formed mutagenic reaction products from NO\textsubscript{2} and hydrocarbons.

The effects of exposure to mixtures of NO\textsubscript{2} and other pollutants are dependent on the exposure regimen, species and end-point measured. Most mixture research involves NO\textsubscript{2} and O\textsubscript{3} and shows that additivity and synergism can occur. A similar conclusion can be drawn from the more limited research with NO\textsubscript{2} and sulfuric acid. Findings of either additivity or synergism are of concern because of the ubiquitous co-occurrence of NO\textsubscript{2} and O\textsubscript{3}. Extrapolation of these findings is not currently possible.

NO is a potent vasodilator and effects can be demonstrated with inhaled concentrations of approximately 6130 $\mu$g/m$^3$ (5 ppm) in sheep and guinea-pigs. NO also reduces resistance to bacterial infection via the inhalation route in female mice exposed to 2452 $\mu$g/m$^3$ (2 ppm). Morphological alterations in the alveoli and thickening of the alveolocapillary membrane are seen in rabbits at 6130 $\mu$g/m$^3$. Methaemoglobin formation is seen at concentrations above 12 260 $\mu$g/m$^3$ (10 ppm).

NO\textsubscript{2} acts as a strong oxidant. Unsaturated lipids are readily oxidized with peroxides as the dominant product. Both ascorbic acid and alpha-tocopherol inhibit the peroxidation of unsaturated lipids. When ascorbic acid is sealed within bi-layer liposomes, NO\textsubscript{2} rapidly oxidizes the sealed ascorbic acid. The protective effects of alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C) in animals and humans are due to the inhibition of NO\textsubscript{2} oxidation. NO\textsubscript{2} also oxidizes membrane proteins. The oxidation of either membrane lipids or proteins results in the loss of cell permeability control. The lungs of NO\textsubscript{2}-exposed humans and experimental animals have larger amounts of protein within the lumen. The recruitment of inflammatory cells and the remodelling of the lung are a consequence of these events.

The oxidant properties of NO\textsubscript{2} also induce the peroxide detoxification pathway of glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase. Increases in the peroxide detoxification pathway occur in animals in a roughly dose-response relationship following NO\textsubscript{2} exposure.
The mechanism of action of NO is less clear. NO is readily oxidized to NO₂ and then peroxidation occurs. Because of concomitant exposure to some NO₂ in NO exposures, it is difficult to discriminate NO effects from those of NO₂. NO is, however, a potent second messenger modulating a wide variety of essential cellular functions.

Peroxyacetyl nitrate (PAN) decomposes in water generating hydrogen peroxide. Little is known of the mechanism of action, but oxidative stress is likely for PAN and its congeners.

Inorganic nitrates may act by alterations in intracellular pH. Nitrate ion is transported into Type 2 cells, acidifying the cell. Nitrate also mobilizes histamine from mast cells. Nitrous acid could also act to alter intracellular pH, but this mechanism is unclear.

The mechanisms of action of the other nitrogen oxides are unknown at present.
6. CONTROLLED HUMAN EXPOSURE STUDIES OF NITROGEN OXIDES

6.1 Introduction

The effects of nitrogen oxides (NOx) on human volunteers exposed under controlled exposure conditions are evaluated in this chapter. Of the NOx species typically found in the ambient air, NO2 has been the most extensively studied. Nitric oxide (NO), nitrates, nitrous acid and nitric acid also have been evaluated and are discussed here, as are investigations of mixtures of NOx and other co-occurring pollutants. A more extensive detailed review of this literature can be found in US EPA (1993).

Most volunteers for human clinical studies are young, healthy adult males, but other potentially susceptible subpopulations, especially asthmatics, patients with chronic obstructive pulmonary disease (COPD), children and the elderly have also been studied. Many exposures are conducted while the volunteer performs some form of controlled exercise. The exercise increases ventilation, which increases the mass of pollutant inhaled per unit time and may alter the distribution of the dose within the lung. More information on NO2 dosimetry is presented in chapter 5. Important methodological and experimental design considerations for controlled human studies have been discussed in greater detail by Folinsbee (1988).

In many human clinical studies of NO2 exposure, both pulmonary function and airway responsiveness to bronchoconstrictors have been measured. Spirometric measurements of lung volume, as well as measurements of airway resistance, ventilation volume, breathing pattern, and other tests provide information about some of the basic physiological functions of the lung. Dynamic spirometry tests (forced expiratory tests such as forced expiratory volume in 1 second (FEV1), maximal and partial flow-volume curves (including those using gases of different densities such as helium), peak flow measurements, etc.), and measurements of specific airway resistance/conductance (SRw, SGw) are also used. Most of these tests evaluate large airway function. However, since NO2 deposition occurs primarily around the junction of the tracheobronchial and pulmonary regions (section 5.2.1), many of these tests may not provide the necessary information to evaluate fully the effects of NO2. Other tests that may evaluate small airway function (e.g., multiple breath nitrogen washout tests,
closing volume tests, aerosol deposition/distribution tests, density
dependence of flow-volume curves, and frequency dependence of
dynamic compliance) are less frequently used, and the extent to
which they indicate small airways function is not clearly
established. As discussed below, NO₂ can increase airway
responsiveness to chemicals that cause bronchoconstriction, such
as histamine or cholinergic agonists (i.e., acetylcholine, carbachol
or methacholine). Other challenge tests use allergens, exercise,
hypertonic saline or cold-dry air. Responses are usually measured
by evaluating changes in airway resistance (R₉₀,) or spirometry (e.g.
FEV₁) after each dose of the challenge is administered. Generally,
asthmatics are significantly more responsive than healthy normal
subjects to these types of airway challenge (O'Connor et al., 1987).
However, there is some overlap between the most responsive
healthy subjects and the least responsive (to histamine) asthmatics
(Pattemore et al., 1990).

In the following sections, the changes in pulmonary function
and airway responsiveness after NO₂ exposure in healthy subjects
are discussed. Responses of asthmatics and patients with chronic
obstructive pulmonary disease (COPD) are then evaluated. A brief
note regarding age-related susceptibility is followed by a review
of the effects of NO₂ on pulmonary host defences and on bio-
chemical markers in lung lavage fluid or in the blood. The effects
of two other oxidized nitrogen compounds, NO and nitric acid
vapour are also discussed. Finally, the effects of mixtures of
oxidized nitrogen compounds (NO₂, NO, HNO₃) with other
gaseous or particulate pollutants are considered. An overall
summary is presented at the end of the chapter.

6.2 Effects of nitrogen dioxide

6.2.1 Nitrogen dioxide effects on pulmonary function and airway
responsiveness to bronchoconstrictive agents

Much research has focused on NO₂-induced changes in pul-
monary function and airway responsiveness to bronchoconstrictive
agents. Healthy adults do not typically respond to low levels of
NO₂ (< 1880 μg/m³, 1 ppm). However, asthmatics appear to be the
most susceptible members of the population (section 6.2.1.2).
Asthmatics are generally much more sensitive to inhaled broncho-
constrictors. The potential addition of an NO₂-induced increase
in airway response to the already heightened responsiveness to
other substances raises the possibility of exacerbation of asthma by
Controlled Human Exposure Studies of Nitrogen Oxides

NO\textsubscript{2}. Another potentially susceptible group includes patients with COPD (section 6.2.1.3). A major concern with COPD patients is the absence of an adequate pulmonary reserve, so that even a relatively small alteration in lung function in these individuals could potentially cause serious problems. In addition, both adolescents and the elderly have been evaluated, to determine whether differential age-related susceptibility exists (section 6.2.1.4).

6.2.1.1 Nitrogen dioxide effects in healthy subjects

The effects of NO\textsubscript{2} levels greater than 1880 \(\mu g/m^3\) (1.0 ppm) on respiratory function in healthy subjects have been examined in several studies (Table 39). Early work indicated that NO\textsubscript{2} increased \(R\text{\textsubscript{T}}\) or total respiratory resistance (\(R\text{\textsubscript{T}}\)) at concentrations above 2820 \(\mu g/m^3\) (1.5 ppm) in healthy volunteers (Abe, 1967; Von Nieding et al., 1970, 1973a, 1979; Von Nieding & Wagner, 1977). Although Bell & Ulmer (1976) found a small but statistically significant increase in \(R\text{\textsubscript{T}}\) after a 2-h exposure to \(\geq 4700 \mu g/m^3\) (\(\geq 2.5\) ppm) NO\textsubscript{2}, the response was not appreciably increased by raising the NO\textsubscript{2} concentration to 9400 or 14 100 \(\mu g/m^3\) (5.0 or 7.5 ppm). Also, airway responsiveness to acetylcholine was increased after exposure to 14 100 \(\mu g/m^3\) for 2 h or to 9400 \(\mu g/m^3\) for 14 h, but not after the 2-h exposures to \(\leq 9400 \mu g/m^3\).

In contrast, some investigators found no effects at high concentrations. For example, a 75-min exposure with light and heavy exercise to 7520 \(\mu g/m^3\) (4.0 ppm) NO\textsubscript{2} did not affect \(R\text{\textsubscript{T}}\) (Linn et al., 1985b), and a 1-h resting exposure to 3760 \(\mu g/m^3\) (2 ppm) did not cause a change in lung volume, flow-volume characteristics on either full or partial expiratory flow-volume (PEFV) curves, or SG\textsubscript{av} (Möhnenin, 1987b, 1988). However, NO\textsubscript{2} did increase airway responsiveness to methacholine (Möhnenin, 1987b, 1988).

Goings et al. (1989) found no effects of exposure to NO\textsubscript{2} at 1880, 3760 or 5640 \(\mu g/m^3\) (1, 2 or 3 ppm; for 2 h/day on 3 consecutive days) on respiratory symptoms, lung function or airway reactivity to methacholine. Laboratory-induced influenza virus infection did not alter airway responsiveness in either sham (clean air) or NO\textsubscript{2} exposure groups. The infectivity portion of this study is discussed in section 6.2.2.

The influence of exposure pattern was examined by Frampton et al. (1991), using healthy subjects exposed for 3 h to either
Table 39. Effects of nitrogen dioxide (NO\textsubscript{2}) on lung function and airway responsiveness of healthy subjects

<table>
<thead>
<tr>
<th>NO\textsubscript{2} concentration</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/ gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu g/m^3)</td>
<td>ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>0.1</td>
<td>60</td>
<td></td>
<td>15 M</td>
<td>23-29 years, No symptoms; no odour detection; Hazucha et al. (1982, 1983)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>240</td>
<td>6</td>
<td>Normal adults</td>
<td>No effects of NO\textsubscript{2}</td>
<td></td>
<td>Seckner et al. (1960)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>226</td>
<td>0.12</td>
<td>60</td>
<td></td>
<td>4 M/6 F</td>
<td>13-18 years</td>
<td>No effects on lung function.</td>
<td></td>
</tr>
<tr>
<td>230</td>
<td>0.12</td>
<td>40</td>
<td>10</td>
<td>32.5</td>
<td>3 M/7 F</td>
<td>14-19 years</td>
<td>No effects on R\textsubscript{t} or spirometry.</td>
</tr>
<tr>
<td>338</td>
<td>0.18</td>
<td>40</td>
<td></td>
<td>4 M/6 F</td>
<td>15-19 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>230</td>
<td>0.12</td>
<td>20</td>
<td></td>
<td>5 M/4 F</td>
<td>20-36 years, NS</td>
<td>Suggestion of change in SR\textsubscript{aw} in normals; SR\textsubscript{aw} tended to increase at 476 (\mu g/m^3) and tended to decrease at 910 (\mu g/m^3). Analysis of variance indicates no significance. No effects on bronchial reactivity. Median odour threshold 75 (\mu g/m^3).</td>
<td></td>
</tr>
<tr>
<td>Study Code</td>
<td>Concentration (mg/m³)</td>
<td>Exposure Duration (h)</td>
<td>Age Group</td>
<td>Gender</td>
<td>Effects on Lung Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>------------</td>
<td>--------</td>
<td>--------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>282</td>
<td>0.15</td>
<td>120</td>
<td>60</td>
<td>50 W</td>
<td>6 M</td>
<td>19-24 years, no symptoms, no pulmonary function effects. Suggested individual changes in $SG_{aw}$.</td>
<td>Kagawa &amp; Tsuru (1979), Johnson et al. (1990)</td>
</tr>
<tr>
<td>338</td>
<td>0.18</td>
<td>30</td>
<td>L = 25</td>
<td>18 M</td>
<td>9 M</td>
<td>18-23 years, &quot;collegiate athletes&quot;</td>
<td>No change in lung function.</td>
</tr>
<tr>
<td>364</td>
<td>0.3</td>
<td>60</td>
<td>Healthy</td>
<td>50 M</td>
<td>6</td>
<td>19-25 years</td>
<td>Possible small increase in $R_{aw}$ at 508 mg/m³ (0.27 ppm).</td>
</tr>
<tr>
<td>508</td>
<td>0.27</td>
<td>120</td>
<td>60</td>
<td>50 W</td>
<td>6</td>
<td>19-25 years</td>
<td>No effect on $SG_{aw}$.</td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>225</td>
<td>30 (3 x 10)</td>
<td>30</td>
<td>10 M/10 F</td>
<td>20-49 years, (FEV₁/FVC 76-95%)</td>
<td>No symptom, lung function or airway reactivity responses to carbachol for either of the 20-49 year or the 49-69 year age groups.</td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>225</td>
<td>21 (3 x 7)</td>
<td>30-40</td>
<td>10 M/10 F</td>
<td>49-69 years, (FEV₁/FVC 72-84%)</td>
<td>No change in lung function or airway reactivity responses to carbachol for either of the 20-49 year or the 49-69 year age groups.</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>120</td>
<td>Light/ moderate</td>
<td>15</td>
<td>10</td>
<td>Healthy, three ex-smokers in group</td>
<td>Decreased quasistatic compliance. Non-random exposure sequence air-NO₂. No change in spirometry or resistance. Apparent compliance change may be due to exposure order.</td>
</tr>
</tbody>
</table>
Table 39 (contd).

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>Exposure duration</th>
<th>Exercise duration</th>
<th>Exercise ventilation</th>
<th>Number of subjects</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³</td>
<td>(min)</td>
<td>(min)</td>
<td>(litres/min)</td>
<td>gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>940 ppm</td>
<td>0.5</td>
<td>120</td>
<td>15</td>
<td>10</td>
<td>Normal adults</td>
<td>Decreased static lung compliance.</td>
<td>Kulle (1982)</td>
</tr>
<tr>
<td>940 ppm</td>
<td>0.5</td>
<td>240</td>
<td>30</td>
<td>55</td>
<td>10 M</td>
<td>No significant effects on spirometry or R_ex.</td>
<td>Stacy et al. (1983)</td>
</tr>
<tr>
<td>1128 ppm</td>
<td>0.6</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>8 M/8 F</td>
<td>No statistically significant changes in lung function due to NO₂ exposure in either age group.</td>
<td>Drechsler-Parks et al. (1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 M/8 F</td>
<td>18-26 years, NS</td>
<td></td>
</tr>
<tr>
<td>1128 ppm</td>
<td>0.6</td>
<td>180</td>
<td>60</td>
<td>40</td>
<td>7 M/2 F</td>
<td>No change in spirometry, R_ex or carbachol reactivity.</td>
<td>Frampton et al. (1989a)</td>
</tr>
<tr>
<td>94 with 3760 spikes</td>
<td>0.05 with 2.0 spikes</td>
<td>135</td>
<td>60</td>
<td>11 M/4 F</td>
<td>Non-reactive (carbachol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 39 (contd).

| (1) 1128 | (1) 0.6 | 180 | 60 | 39 | 6 M/2 F | 30.3 ± 1.4 years, NS | There were no changes in airway mechanics (FVC, FEV₁, SGₐ). Responsiveness to carbachol was significantly increased after 2820 µg/m³ NO₂ (Group 3) but not after the other exposures (Groups 1 and 2). Degree of baseline responsiveness to carbachol was not related to response after 2820 µg/m³. Frampton et al. (1991) |
| (2) Var. | (2) Var. (0.05 background with 3 × 15 min at 2.0 ppm) | 180 | 50 | 43 | 11 M/4 F | 25.3 ± 1.2 years, NS |
| (3) 2820 | (3) 1.5 | 180 | 60 | 39 | 12 M/3 F | 32.6 ± 1.6 years, NS |
| 1128 | 0.6 | 120/day for 4 days | 60 | = 30-40 | 4 M/1 F | NS, 21-36 years, FEV₁/PVC% range 73-83%, "normal" methacholine responsiveness |
| 1128 | 0.6 | 60 | 60 | 70 | 50 | 20 M | Healthy | No effect of NO₂ on spirometry or airway resistance. Adams et al. (1987) |
| 1166 | 0.62 | 120 | 15 | 33 | 5 M | Healthy | No significant pulmonary function responses attributed to NO₂ exposure. Folinabbee et al. (1978) |
Table 39 (contd).

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>NO₂ concentration</th>
<th>Exercise duration</th>
<th>Exercise duration</th>
<th>Number of subjects/gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³ ppm</td>
<td>µg/m³ ppm</td>
<td>(min)</td>
<td>(min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1516-3760</td>
<td>0.7-2.0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>Increased resistance 10 min after exposure.</td>
<td>Increased resistance 10 min after exposure.</td>
<td>Suzuki &amp; Ishikawa (1965)</td>
</tr>
<tr>
<td>1316</td>
<td>0.7</td>
<td>60</td>
<td>5</td>
<td>19-22 years, 3 of 5 were investigators</td>
<td>No effects on airway conductance.</td>
<td>No effects on airway conductance.</td>
<td>Toyama et al. (1981)</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>120 (2 consecutive days)</td>
<td>60</td>
<td>16</td>
<td>Healthy</td>
<td>Air-NO₂-NO₂ fixed exposure sequence. 1.5% decrease in FVC after second day of NO₂. Not clear that the decreased FVC is an NO₂ effect or an order effect. No other effects.</td>
<td>Air-NO₂-NO₂ fixed exposure sequence. 1.5% decrease in FVC after second day of NO₂. Not clear that the decreased FVC is an NO₂ effect or an order effect. No other effects.</td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>120/day</td>
<td>60</td>
<td>22</td>
<td>Healthy</td>
<td>Overall trend for a slight decrement in FEV₁ with NO₂ exposure (≤ 1%). No change in methacholine responsiveness as a result of NO₂ exposure or viral infection status.</td>
<td>Overall trend for a slight decrement in FEV₁ with NO₂ exposure (≤ 1%). No change in methacholine responsiveness as a result of NO₂ exposure or viral infection status.</td>
</tr>
<tr>
<td>Concentration</td>
<td>Dose</td>
<td>Time</td>
<td>Duration</td>
<td>Airway Changes</td>
<td>Notes</td>
<td></td>
<td></td>
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<td>---------------</td>
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</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>120</td>
<td></td>
<td>16</td>
<td>11 S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4700</td>
<td>2.5</td>
<td>120</td>
<td></td>
<td>16</td>
<td>5 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>120</td>
<td></td>
<td>16</td>
<td>8 S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14100</td>
<td>7.5</td>
<td>120</td>
<td></td>
<td>16</td>
<td>8 S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400</td>
<td>5.0</td>
<td>840</td>
<td></td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>60</td>
<td></td>
<td>8 M/3 F</td>
<td>18-36 years, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>120</td>
<td></td>
<td>13 M/5 F</td>
<td>Normal, NS, 18-33 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520-9400</td>
<td>4.0-5.0</td>
<td>10</td>
<td></td>
<td>Bag exposure technique, Airway resistance increased 30 min after end of exposure. No change in spirometry.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>75</td>
<td></td>
<td>16 M/9 F</td>
<td>18-45 years, NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After 14 100 µg/m³ (120 min) and 9400 µg/m³ (14 h), responsiveness to acetylcholine increased. Resistance increased after all but the 1880 µg/m³ exposure.

Vitamin C blocked NO₂-induced increase in airway reactivity to methacholine.

No symptoms; no lung function changes. Increased methacholine reactivity.

Bag exposure technique. Airway resistance increased 30 min after end of exposure. No change in spirometry.

No change in SRaw associated with NO₂. Small but significant decrease in blood pressure; some mild increase in symptoms.
Table 39 (cont'd).

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Number of subjects/gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9400 ppm</td>
<td>5.0</td>
<td>15</td>
<td>16</td>
<td>Healthy</td>
<td>Decreased O₂CO 18%.</td>
<td>Von Nieding et al. (1973a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased resistance 60%.</td>
<td>Von Nieding et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Remained elevated for 60 min.</td>
<td>Von Nieding et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible decrease in PaO₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible decrease in earlobe PO₂</td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

Abbreviations:
M = Male; F = Female; S = Active smoker; NS = Non-smoker; FEV₁ = Forced expiratory volume in 1 second; FVC = Forced vital capacity; SRₐ = Specific airway resistance; Var = Variable; Rₐ = Airway resistance; SGₚ = Specific airway conductance; W = Watts; L = Light; H = Heavy; Rₜ = Total respiratory resistance; O₂CO = Diffusing capacity for carbon monoxide; PaO₂ = Arterial partial pressure of oxygen; PO₂ = Partial pressure of oxygen
1128 µg/m³ (0.60 ppm), 2820 µg/m³ (1.5 ppm) or a variable concentration protocol where three 15 min peaks of 3760 µg/m³ (2.0 ppm) were added to a background level of 94 µg/m³ (0.05 ppm). Nitrogen dioxide did not affect airway mechanics (forced vital capacity (FVC), FEV₁, SG). However, after exposure to 2820 µg/m³, but not to the other concentrations, there was a small but statistically significant increase in airway responsiveness to carbachol. This study supported the earlier observations by Mohsenin (1987b, 1988) of increased airway responsiveness after a 1-h exposure to 3760 µg/m³. Mohsenin (1987b) further observed that the NO₂-induced increase in airway responsiveness could be blocked by elevation of serum ascorbate level through pretreatment with the antioxidant ascorbic acid (vitamin C).

At concentrations below 1880 µg/m³ (1.0 ppm) NO₂, pulmonary function and airway responsiveness have generally not been found to be affected in healthy adult subjects (Bell & Ulmer, 1976; Folinsbee et al., 1978; Hackney et al., 1978; Kerr et al., 1979; Sackner et al., 1980; Toyama et al., 1981; Kulle, 1982; Hazucha et al., 1982, 1983; Stacy et al., 1983; Kagawa, 1986; Adams et al., 1987; Drechsler-Parks et al., 1987; Drechsler-Parks, 1987; Boushey et al., 1988; Morrow & Utell, 1989; Frampton et al., 1989a, 1991; Kim et al., 1991). Although some investigators have at times reported statistically significant effects, there does not appear to be a consistent pattern of acute responses in healthy subjects at these low NO₂ concentrations.

Kagawa & Tsuru (1979) reported the lowest NO₂ exposure concentration that appeared to cause an effect. Healthy men were exposed to 282 µg/m³ (0.15 ppm) NO₂ for 2 h while performing light, intermittent exercise. The authors suggested that NO₂ caused some statistically significant changes, i.e. a 0.5% decrease in vital capacity (VC) and a 16% decrease in an index of small airway function (i.e. FEF75, FEF75/H, the ratio of forced expiratory flow at 75% FVC expired while breathing a helium-oxygen mixture compared to FEF75 while breathing air). These findings should be interpreted with the consideration that multiple t-tests were used in the statistical analysis of these data. Rehn et al. (1982) reported a small (17%) increase in SR in men exposed to 500 µg/m³ (0.27 ppm) for 1 h, but a higher concentration (2000 µg/m³, 1.06 ppm) did not cause an effect.

Bylin et al. (1985) reported that the SR of normal subjects exposed to 230, 460 and 910 µg/m³ (0.12, 0.24 and 0.48 ppm) for 20 min was unaffected. Specific comparisons revealed a significant
11% increase in SR\textsubscript{aw} at 460 \, \mu g/m\textsuperscript{3} (0.24 ppm) and a 9% decrease in SR\textsubscript{aw} at 910 \, \mu g/m\textsuperscript{3}. Bronchial responsiveness to histamine was increased by 910 \, \mu g/m\textsuperscript{3} NO\textsubscript{2}.

Symptomatic responses of subjects exposed to NO\textsubscript{2} were evaluated in several of the above studies. None of these studies, including exposures for as long as 75 min to 7520 \, \mu g/m\textsuperscript{3} (4.0 ppm) NO\textsubscript{2} (Linn & Hackney, 1983; Linn et al., 1985b), resulted in a significant increase in respiratory symptoms. In studies of sensory effects, subjects were unable to detect the odour of 188 \, \mu g/m\textsuperscript{3} (0.1 ppm) NO\textsubscript{2} (Hazucha et al., 1983), but Bylin et al. (1985) observed an odour threshold of 75 \, \mu g/m\textsuperscript{3} (0.04 ppm) for normal subjects and 150 \, \mu g/m\textsuperscript{3} (0.08 ppm) for asthmatics.

### 6.2.1.2 Nitrogen dioxide effects on asthmatics

Studies of the effects of exposures to NO\textsubscript{2} on respiratory function and airway responsiveness of asthmatics are summarized in Table 40. Asthmatics are generally more responsive than healthy subjects to NO\textsubscript{2}. However, as can be seen in Table 40, there is substantial variability in observed responses between and even within laboratories. This variability is illustrated in Fig. 22 and 23, in which changes in airway resistance and FEV\textsubscript{1} are related to the “exposure dose” of NO\textsubscript{2} (calculated as ppm \times litres of air breathed over the duration of exposure) (US EPA, 1993). The individual investigations that yielded the data used to develop these illustrations will be discussed in more detail below. Other studies, not discussed separately, are also summarized in Table 40. The review by the US EPA (1993) provides more detail on many of these studies. Although differences in exposure protocols may explain some of the differences between studies, the explanation most often invoked is that there may be differences in the severity of asthma among the subject groups tested. There are numerous definitions of “asthma severity” (see, for example, National Institutes of Health, 1991). Those applied to the key asthma studies discussed here (based on the data available) are: (1) mild: controlled by bronchodilators and avoidance of known precipitating factors, does not interfere with normal activities; and (2) moderate: often requires periodic use of inhaled steroids in treatment and may interfere with work or school activities. Those with severe asthma are seldom used as subjects for NO\textsubscript{2} studies because their disease can include life-threatening episodes. Typical volunteers for the studies described here had mild allergic asthma.
Fig. 22. Percent change (post-air vs. post-NO₂) in FEV₁ vs. NO₂ "dose" in parts per million x litres in asthmatics (source: Modified from US EPA, 1993)
Fig. 23. Percentage change (post-NO$_2$ - post-air + post-air) in resistance ($R_{aw}$, $SR_{aw}$, or $R_1$) versus NO$_2$ "dose" (ppm x litres) in asthmatics (modified from: US EPA, 1993)
Table 40. Effects of nitrogen dioxide (NO$_2$) on lung function and airway responsiveness of asthmatic

<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm (litres/mm)</td>
<td></td>
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</tr>
<tr>
<td>188</td>
<td>0.1</td>
<td>60</td>
<td>9</td>
<td>20-51 years, &quot;history of bronchial asthma&quot;</td>
<td>No effect of NO$_2$ on FEV$<em>1$, SG$</em>{aw}$ or on bronchial reactivity to ragweed antigen, either immediately or 24 h after exposure.</td>
<td>Ahmed et al. (1983a)</td>
</tr>
<tr>
<td>188</td>
<td>0.1</td>
<td>60</td>
<td>20 M/34 F</td>
<td>18-39 years</td>
<td>No significant effect on SG$_{aw}$, FEV$<em>1$, V$</em>{aw}$; variable effect on carbachol reactivity. No information on controlled exposure.</td>
<td>Ahmed et al. (1983b)</td>
</tr>
<tr>
<td>188</td>
<td>0.1</td>
<td>60</td>
<td>15 M</td>
<td>21-46 years, mild or inactive disease</td>
<td>No significant changes in R$_p$ or responsiveness to methacholine associated with NO$_2$ exposure.</td>
<td>Hazucha et al. (1982, 1983)</td>
</tr>
<tr>
<td>207</td>
<td>0.11-0.16</td>
<td>60</td>
<td>6 M/1 F</td>
<td>1 Smoker, 3 asthmatic, 4 allergic</td>
<td>No change in SR$_{aw}$ or in responsiveness to grass pollen in 3 allergic asthmatics and 4 allergic subjects.</td>
<td>Orehek et al. (1981)</td>
</tr>
<tr>
<td>210</td>
<td>0.09-0.13</td>
<td>60</td>
<td>13 M/7 F</td>
<td>15-44 years, 13 mild/7 mod asthmatics;</td>
<td>13/20 subjects had enhanced responses to carbachol after 210 µg/m$^2$ NO$_2$. Post hoc statistical analysis questionable.</td>
<td>Orehek et al. (1976)</td>
</tr>
</tbody>
</table>

$n=20$
Table 40 (contd).

<table>
<thead>
<tr>
<th>NO₂ concentration (µg/m³)</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>489</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65 years</td>
<td>1/4 subjects had enhanced responses to carbachol after 489 µg/m³ NO₂</td>
</tr>
<tr>
<td>226</td>
<td>0.12</td>
<td>60</td>
<td>4 M/6 F</td>
<td></td>
<td></td>
<td>12-18 years, asympt., extrinsic asthmatics</td>
<td>No significant effects on pulmonary function due to NO₂ increased symptoms after NO₂ exposures.</td>
</tr>
<tr>
<td>338</td>
<td>0.18</td>
<td>40</td>
<td>4 M/6 F</td>
<td>10</td>
<td>39</td>
<td>12-18 years, asympt., extrinsic allergic asthmatics</td>
<td>No change in FEV₁, R, increased 10.4% (NS), 3% decrease in FEV₁ (p &lt; 0.05).</td>
</tr>
<tr>
<td>230</td>
<td>0.12</td>
<td>20</td>
<td>6 M/2 F</td>
<td>17-45 years, very mild asympt.</td>
<td></td>
<td>No significant change in SR, at any NO₂ levels. Histamine reactivity tended to increase.</td>
<td>Bylin et al. (1986)</td>
</tr>
<tr>
<td>Exposures</td>
<td>Concentration</td>
<td>Duration</td>
<td>Age Details</td>
<td>Reproductive Details</td>
<td>Observations</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
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<td>------------</td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>0.14</td>
<td>30</td>
<td>8 M/12 F</td>
<td>17-56 years, very mild asympt.</td>
<td>Overall trend for (S_{R_{\infty}}) to decline during exposure period, not related to NO(_2) concentration. Histamine bronchial reactivity tended to increase after 260 and 510 (\mu g/m^3) NO(_2) exposure.</td>
<td>Bylin et al. (1988)</td>
<td></td>
</tr>
<tr>
<td>510</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>0.2</td>
<td>120</td>
<td>12 M/19 F</td>
<td>18-55 years, wide range of asthma severity</td>
<td>No effects on spirometry or airway resistance. Airway reactivity to methacholine results variable—tended to increase with exposure.</td>
<td>Kleinman et al. (1983)</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>0.25</td>
<td>30</td>
<td>30 M/2 F</td>
<td>18-55 years, mild asympt.</td>
<td>Mouthpiece exposure system. No changes in methacholine responsiveness were observed after NO(_2) exposure.</td>
<td>Joerres &amp; Magnussen (1991)</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>0.25</td>
<td>30</td>
<td>10 M/4 F</td>
<td>20-55 years, mild asthma, most sympt.</td>
<td>After NO(_2) exposure, responsiveness to inhaled SO(<em>2) was increased. No effect of NO(<em>2) alone on (S</em>{R</em>{bw}}).</td>
<td>Joerres &amp; Magnussen (1990)</td>
<td></td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>30</td>
<td>30 M/4 F</td>
<td>23-34 years</td>
<td>No changes in (S_{R_{bw}}), FVC, FEV(_1), SBN(_2) or symptoms after NO(_2) exposure. NO(_2) exposure did not increase airway responsiveness to SO(_2).</td>
<td>Rubinstein et al. (1990)</td>
<td></td>
</tr>
</tbody>
</table>
Table 40 (contd).

<table>
<thead>
<tr>
<th>NO₂ concentration (µg/m³)</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>564 ppm</td>
<td>0.3</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>20-45 years, mild asymp.</td>
<td>Resting 20 min exposures produced no effects. Slight excess decrease in FEV₁ and PEFR in NO₂ plus exercise above that caused by exercise alone. PEFR, -16% (air), -28% (NO₂); FEV₁, -5.5% (air), -9.3% (NO₂). Significantly increased response to cold air after NO₂ exposure.</td>
<td>Bauer et al. (1986)</td>
</tr>
<tr>
<td>564 ppm</td>
<td>0.3</td>
<td>225</td>
<td>30</td>
<td>30-40</td>
<td>10 M/10 F</td>
<td>19-54 years</td>
<td>Group finding indicated no significant responses. No change in lung function, symptoms, carbachol reactivity. Subjects studied previously (Bauer et al., 1986) showed possible responses to NO₂. New subject subgroup showed significantly greater response in air exposures.</td>
</tr>
<tr>
<td>564 ppm</td>
<td>A. 03</td>
<td>110</td>
<td>50</td>
<td>42</td>
<td>A. 13 M</td>
<td>19-35 years, mild asthmatics</td>
<td>FEV₁ decreased 11% in NO₂ but only 7% in air, after first 10 min of exercise. Smaller changes later in exposure.</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂</td>
<td>Time</td>
<td>Age</td>
<td>Gender</td>
<td>NO₂Exposure</td>
<td>Data Description</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----</td>
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<td>-----</td>
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<td>--------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>282</td>
<td>B. 0.15</td>
<td>75</td>
<td>30</td>
<td>42</td>
<td>B. 21</td>
<td>No increase in airway reactivity to methacholine 2 h after exposure. No change in FEV₁ or SRmax as a result of NO₂ exposure.</td>
<td></td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>180</td>
<td>90</td>
<td>30</td>
<td>24 M/10 F</td>
<td>After 60 min of exposure, FEV₁, FVC and PEFR (−3.4, −4.0 and −5.6%, respectively) were significantly reduced. No change in airways responsiveness to cold air challenge. SRmax increased 17% after NO₂ exposure. After 180 min of exposure, the responses had returned to baseline levels.</td>
<td></td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>120</td>
<td>60</td>
<td>40</td>
<td>27 M/32 F</td>
<td>Exercise-related increases in symptoms. Possible NO₂-related decrease in FEV₁, PEFR. Increased cold air response after 564 μg/m³.</td>
<td></td>
</tr>
<tr>
<td>1128</td>
<td>0.6</td>
<td>120</td>
<td>60</td>
<td>41</td>
<td></td>
<td>More consistent increases in SRmax at 1128 μg/m³ but not significantly different from air and 564 μg/m³.</td>
<td></td>
</tr>
<tr>
<td>564</td>
<td>0.3</td>
<td>60</td>
<td>30</td>
<td>41</td>
<td>15 M/6 F</td>
<td>No effect of NO₂. Exercise-related increase in SRmax under all conditions.</td>
<td></td>
</tr>
<tr>
<td>1880</td>
<td>1.0</td>
<td>60</td>
<td>30</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>60</td>
<td>30</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 40 (contd).

<table>
<thead>
<tr>
<th>NO&lt;sub&gt;2&lt;/sub&gt; concentration</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt; concentration (µg/m&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>ppm</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/ gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940</td>
<td>0.5</td>
<td>120</td>
<td>15</td>
<td>9 M/4 F</td>
<td>19-50 years, 3 Smokers</td>
<td>Increased respiratory symptoms in 4/13 subjects. Also, increased static lung compliance. Impossible to determine amount of effect due to NO&lt;sub&gt;2&lt;/sub&gt;.</td>
<td>Kulle (1982)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>60</td>
<td>10</td>
<td>22-44 years, mild asthmatics</td>
<td>No change in symptoms. Significant group mean increase in responsiveness to methacholine after NO&lt;sub&gt;2&lt;/sub&gt; exposure. No other function changes.</td>
<td>Mohsenin (1997b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>857</td>
<td>0.3 ppm</td>
<td>60</td>
<td>120</td>
<td>6 M/12 F</td>
<td>33 years, physician-diagnosed asthma</td>
<td>No significant effect on spirometry, heart rate, skin conductance. Small decrease in systolic blood pressure.</td>
<td>Linn et al. (1980a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7520</td>
<td>4.0</td>
<td>75</td>
<td>12 M/11 F</td>
<td>18-34 years, physician-diagnosed asthma</td>
<td>No NO&lt;sub&gt;2&lt;/sub&gt; effects on SR&lt;sub&gt;asth&lt;/sub&gt; symptoms, heart rate, skin conductance. Small decrease in systolic blood pressure.</td>
<td>Linn &amp; Hackney (1984); Linn et al. (1985a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

M = male; F = female; SG<sub>aw</sub> = specific airway conductance; FEV<sub>1</sub> = forced expiratory volume in 1 second; V<sub>ISO</sub> = volume of isoflow; PEFR = peak expiratory flow; SR<sub>aw</sub> = specific airway resistance; FVC = forced vital capacity; Asympt. = asymptomatic; R<sub>t</sub> = total respiratory resistance; NS = not significant; SO<sub>2</sub> = sulfur dioxide; SBN<sub>2</sub> = single breath nitrogen washout.
Avol et al. (1988) studied a group of moderate-to-severe asthmatics exposed to 564 and 1128 μg/m³ (0.3 and 0.6 ppm) NO₂ for 2 h with moderate intermittent exercise. NO₂ did not cause significant changes in SR₉₀ or FEV₁. Results of tests of airway responsiveness to cold air suggested a slightly increased response after exposure to 564 μg/m³, but not after 1128 μg/m³. A post hoc analysis of a subgroup of subjects with the most abnormal lung function (i.e., FEV₁/FVC ratios < 0.65) did not find enhanced susceptibility. In a subsequent study using 564 μg/m³ NO₂, Avol et al. (1989) found decreases in FEV₁, FVC and peak expiratory flow rate (PEFR), but no change in responsiveness to cold air challenge.

Roger et al. (1990) reported the effects of NO₂ exposure on mild asthmatics. Their first study was a pilot study of 12 mild asthmatics exposed to 564 μg/m³ (0.3 ppm) for 110 mm, including three 10-min periods of exercise. After the first 10 min of exercise in NO₂, there was a decrease in FEV₁ that persisted for the remainder of the exposure period, although the overall responses were progressively less with successive periods of exercise, as is common with exercise-induced asthma when the exercise is intermittent. Their subsequent concentration-response study of twenty-one subjects included six responsive subjects from the pilot study; volunteers were exposed to 282, 564 and 1128 μg/m³ (0.15, 0.30 and 0.60 ppm) NO₂ for 75 min, with three 10-min exercise periods. In contrast to the pilot study, there were no effects of NO₂ on pulmonary function or airway responsiveness to methacholine, tested 2 h after exposure ceased. The authors suggested that the differences between the pilot and the main study may have been due to more reactive airways in the pilot study asthmatics. Because the studies were conducted during different seasons, seasonal differences in temperature, air pollution, ambient aeroallergens or other factors may have contributed to some of the variability in response.

Asthmatics exposed to 230, 460 and 910 μg/m³ (0.12, 0.24 and 0.48 ppm) NO₂ for 20 min were studied by Bylin et al. (1985). Changes in SR₉₀ during the four exposures averaged +3% after air and +9%, -2% and -14% after the three levels of NO₂, respectively; these changes were not significantly different. There was a tendency for an increase in thoracic gas volume (TGV) after NO₂ exposures (9 to 10%), but differences in pre-exposure values for TGV were probably responsible, rather than NO₂. There were no significant changes in tidal volume or respiratory rate. At the highest concentration tested (910 μg/m³, 0.48 ppm), histamine bronchial responsiveness was increased.
In mild asthmatics exposed for 30 min to 260, 510 and 1000 μg/m³ (0.14, 0.27 and 0.53 ppm), there were no significant changes in SR\textsubscript{S}, although there was a general trend for SR\textsubscript{S} to fall throughout the period of exposure at all NO\textsubscript{2} concentrations (Bylin et al., 1988). There was, however, a significant increase \((p = 0.03)\) in airway responsiveness to histamine after 30 min of exposure to 510 μg/m³ (0.27 ppm) only. The absence of a concentration-related increase in responsiveness is not inconsistent with other studies. This observation contrasts with earlier results (Bylin et al., 1985) that suggested a possible increased responsiveness after exposure to 910 μg/m³ (0.48 ppm). Because of the use of a non-parametric pair comparison test that was not adjusted for multiple comparisons, the raw data presented in the paper were subjected to reanalysis (US EPA, 1993) using a Friedman non-parametric analogue of an F test, which is probably more appropriate for these data than a series of Wilcoxon matched pairs signed rank tests. This analysis showed no statistically significant change in histamine responsiveness due to NO\textsubscript{2} exposure.

Asthmatics exposed to 564 μg/m³ (0.3 ppm) NO\textsubscript{2} by mouthpiece for 20 min at rest followed by 10 min of exercise (30 litres/min) experienced a statistically significant spirometric response to NO\textsubscript{2} (Bauer et al., 1986). After NO\textsubscript{2} exposure, 9 out of 15 asthmatics had a decrease in FEV\textsubscript{1}; both the pre-post exposure difference on the NO\textsubscript{2} day (10.1%) and the pre-post NO\textsubscript{2} minus the pre-post air (i.e., delta-delta) differences (6%) were significant using a paired t-test. Maximum expiratory flow at 60% total lung capacity (PEFV curve) was also decreased, but FVC and SG\textsubscript{S} were not altered. Nine out of twelve subjects experienced an increase in airway responsiveness to cold air. The mouthpiece exposure system used in this study contained relatively dry air (relative humidity, RH, of 9 to 14% at 20 °C) and airway drying may have interacted with NO\textsubscript{2} to cause greater responses. However, Bauer et al. (1986) controlled for the airway drying effect by exposing subjects to clean air at the same temperature and RH. Nevertheless, air temperature and humidity effects may be an important consideration for NO\textsubscript{2} effects in winter in the temperate regions of the world.

Linn et al. (1985b) and Linn & Hackney (1984) exposed mild asthmatics to 7520 μg/m³ (4.0 ppm) NO\textsubscript{2} for 75 min, with two 15-min exercise periods. There was no significant difference in lung function that could be attributed to NO\textsubscript{2} if anything, SR\textsubscript{S} tended to be slightly lower with the NO\textsubscript{2} exposures.
The reasons for the differences between the group of asthmatics exposed to 7520 μg/m³ (4 ppm) for 75 min (with exercise) (Linn et al., 1985b) and the group exposed to 564 μg/m³ (0.30 ppm) for 30 min with exercise studied by Bauer et al. (1986) are not clear. The subjects of Bauer et al. were exposed to NO₂ in dry air through a mouthpiece which could have caused some drying of the upper airways; Linn et al. (1985b) used a chamber exposure. Second, the subjects in the Linn et al. (1985b) study tended to have milder asthma than the subjects in the Bauer et al. (1986) study. There were differences in the season in which the two studies were conducted, and there may have been a difference in background exposure to NO₂ (outdoors and/or indoors). In addition, increased bronchial reactivity to cold air was an important finding in the Bauer et al. (1986) study, but it was not measured by Linn et al. (1985b).

Further research was conducted by Linn et al. (1986) on mild asthmatics exposed to 564, 1880 and 5640 μg/m³ (0.30, 1.0 and 3.0 ppm) NO₂ for 1 h. The exposures included intermittent, moderate exercise. As in the previous study with 7520 μg/m³ (4.0 ppm) NO₂, there were no significant effects of NO₂ on spirometry, SRaw or symptoms. Furthermore, there was no significant effect on airway responsiveness to cold air. In order to examine the suggestion that the severity of response to NO₂ may be related to the clinical severity of asthma, the authors selected three subjects characterized as having more severe illness. Although they experienced markedly larger changes in resistance than other milder asthmatics under all exposure conditions, there was no indication that the responses of these subjects were related to NO₂ exposure.

Mohsenin (1987a) found no changes in symptoms, spirometry, or plethysmography in mild asthmatics exposed to 940 μg/m³ (0.5 ppm) NO₂ for 1 h at rest. However, airway responsiveness to methacholine increased after the NO₂ exposure.

The effects of previous NO₂ exposure on SO₂-induced bronchoconstriction has been examined by Joerres & Magnussen (1990) and Rubinstein et al. (1990). Neither study found changes in pulmonary function after NO₂ exposure. Joerres & Magnussen (1990) exposed mild-to-moderate asthmatic subjects to 470 μg/m³ (0.25 ppm) NO₂ for 30 min while breathing through a mouthpiece at rest. After the NO₂ exposure, airway responsiveness to 1965 μg/m³ (0.75 ppm) SO₂ was increased. Rubinstein et al. (1990) exposed asthmatics to 564 μg/m³ (0.30 ppm) NO₂ for 30 min...
(including 20 min light exercise). No mean change in responsiveness to SO$_2$ occurred, but one subject showed a tendency toward increased responsiveness. The reasons for the different findings in these two studies is not clear, especially as the subjects of Rubinstein et al. (1990) were exposed to a higher NO$_2$ concentration and exercised during exposure. However, Joerres & Magnussen’s subjects appeared to have had slightly more severe asthma and were somewhat older. The modest increase in SR caused by exercise in the Rubinstein et al. (1990) study may have induced a refractory state to SO$_2$. Finally, the different method of administering the SO$_2$ bronchoprovocation test may have had an influence. Joerres & Magnussen (1990) increased minute ventilation ($\hat{V}$) at a constant SO$_2$ concentration, whereas Rubinstein et al. (1990) increased SO$_2$ concentration at constant $\dot{V}$.

A number of studies of the effects of NO$_2$ exposure in asthmatics on changes in airway responsiveness to bronchoconstrictors have been presented in Table 40, but not evaluated in the text. Various types of inhalation challenge tests have been used (methacholine, histamine, cold air, etc.). Some exposures were conducted at rest and others while performing some exercise. For twenty studies for which individual data were available, a meta analysis (Folinsbee, 1992) was performed to assess the changes in airway responsiveness in asthmatics exposed to NO$_2$. The aim of the meta analysis was to examine the diversity of response seen in the various studies and to examine factors such as NO$_2$ concentration, exercise, and airway challenge method that could help explain some of the variability in response. Such questions could not be adequately addressed using individual studies. The analysis provides only a qualitative examination of concentration-response relationships. For this analysis, the directional change (i.e., increased or decreased) in airway responsiveness after NO$_2$ exposure was determined for each subject. The data were then organized by exposure concentration range and whether or not exposures included exercise. Within each exposure category the fraction of subjects with increased airway responsiveness was determined (see Table 41). For the total of 355 individual NO$_2$ exposures, 59% of the asthmatics had increased responsiveness. If the response was not associated with NO$_2$ exposure, the fraction would be expected to approach 50%. The excess increase in responsiveness can be attributed primarily to the NO$_2$ exposures conducted at rest (fraction was 69%). There was a larger fraction of increased responsiveness during the resting exposures in all three concentration ranges (see Table 41). In the exercising studies, however, there was no effect because only 51%
Table 41. Fraction of nitrogen dioxide-exposed subjects with increased airway responsiveness

<table>
<thead>
<tr>
<th>Nitrogen dioxide concentration (ppm)</th>
<th>All exposures</th>
<th>Exposures with exercise</th>
<th>Exposure at rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All NO&lt;sub&gt;2&lt;/sub&gt; concentrations</td>
<td>0.59 (355)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 (222)</td>
<td>0.69 (154)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.47 (36)</td>
<td>0.73 (15)</td>
<td>0.86 (14)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.79 (29)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 (15)</td>
<td>0.86 (14)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are fraction of subjects with an increase in airways responsiveness above the value for clean air. Numbers in parenthesis indicate actual number of subjects in each category. Total number = 355. Ties (i.e. no change) were excluded.

<sup>a</sup> p < 0.01 two-tailed sign test
<sup>b</sup> p < 0.05 two-tailed sign test

had an increase in airway responsiveness. There was a trend for a slightly larger percentage (~75%) of subjects to have increased airway responsiveness after NO<sub>2</sub> exposures above 376 µg/m<sup>3</sup> (0.20 ppm) and under resting conditions. Of those six studies independently reporting a statistically significant response (Kleinman et al., 1983; Bylin et al., 1985, 1988; Bauer et al., 1986; Mohsenin, 1987a; Joerres & Magnussen, 1990), four were resting exposures, and in four the exposure duration was 30 min or less. Although the authors offered various hypotheses for this apparent effect of low-level NO<sub>2</sub> resting exposures, the mechanisms are unknown. Changes in responsiveness were seen with relatively brief exposures. One possible explanation for the absence of response in the exercising exposures is that exercise-induced bronchoconstriction may interfere with the NO<sub>2</sub>-induced response or that prior exercise may cause the airways to become refractory to the effects of NO<sub>2</sub>. Possible confounding influences of nitric oxide, not measured in most studies, cannot be determined.

305
A similar meta-analysis for healthy subjects indicated increased airway responsiveness after exposure to NO\textsubscript{2} concentrations greater than 1880 µg/m\textsuperscript{3} (1 ppm). Exercise during exposure did not appear to influence the responses as much in the healthy subjects as in the asthmatics, but a similar trend was evident.

### 6.2.1.3 Nitrogen dioxide effects on patients with chronic obstructive pulmonary disease

Patients with COPD represent an important potentially sensitive population group. Studies evaluating NO\textsubscript{2} effects on respiratory function in COPD subjects are summarized in Table 42. The results of two NO\textsubscript{2} exposure studies (9400 to 15 040 µg/m\textsuperscript{3}, 5 to 8 ppm NO\textsubscript{2} for up to 5 min) were discussed by Von Nieding et al. (1980), who found that the responses of bronchitics were generally similar to those of healthy subjects. There was a tendency for the response to NO\textsubscript{2} to be greater in the subjects with the highest baseline R\textsubscript{aw}. Percentage changes ranged from approximately 25 to 50%. In a review of their studies, Von Nieding & Wagner (1979) showed that R\textsubscript{aw} increased in chronic bronchitics exposed to ≥ 3760 µg/m\textsuperscript{3} (2.0 ppm) NO\textsubscript{2}.

The responses of COPD patients were affected by exposure (with mild exercise) to 564 µg/m\textsuperscript{3} (0.3 ppm) NO\textsubscript{2} for 3.75 h (Morrow & Utell, 1989). Forced vital capacity showed progressive and significant decreases during and following NO\textsubscript{2} exposure, the largest change of -9.6% occurring after 3.75 h of exposure. Smaller decrements in FEV\textsubscript{1} (-5.2%) occurred at the end of exposure. There was no effect of NO\textsubscript{2} on SG\textsubscript{aw} or diffusing capacity. The severity of disease (based on impairment of lung function: FEV\textsubscript{1} < 60% predicted vs. ≥ 60% predicted) generally did not influence the magnitude of response to NO\textsubscript{2}. The COPD patients showed a decrement in FEV\textsubscript{1} compared to the healthy, elderly non-smokers who experienced an improvement in FEV\textsubscript{1}. In contrast, Linn et al. (1985a) found no effects from a 1-h exposure (with exercise) to 940, 1880 and 3760 µg/m\textsuperscript{3} (0.5, 1.0 and 2.0 ppm) NO\textsubscript{2} in a diverse group of COPD patients. The reasons for the marked difference in responses between the two studies are not known. Ambient exposure to air pollution in general and NO\textsubscript{2} in particular was probably much higher for the subjects in the Linn et al. (1985a) study. Thus, attenuation of physiological responses may have been a factor.

Hackney et al. (1992) studied effects of field exposure to ambient air and chamber exposure to 564 µg/m\textsuperscript{3} (0.3 ppm) NO\textsubscript{2} in...
<table>
<thead>
<tr>
<th>NO$_2$ concentration</th>
<th>Exposure duration</th>
<th>Exercise duration</th>
<th>Exercise ventilation</th>
<th>Number of subjects/gender</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>564 µg/m$^3$ ppm</td>
<td>0.3</td>
<td>225 (3 × 7)</td>
<td>21</td>
<td>25</td>
<td>13 M/7 F</td>
<td>47-70 years, 8 mild, 12 moderate</td>
</tr>
<tr>
<td>564 µg/m$^3$ ppm</td>
<td>0.3</td>
<td>240 (4 × 7)</td>
<td>28</td>
<td>25</td>
<td>15 M/11 F</td>
<td>47-69</td>
</tr>
<tr>
<td>940 µg/m$^3$ ppm</td>
<td>0.5</td>
<td>120</td>
<td>15</td>
<td>25</td>
<td>7</td>
<td>24-53 years, daily cough for 3 months</td>
</tr>
<tr>
<td>940 µg/m$^3$ ppm</td>
<td>0.5</td>
<td>60</td>
<td>30</td>
<td>16</td>
<td>13 M/9 F</td>
<td>48-69 years, some with emphysema, some with chronic bronchitis</td>
</tr>
<tr>
<td>1880 µg/m$^3$ ppm</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3760 µg/m$^3$ ppm</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ concentration (µg/m³)</td>
<td>Exposure duration (min)</td>
<td>Exercise duration (min)</td>
<td>Number of subjects/gender</td>
<td>Subject characteristics</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>---------------------------</td>
<td>------------------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>940-9400 0.5-5 ppm</td>
<td>15</td>
<td>88</td>
<td>Decrease in earlobe blood P0₂ at ≥ 7500 µg/m³. Increased Rₑₑ at ≥ 3006 µg/m³.</td>
<td>Von Nieding et al. (1971, 1970)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1880-9400 1-5 30 breaths (15 min)</td>
<td>84 M 30-72 years, chronic non-specific disease</td>
<td>Increase in Rₑₑ related to NO₂ concentration. No effect on Rₑₑ below 2820 µg/m³.</td>
<td>Von Nieding et al. (1973a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9400 5 60</td>
<td>Changes in PO₂ of earlobe capillary blood. Change occurred in first 15 min, effect did not increase with further exposure.</td>
<td>Von Nieding &amp; Wagner (1979)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1890-15 040 1-8 ppm 5-60</td>
<td>At 7520-9400 µg/m³ for 15 min, PaO₂ decreased (arterialized capillary blood). Rₑₑ increased with exposure to ≥ 3006 µg/m³.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

Abbreviations: FVC = Forced vital capacity; FEV₁ = Forced expiratory volume in 1 second; PaO₂ = Arterial partial pressure of oxygen; PO₂ = Partial pressure of oxygen; Rₑₑ = Airway resistance; SRₑₑ = Specific airway resistance; SaO₂ = Arterial oxygen saturation.
Controlled Human Exposure Studies of Nitrogen Oxides

older adults with evidence of COPD and a history of heavy smoking. They reported only slight adverse effects of NO\textsubscript{2}. The study did not strongly confirm the findings of Morrow & Utell (1989) and Morrow et al. (1992), and the authors speculated that ambient exposure history may have been responsible for differences between these studies.

6.2.1.4 Age-related differential susceptibility

Studies evaluating possible age-related differences in susceptibility to NO\textsubscript{2} effects on respiratory function in healthy subjects are summarized in Table 39. Research on asthmatics is summarized in Table 40. Spirometry measurements of young (18 to 26 years old) and older (51 to 76 years old) men and women were not affected by exposure to 1128 µg/m\textsuperscript{3} (0.6 ppm) NO\textsubscript{2} with light intermittent exercise (Drechsler-Parks et al., 1987; Drechsler-Parks, 1987). In addition, Morrow & Utell (1989) did not observe any pulmonary function or airway responsiveness effects due to a lower level of NO\textsubscript{2} (564 µg/m\textsuperscript{3}, 0.3 ppm) in young or elderly healthy subjects.

Koenig et al. (1985) found no “consistent significant changes in pulmonary functional parameters” after 1-h resting exposures of asthmatic adolescents to 226 µg/m\textsuperscript{3} (0.12 ppm) NO\textsubscript{2}. Subsequent mouthpiece exposures to 226 µg/m\textsuperscript{3} NO\textsubscript{2} with exercise, caused increases in R\textsubscript{T} and decreases in FEV\textsubscript{1}, after both air and NO\textsubscript{2} exposure, which were apparently due to exercise alone (Koenig et al., 1987a,b). When subjects were exposed to a higher level of NO\textsubscript{2} (338 µg/m\textsuperscript{3}, 0.18 ppm), no differences in R\textsubscript{T} occurred. Decreases in FEV\textsubscript{1}, were -1.3 and -3.3% for air and NO\textsubscript{2}, respectively; this difference (p = 0.06) may indicate a possible response trend.

6.2.2 Nitrogen dioxide effects on pulmonary host defences and bronchoalveolar lavage fluid biomarkers

Nitrogen dioxide can enhance susceptibility to infectious pulmonary disease, as clearly demonstrated in the animal toxicological literature (chapter 5). Epidemiological studies (chapter 7) suggest similar effects. Human clinical studies of NO\textsubscript{2} effects on host defences are summarized in Table 43.

Kulle & Clements (1988) and Goings et al. (1989) (two reports of the same study) examined the effect of NO\textsubscript{2} exposure on
Table 43. Effects of nitrogen dioxide on host defences of humans

<table>
<thead>
<tr>
<th>NO₂ concentration</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³ ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>508</td>
<td>0.27</td>
<td>60</td>
<td>M</td>
<td>Healthy, young</td>
<td>No change in nasal or tracheobronchial clearance.</td>
<td>Rehn et al. (1982)</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) 1128</td>
<td>(1) 0.6</td>
<td>180</td>
<td>60</td>
<td>39</td>
<td>6 M/2 F</td>
<td>Total NO₂ uptake (1) 3.4 mg (2) 5.6 mg, (3) =3.3 mg (4) 8.1 mg. BAL fluid analysis showed no significant effect on total protein or albumin. Apparent increase in alpha-2-macroglobulin 3.5 h after exposure to 0.6 ppm (Group 1) but not after the other protocols. No changes in percentage of lymphocytes or neutrophils. Concluded that NO₂ at these concentrations neither altered epithelial permeability nor caused inflammatory cell influx.</td>
<td>Frampton et al. (1989b)</td>
</tr>
<tr>
<td>(2) Var</td>
<td>(2) Var</td>
<td>180</td>
<td>60</td>
<td>43</td>
<td>11 M/4 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(94 background with 3 = 15 min at 2.0 ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) 1128</td>
<td>(3) 0.6</td>
<td>180</td>
<td>60</td>
<td>40</td>
<td>5 M/3 F</td>
<td>32.6 ± 1.6 years, Healthy, NS</td>
<td></td>
</tr>
<tr>
<td>(4) 2820</td>
<td>(4) 1.5</td>
<td>180</td>
<td>60</td>
<td>39</td>
<td>12 M/3 F</td>
<td>23.5 ± 0.7 years, Healthy, NS</td>
<td></td>
</tr>
</tbody>
</table>
Table 43 (contd).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose</th>
<th>Duration</th>
<th>Dosing Schedule</th>
<th>Gender</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1128</td>
<td>0.6</td>
<td>120/day</td>
<td>60 = 30-40</td>
<td>M/F</td>
<td>4 M/1 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for 4 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21-36 years, Healthy, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FEV/FVC%, range 73-83%, &quot;normal&quot; methacholine responsiveness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slight increase in circulating (venous) lymphocytes: 1792 ± 544 per mm$^3$ (post-NO$_2$) vs. 1598 ± 549 per mm$^3$ (baseline). No change in BAL lymphocytes except an increase in natural killer cells: 7.2 ± 3.1% (post-NO$_2$) vs. 4.2 ± 2.4% (baseline). No change observed in IL-1 or TNF.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frampton et al. (1989a)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose</th>
<th>Duration</th>
<th>Dosing Schedule</th>
<th>Gender</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1128</td>
<td>0.6</td>
<td>180</td>
<td>60 = 40</td>
<td>M/F</td>
<td>7 M/2 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No change in cell recovery or differential counts. Possible decrease in macrophage inactivation of virus in vitro. Possible sensitive subgroup.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frampton et al. (1989a)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose</th>
<th>Duration</th>
<th>Dosing Schedule</th>
<th>Gender</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 with 3760 spikes</td>
<td>0.05 with 0.05 spikes</td>
<td>120/day 3 x 15</td>
<td>21, 22</td>
<td>11 M/4 F</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 (6 x 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonreactive (carbachol), no recent upper resp. infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jorres et al. (1992)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose</th>
<th>Duration</th>
<th>Dosing Schedule</th>
<th>Gender</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1880</td>
<td>1.0</td>
<td>180</td>
<td>Intermittent</td>
<td>M/F</td>
<td>3 M/5 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No responses.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jorres et al. (1992)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose</th>
<th>Duration</th>
<th>Dosing Schedule</th>
<th>Gender</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1880</td>
<td>1.0</td>
<td>120/day</td>
<td></td>
<td>M/F</td>
<td>22 M/2 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy, NS, seronegative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study conducted over 3-year period. NO$_2$ did not significantly increase viral infectivity, although a trend was observed. This study had a low power to detect small differences in infection rate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Goings et al. (1989)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 43 (contd).

<table>
<thead>
<tr>
<th>NO&lt;sub&gt;2&lt;/sub&gt; concentration</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/ gender</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3760</td>
<td>2.0</td>
<td>240</td>
<td>120</td>
<td>50</td>
<td>10</td>
<td>Healthy, NS</td>
<td>Increased bronchial PMN's and decreased macrophage phagocytosis</td>
</tr>
<tr>
<td>3760</td>
<td>2.0</td>
<td>360</td>
<td>Intermittent</td>
<td>12</td>
<td>Healthy, NS</td>
<td>Immediate and 18-h post-BAL increase in PMN.</td>
<td>Frampton et al. (1992)</td>
</tr>
<tr>
<td>4230</td>
<td>2.25</td>
<td>20</td>
<td>20</td>
<td>= 35</td>
<td>8</td>
<td>Healthy, NS</td>
<td>Increased levels of mast cells in BAL fluid at all concentrations. Increased numbers of lymphocytes at ≥ 7520 µg/m&lt;sup&gt;3&lt;/sup&gt; (BAL 24-h post-exposure).</td>
</tr>
</tbody>
</table>
Table 43 (contd).

| 7520 | 4.0 | 20 min- alternate days for 12 days | 20 = 35 | 8 | Healthy, NS | Total cell counts were reduced. Alveolar macrophages had enhanced phagocytic activity but fewer were present. Decreased numbers of mast cells, T and B lymphocytes, and natural killer cells (BAL 24-h post-exposure). | Sandstroem et al. (1990a) |

* Modified from US EPA (1991)

Abbreviations: M = Male; F = Female; NS = Non-smoker; FEV₁ = Forced expiratory volume in 1 second; FVC = Forced vital capacity; BAL = Bronchoalveolar lavage; IL-1 = Interleukin-1; TNF = Tumour necrosis factor; VAR = Variable
susceptibility to attenuated influenza virus. Healthy adults were exposed for 2 h/day for 3 days to either clean air or 1880, 3760 or 5640 µg/m³ (0, 1.0, 2.0 or 3.0 ppm) NO₂. The virus was administered intranasally after the second day of exposure, and infectivity was defined as the presence of virus in nasal washes, a rise in either nasal wash or serum antibody titre to the virus, or both. Although the rates of infection were elevated after NO₂ exposure in some of the NO₂-exposed groups (91% of subjects exposed to 1880 or 3760 µg/m³ (1 or 2 ppm) infected vs. 71% of controls), the changes were not significant. The investigators concluded that the results of the study were inconclusive, rather than negative, because the experimental design had a low power to detect a 20% difference in infection rate, decreasing the possibility of statistical significance.

Others investigated the effects of NO₂ on cells and fluids in bronchoalveolar lavage (BAL) of healthy adults. Frampton et al. (1989a) used two different exposure protocols that had the same concentration x time product. One group was exposed for 3 h to 1128 µg/m³ (0.6 ppm), whereas the other was exposed to a background level of 94 µg/m³ (0.05 ppm) with three 15-min spikes of 3760 µg/m³ (2.0 ppm). Both exposures included exercise. Pulmonary function and airway responsiveness were not affected. Alveolar macrophages (AM) obtained by BAL after exposure to 1128 µg/m³ NO₂ tended to inactivate virus less effectively than AM collected after air exposure. The AMs that showed the impairment of virus inactivation also showed an increase in interleukin-1 production, not seen in the AMs from other subjects. Interleukin-1 is a proinflammatory protein produced by AMs, which performs a number of immunoregulatory functions, including induction of fibroblast proliferation, activation of lymphocytes, and chemotaxis for monocytes. The study had relatively low statistical power to detect an effect. Becker et al. (1993) reported no change in virus inactivation properties of alveolar macrophages lavaged from subjects exposed to 3760 µg/m³ (2 ppm) for 4 h.

Using exposures similar to the above, with the addition of two groups exposed to 2820 µg/m³ (1.5 ppm) NO₂ for 3 h, one with BAL at 3.5 h post-exposure and the other with BAL at 18 h post-exposure, Frampton et al. (1989b) examined changes in protein in BAL fluid. The total protein and albumin content of BAL fluid obtained at either 3.5- or 18-h post-exposure was not changed. In BAL fluid obtained 3.5 h after exposure to 1128 µg/m³ (0.60 ppm) there was an increase in alpha-2-macroglobulin, a regulatory
protein that has antiprotease activity and immunoregulatory effects. This response was not seen in the group lavaged at 18 h post-exposure and no such effect occurred at a higher \( \text{NO}_2 \) concentration (2820 \( \mu g/m^3 \)).

Sandstroem et al. (1989) exposed healthy subjects to 4230, 7520 and 10 340 \( \mu g/m^3 \) (2.25, 4.0 and 5.5 ppm) for 20 min (with moderate exercise) and performed BAL 24 h after exposure. Increased numbers of mast cells were observed at all \( \text{NO}_2 \) concentrations; numbers of lymphocytes were increased only at \( \geq 7520 \mu g/m^3 \). In order to determine the time course of this response, Sandstroem et al. (1990a) exposed four groups of healthy subjects to 7520 \( \mu g/m^3 \) \( \text{NO}_2 \) for 20 min (mild exercise) and then performed BAL 4, 8, 24 or 72 h after exposure. Increased numbers of mast cells and lymphocytes were observed at 4, 8 and 24 h but not at 72 h. There was no change in the numbers of AMs, eosinophils, polymorphonuclear leukocytes, T cells or epithelial cells, or in the albumin concentration of lavage fluid. The authors interpreted the increased numbers of mast cells and lymphocytes as a nonspecific inflammatory response.

Sandstroem et al. (1990b) also evaluated responses to repeated \( \text{NO}_2 \) exposures. Healthy subjects were exposed to 7520 \( \mu g/m^3 \) (4.0 ppm) \( \text{NO}_2 \) for 20 min/day (with moderate exercise) on alternate days over a 12-day period (seven exposures in all); BAL was performed 24 h after the last exposure. The first 20 ml of BAL fluid was treated separately and presumed to represent primarily bronchial cells and secretions; subsequent fractions presumably were from the alveolar region. In the first fraction, there was a reduction in the numbers of mast cells and AMs; AM phagocytic activity (on a per cell basis) was increased. In addition, there were reduced numbers of T-suppressor cells, B cells and natural killer (NK) cells in the alveolar portion of the BAL. This pattern of cellular response contrasts with that after single \( \text{NO}_2 \) exposure (Sandstroem et al., 1990a).

Rubinstein et al. (1991) studied five healthy volunteers exposed for 2 h/day for 4 days to 1128 \( \mu g/m^3 \) (0.60 ppm) \( \text{NO}_2 \) with intermittent exercise. A slight increase in circulating (venous blood) lymphocytes was observed. The only change observed in BAL cells was a modest increase in the percentage of NK cells, suggesting a possible increase in immune surveillance.

Three recent studies examined the effects of longer exposures to 1880 or 3760 \( \mu g/m^3 \) (1.0 to 2.0 ppm) \( \text{NO}_2 \) on lavaged cells and
mediators. Devlin et al. (1992) (also Becker et al., 1993) studied healthy subjects exposed to 3760 μg/m³ NO₂ for 4 h with alternating 15-min periods of rest and moderate exercise. One of the main findings after NO₂ exposure was that there was a three-fold increase in PMNs in the first lavage sample, representing predominantly bronchial cells and fluid. In addition, macrophages recovered from the predominantly alveolar fraction showed a 42% decrease in ability to phagocytose *Candida albicans* and a 72% decrease in release of superoxide anion. In another study, Frampton et al. (1992) exposed exercising subjects to 3760 μg/m³ NO₂ for 6 h. Bronchoalveolar lavage was performed either immediately or 18 h after exposure. There was a modest increase in lavage fluid PMN levels (< two-fold increase) but no change in lymphocytes. Alveolar macrophage production of superoxide anion was not altered in these subjects. These two studies suggest that NO₂ exposure may induce a mild bronchial inflammation and may also lead to impaired macrophage function. On the other hand, Joerres et al. (1992) examined both healthy and asthmatic subjects exposed to 1880 μg/m³ NO₂ for 3 h, but observed no changes in cells or mediators in BAL fluid or in the appearance of bronchial mucosal biopsies after this exposure. Neither macrophage function nor a specific bronchial washing were examined in this study.

Rehn et al. (1982) reported that a 1-h exposure to either 500 or 2000 μg/m³ (0.27 or 1.06 ppm) NO₂ did not alter nasal or tracheobronchial mucociliary clearance rates.

### 6.2.3 Other classes of nitrogen dioxide effects

There have been isolated reports that higher levels of NO₂ (> 7520 μg/m³, 4.0 ppm) can decrease arterial oxygen partial pressure (PaO₂) (Von Nieding & Wagner, 1977; Von Nieding et al., 1979) and cause a small decrease in systemic blood pressure (Linn et al., 1985b). However, the impact of such changes is not clear, especially considering the high concentrations of NO₂ required.

The effects of NO₂ on the constituents of BAL fluid, blood and urine have been examined in very few studies and are reviewed in more detail elsewhere (US EPA, 1993). The general purpose of this research was to examine mechanisms of pulmonary effects or to determine whether NO₂ exposure could result in systemic effects. Investigations of the effects of NO₂ on levels of serum enzymes and antioxidants have been conducted, but few effects were found and they cannot be interpreted (Posin et al., 1978;
Chaney et al., 1981). For example, Chaney et al. (1981) found an increase in glutathione levels, but Posin et al. (1978), using a higher NO2 concentration, did not find such an effect. Studies of exposure to NO2 concentrations between 2820 and 7520 µg/m³ (1.5 and 4.0 ppm) found either slight or no changes in BAL levels of α-1-antitrypsin, which inhibits protease activity (Mohsenin & Gee, 1987; Johnson et al., 1990; Mohsenin, 1991). Healthy subjects exposed to 7520 µg/m³ NO2 (Mohsenin, 1991) at rest for 3 h showed increased lipid peroxidation products in BAL fluid obtained immediately after exposure. In addition, the activity or the elastase inhibitory capacity (EIC) of alpha-1-protease inhibitor (α-1-PI) was decreased after NO2 exposure. However, vitamin C supplementation for 4 weeks prior to NO2 exposure markedly attenuated the EIC response and resulted in a lower level of lipid peroxidation products. The author suggested that the reduced activity of α-1-PI may have implications for the pathogenesis of emphysema, especially in smokers. At a lower NO2 concentration (3760 µg/m³, 2.0 ppm, for 4 h), Becker et al. (1993) reported no change in α-1-antitrypsin. Potential effects of NO2 on collagen metabolism have been investigated by examining urinary excretion of collagen metabolites after a 3-day (4 h/day) exposure to 1128 µg/m³ (0.6 ppm) NO2, but no effects were found (Muellenaer et al., 1987).

6.3 Effects of other nitrogen oxide compounds

Relatively few controlled human exposure studies have been conducted that evaluate NOx species other than NO2. Such studies are summarized in Table 44 and concisely discussed here.

Von Nieding et al. (1973b) exposed healthy subjects and smokers to 12 300 to 47 970 µg/m³ (10 to 39 ppm) NO for 15 min. Total respiratory resistance increased significantly (≈ 10–12%) after exposure to ≥ 24 600 µg/m³ (≥ 20 ppm) NO. Diffusing capacity was not changed, but a small decrease (7 to 8 torr) in PaO2 was noted between 18 450 and 36 900 µg/m³ (15 to 30 ppm). Kagawa (1982) examined the effects of a 1230 µg/m³ (1 ppm) NO exposure for 2 h in normal subjects. A few individuals had increases in SGaw, and a few had decreases. Analysis of the group mean data produced only one apparently statistically significant change: an 11% decrease in flow at 50% FVC in a helium-air mixture compared to this flow in air. However, because the data were analysed by multiple t-tests the results should be interpreted with this in mind.
<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{HNO}_2$</td>
<td>0.004 0.077 0.395</td>
<td>210</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy 22-57 years</td>
<td>A dose-dependent vasodilation in bulbar conjunctiva. Significant increase of polymorphonuclear neutrophils, cuboidal and squamous epithelium cell counts in the tear fluid</td>
<td>Kjærgaard et al. (1993)</td>
</tr>
<tr>
<td>$\text{HNO}_3$</td>
<td>129 0.050 200</td>
<td>40 10</td>
<td>25-30</td>
<td>5 M/4 F</td>
<td>12-17 years, asthmatic</td>
<td>FEV$\text{i}$ decreased - 4.4% after $\text{HNO}_3$ and - 1.7% after $\text{HNO}_2$ plus air exposure. $R_I$ increased +22.5% after $\text{HNO}_2$ and +7.4% after air exposure.</td>
<td>Koenig et al. (1999a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td>In BAL, increase in AM phagocytosis and AM infection resistance.</td>
<td>Becker et al. (1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td>No effect on FEV$\text{i}$, FVC, $SR_{aw}$ or BAL cells.</td>
<td>Aris et al. (1991)</td>
</tr>
</tbody>
</table>
Table 44 (contd).

<table>
<thead>
<tr>
<th>NO</th>
<th>1230</th>
<th>1.0</th>
<th>120</th>
<th>60</th>
<th>50 W</th>
<th>8 M</th>
<th>19-24 years</th>
<th>Suggested change in density dependent of expired flow.</th>
<th>Kagawa (1982)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 300-</td>
<td>10-39</td>
<td>15</td>
<td>191</td>
<td>Healthy</td>
<td>20-50 years</td>
<td>Increase in total respiratory resistance at ≥ 24 600 µg/m³ and a decrease in PaO₂ at ≥ 18 450 µg/m³.</td>
<td>Von Nieding et al. (1973b)</td>
<td></td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>200</td>
<td>(1.1 MMAD)</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>19</td>
<td>Normal Asthmatic</td>
<td>No significant changes due to NH₄NO₃ in normals or asthmatics except possible decrease in Rₜ. No symptoms and effects.</td>
<td>Kleinman et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>80 + 940</td>
<td>(0.55 MMAD)</td>
<td>240</td>
<td>30</td>
<td>55</td>
<td>12</td>
<td>Normal</td>
<td>No effects.</td>
<td>Stacy et al. (1983)</td>
</tr>
</tbody>
</table>

| NaNO₂ | 10, 100 | (0.2 MMAD) | 10 | 5 | Normal Asthmatic | No effects. | Sedlner et al. (1979) |
|       | 1000 | | 5 | 6 | Normal Asthmatic | | |
|       | 1000 | | 6 | 6 | Normal Asthmatic | | |
Table 44 (contd).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³ ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7000</td>
<td>(0.48) 16 (± 2) MMAD)</td>
<td>32 (total)</td>
<td></td>
<td>10</td>
<td>Normal</td>
<td>No effects.</td>
<td>Utell et al. (1979)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>Mild asthmatics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7000</td>
<td>(0.49) 16 (± 2) MMAD)</td>
<td>32 (total)</td>
<td></td>
<td>11</td>
<td>influenza patients</td>
<td>No symptoms. SGmax decrease 17% and VEmax 40% TLC decreased by 12% after nitrate, within 2 days of onset of illness. Similar effects 1 week later but not 3 weeks later.</td>
<td>Utell et al. (1980)</td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

Abbreviations:

W = Watt; M = Male; PaO₂ = Arterial partial pressure of oxygen; HNO₃ = Nitric acid; FEV₁ = Forced expiratory volume in 1 second; FVC = Forced vital capacity; SRaw = Specific airway resistance; BAL = Bronchoalveolar lavage; AM = Alveolar macrophage; F = Female; R₉ = Total respiratory resistance; NS = Not significant; MMAD = Mass median aerodynamic diameter; SGmax = Specific airway conductance; VEmax 40% TLC = Maximum expiratory flow at 40% of total lung capacity on a partial expiratory flow-volume curve.
NO is naturally formed in the body from the amino acid L-arginine and performs a second messenger function in several organ systems. It has been measured in expired air (Gustafsson et al., 1991) and causes vasodilation in the pulmonary circulation. Recently, NO has been used clinically to treat pulmonary hypertension in COPD patients and in infants with persistent pulmonary hypertension of the newborn (Zapol et al., 1994).

In healthy volunteers made hypoxic by breathing 12% oxygen in nitrogen, the inhalation of 49,403 μg/m³ (40 ppm) NO prevented the hypoxia-induced increase in pulmonary artery pressure (Frostell et al., 1993). Systemic arterial pressure was not changed. No evaluation of effects on lung function were performed. Adnot et al. (1993) studied a group of COPD patients who had pulmonary artery pressures averaging 32 mmHg. They breathed 6130 to 49403 pg/m³ (5 to 40 ppm) NO for successive 10-min periods. There was a dose-dependent decrease in pulmonary artery pressure during NO inhalation and no alteration of systemic arterial pressure. Moinard et al. (1994) observed a 20% drop in pulmonary artery pressure in COPD patients after breathing 18,391 pg/m³ (15 ppm) NO for 10 min. Based on an improvement in alveolar ventilation in some segments of the lung, the authors postulated that NO may also act as a bronchodilator. Hoegman et al. (1993) suggested a modest bronchodilator effect of 98,080 pg/m³ (80 ppm) NO. Based on findings in animals, which are summarized in chapter 5, NO does cause bronchodilation at similar concentrations (Barnes, 1993).

Nitrous acid and nitric acid may be formed from the reaction of NO₂ with water. Nitrous acid may also be produced directly in the combustion process.

Koenig et al. (1989a) examined the responses of adolescent asthmatics to a 40-min exposure to 129 μg/m³ (0.05 ppm) HNO₃ vapour via a mouthpiece exposure system. After 30 min of rest and 10 min of exercise while breathing HNO₃, there was a 4.4% decrease in FEV₁ compared to a 1.7% decrease after air breathing. A 22.5% increase in total respiratory resistance was also observed after HNO₃ exposure, compared to a 7.4% increase after air breathing.

The effects of HNO₃ on BAL endpoints have been reported. Becker et al. (1992) exposed healthy subjects to 200 μg/m³ (0.078 ppm) HNO₃ for 120 min, including 100 min of moderate exercise. Bronchoalveolar lavage performed 18-h after exposure indicated increased phagocytic activity of AMs and increased
resistance to respiratory syncytial virus infection. There were no changes in markers of tissue damage. Aris et al. (1991) exposed healthy subjects to 500 µg/m³ (0.194 ppm) HNO₃ for 4 h, including moderate exercise. No change in lactate dehydrogenase levels, lavage fluid protein or differential cell counts in the BAL were observed. Pulmonary function (FEV₁, FVC and SR₁₁₂) was not significantly affected.

Kjaergaard et al. (1993) studied the effects of nitrous acid on the eyes of 15 healthy non-smokers exposed to 8, 148 or 758 µg/m³ (4, 77 or 395 ppb) for 3.5 h. There was an increase in trigeminal sensitivity (CO₂ induced eye irritation) related to the concentration of nitrous acid. Eye inflammation was increased, as indicated by increased PMNs and epithelial cells in tear fluid.

Neither sodium nitrate (NaNO₃) nor ammonium nitrate caused effects on pulmonary function of normal or asthmatic subjects (Sackner et al., 1979; Utell et al., 1979; Kleinman et al., 1980; Stacy et al., 1983). However, there was a decrease in airway conductance and in PEFV curves in normal subjects with acute influenza exposed to 7 mg/m³ of NaNO₃ aerosol (Utell et al., 1980). This is several orders of magnitude above the nitrate concentrations found in most ambient air.

6.4 Effects of nitrogen dioxide/gas or gas/aerosol mixtures on lung function

Table 45 summarizes studies of human subjects exposed to NO₂-containing pollutant mixtures. Most of the studies have been limited primarily to spirometry and plethysmography. More extensive discussion can be found in US EPA (1993).

With a few exceptions (to be discussed below), most research on interactions either showed no effects of the individual pollutants or the mixture, or it indicated that NO₂ did not enhance the effects of the other pollutant(s) in the mixture (Table 45). Most attention has focussed on NO₂ mixtures with ozone (O₃), although combinations with SO₂, NO, particles, and a mixture of SO₂ plus O₃ have also been tested. Due to the varied exposure protocols in the database, no consistent physiological trends are evident. The generally negative responses could either reflect a true lack of interaction or other important design considerations. For example, asthmatics were not studied. Because pulmonary function studies of NO₂ alone cause variable effects with no clear concentration-responses, detecting interactions would be expected to be difficult unless there was significant synergism.
Table 45. Effects of nitrogen dioxide mixtures on healthy subjects

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Exposure duration</th>
<th>Exercise duration</th>
<th>Exercise ventilation</th>
<th>Number of subjects</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (Amb)</td>
<td>(min)</td>
<td>(min)</td>
<td>(litres/min)</td>
<td>gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg/m³ ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 NO₂</td>
<td>0.04 NO₂</td>
<td>60</td>
<td>60</td>
<td>56</td>
<td>42 M/8 F</td>
<td>Healthy</td>
<td>No apparent effect over and above that of O₃ alone.</td>
</tr>
<tr>
<td>75 NO₂</td>
<td>0.04 NO₂</td>
<td>60</td>
<td>60</td>
<td>22.4</td>
<td>33 M/33 F</td>
<td>Children, 8-11 years</td>
<td>No effects of ambient air exposures.</td>
</tr>
<tr>
<td>103 NO₂</td>
<td>0.055 NO₂</td>
<td>60</td>
<td>60</td>
<td>32</td>
<td>46 M/12 F</td>
<td>Adolescents, 12-15 years</td>
<td>Ambient air exposures effect attributed to O₃</td>
</tr>
<tr>
<td>132 NO₂</td>
<td>0.07 NO₂</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>14 M/20 F</td>
<td>29 years</td>
<td>Small decreases in FVC, FEV₁, in ambient air mostly attributable to O₃</td>
</tr>
<tr>
<td>545 NO₂ + 980 O₃</td>
<td>(a) 0.29 NO₂</td>
<td>240 (2 consecutive days of exposure)</td>
<td>120</td>
<td>= 20</td>
<td>4</td>
<td>Healthy</td>
<td>With each group, minimal alterations in pulmonary function caused by O₃ exposure. Effects were not increased by addition of NO₂ or NO₂ plus CO to test atmospheres.</td>
</tr>
<tr>
<td>545 NO₂</td>
<td>(b) 0.29 NO₂</td>
<td>+ 980 O₃</td>
<td>+ 34 350</td>
<td>+ 30 CO mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 45 (contd).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/sex</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$545\ \text{NO}_2 + 490\ \text{O}_3$ (a)</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>7</td>
<td>Healthy</td>
<td>Little or no change in pulmonary function found with $\text{O}_3$ alone. Addition of $\text{NO}_2$ or of $\text{NO}_2$ plus $\text{CO}$ did not noticeably increase the effect. Seven subjects included; some believed to be unusually reactive to respiratory irritants.</td>
<td>Hackney et al. (1975b)</td>
</tr>
<tr>
<td>$545\ \text{NO}_2 + 490\ \text{O}_3 + 34\ 350\ \text{CO}$ (b)</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>7</td>
<td>Healthy</td>
<td>Little or no change in pulmonary function found with $\text{O}_3$ alone. Addition of $\text{NO}_2$ or of $\text{NO}_2$ plus $\text{CO}$ did not noticeably increase the effect. Seven subjects included; some believed to be unusually reactive to respiratory irritants.</td>
<td>Hackney et al. (1975b)</td>
</tr>
<tr>
<td>$20\ \text{M}$</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>7</td>
<td>Healthy</td>
<td>Little or no change in pulmonary function found with $\text{O}_3$ alone. Addition of $\text{NO}_2$ or of $\text{NO}_2$ plus $\text{CO}$ did not noticeably increase the effect. Seven subjects included; some believed to be unusually reactive to respiratory irritants.</td>
<td>Hackney et al. (1975b)</td>
</tr>
<tr>
<td>$940\ \text{NO}_2 + 980\ \text{O}_3$</td>
<td>120</td>
<td>30</td>
<td>40</td>
<td>10 M</td>
<td>NS</td>
<td>FEV$_1$ decreased 8-14%. No differences between $\text{O}_3$ plus $\text{NO}_2$ and $\text{O}_3$ alone.</td>
<td>Folinsbee et al. (1981)</td>
</tr>
<tr>
<td>$1128\ \text{NO}_2 + 980\ \text{O}_3$</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>8 M/8 F</td>
<td>18-26 years, NS</td>
<td>No significant changes attributable to $\text{NO}_2$.</td>
<td>Drechsler-Parks (1987)</td>
</tr>
<tr>
<td>$1128\ \text{NO}_2 + 980\ \text{O}_3$</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>20 M</td>
<td>Healthy</td>
<td>No additional effect of $\text{NO}_2$ over and above effect of $\text{O}_3$.</td>
<td>Adams et al. (1987)</td>
</tr>
<tr>
<td>Concentration</td>
<td>NO$_2$</td>
<td>NO$_2$</td>
<td>Exposure Duration</td>
<td>NO$_2$ Exposure</td>
<td>Description</td>
<td>Source</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>--------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>-------------</td>
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<td></td>
</tr>
<tr>
<td>1128 ppm NO$_2$</td>
<td>0.60</td>
<td>0.3 ppm O$_3$ (3 h later)</td>
<td>120</td>
<td>60</td>
<td>40</td>
<td>21 F</td>
<td>Healthy, NS</td>
</tr>
<tr>
<td>262 NO$_2$ + 294 O$_3$ + 200 H$_2$SO$_4$</td>
<td>0.15</td>
<td>NO$_2$ + 0.15 O$_3$ + H$_2$SO$_4$</td>
<td>120</td>
<td>60</td>
<td>= 25</td>
<td>6 M</td>
<td>Some smokers</td>
</tr>
<tr>
<td>262 NO$_2$ + 294 O$_3$ + 393 SO$_2$ + 200 H$_2$SO$_4$</td>
<td>0.15</td>
<td>NO$_2$ + 0.15 O$_3$ + H$_2$SO$_4$ + SO$_2$</td>
<td>120</td>
<td>60</td>
<td>= 25</td>
<td>3 M</td>
<td>Some smokers</td>
</tr>
<tr>
<td>564 NO$_2$ + 588 O$_3$ + 200 H$_2$SO$_4$</td>
<td>0.30</td>
<td>NO$_2$ + 0.30 O$_3$ + H$_2$SO$_4$</td>
<td>120</td>
<td>20</td>
<td>= 25</td>
<td>6 M</td>
<td>Some smokers</td>
</tr>
<tr>
<td>282 NO$_2$ + 294 O$_3$ + 393 SO$_2$ + 0.15 SO$_2$</td>
<td>0.15</td>
<td>NO$_2$ + 0.15 O$_3$ + SO$_2$</td>
<td>120</td>
<td>60</td>
<td>= 25</td>
<td>7 M</td>
<td>19-23 years</td>
</tr>
<tr>
<td>Concentrations</td>
<td>Exposure duration</td>
<td>Exercise duration</td>
<td>Number of subjects/characteristics</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
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<td>----------------</td>
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<td></td>
</tr>
<tr>
<td>$301 \text{NO}_2$, $+157 \text{O}_3$, $+891 \text{SO}_2$</td>
<td>0.16 NO$_2$, $+0.06 \text{O}_3$, $+0.34 \text{SO}_2$</td>
<td>480</td>
<td>0</td>
<td>15</td>
<td>16-26 years</td>
<td>No change in FVC, acetylcholine airway reactivity.</td>
<td>Islam &amp; Ulmer (1979b)</td>
</tr>
<tr>
<td>$564 \text{NO}_2$, $+738 \text{NO}$</td>
<td>0.3 NO$_2$, $+0.5 \text{NO}$</td>
<td>120</td>
<td>60</td>
<td>6 F</td>
<td>19-25 years NS</td>
<td>No significant effects on pulmonary function or airway responsiveness to acetylcholine.</td>
<td>Kagawa (1990)</td>
</tr>
<tr>
<td>$940 \text{NO}_2$, $1310 \text{SO}_2$, $+26 \text{Zn(NH}_4\text{)}_2(\text{SO}_4\text{)}_2$, $+330 \text{NaCl}$</td>
<td>$0.50 \text{NO}_2$, $+0.5 \text{SO}_2$, $+\text{Zn(NH}_4\text{)}_2(\text{SO}_4\text{)}_2$, $+\text{NaCl}$</td>
<td>135</td>
<td>60</td>
<td>= 20</td>
<td>11 M/9 F</td>
<td>20-53 years</td>
<td>No effects on function; possible symptom responses. NO$_2$ effects not discernible from mixture.</td>
</tr>
<tr>
<td>$940 \text{NO}_2$, $1310 \text{SO}_2$</td>
<td>$0.50 \text{NO}_2$, $+0.50 \text{SO}_2$</td>
<td>120</td>
<td>60</td>
<td>= 20</td>
<td>10 M/14 F</td>
<td>26 ± 4 years, 21 NS, 3 S</td>
<td>No significant effect on lung function in normals. Trend for a slight decrease in FVC after combined exposure.</td>
</tr>
<tr>
<td>Time course of response</td>
<td>Abe (1967)</td>
<td></td>
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<tr>
<td>-------------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-40 years, 4 NS, 1 S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture had intermediate effects on resistance.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time course of response</th>
<th>Islam &amp; Ulmer (1979a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30 years, 8 M</td>
<td>FVC (-5%), FEV₁₋₅ (-11.7%), decreased with exercise exposure to this mixture in &lt; 30 years group.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time course of response</th>
<th>Von Nieding et al. (1977)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy, 20-38 years, 9 M</td>
<td>No interaction on PaO₂ or Rₜ</td>
</tr>
<tr>
<td>Healthy, 20-38 years, 25 S, 9 NS</td>
<td>No interaction on PaO₂ or Rₜ</td>
</tr>
</tbody>
</table>

Table 45 (contd.)

<table>
<thead>
<tr>
<th>7520-9400 NO₂ + 4920-6150 SO₂</th>
<th>4-5 NO₂ + 4-5 SO₂</th>
<th>10</th>
<th>5 M</th>
<th>21-40 years, 4 NS, 1 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time course of response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture had intermediate effects on resistance.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9400 NO₂ + 1960 O₃ + 13 100 SO₂</th>
<th>5.0 NO₂ + 0.1 O₃ + 5.0 SO₂</th>
<th>120</th>
<th>60</th>
<th>?</th>
<th>8 M</th>
<th>&lt; 30 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time course of response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC decreased with exercise exposure to this mixture in &lt; 30 years group.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9400 NO₂ + 196 O₃ + 13 100 SO₂</th>
<th>5.0 NO₂ + 0.1 O₃ + 5.0 SO₂</th>
<th>120</th>
<th>Intermittent</th>
<th>9 M</th>
<th>Healthy, 20-38 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time course of response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No interaction on PaO₂ or Rₜ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9400 NO₂ + 196 O₃</th>
<th>5.0 NO₂ + 0.1 O₃</th>
<th>120</th>
<th>Intermittent</th>
<th>11 M</th>
<th>Healthy, 20-38 years, 25 S, 9 NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time course of response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No interaction on PaO₂ or Rₜ</td>
</tr>
</tbody>
</table>
Table 45 (contd).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Exposure duration (min)</th>
<th>Exercise duration (min)</th>
<th>Exercise ventilation (litres/min)</th>
<th>Number of subjects/characteristics</th>
<th>Subject characteristics</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 NO2 + 196 O3 + 13 100 SO2</td>
<td>120</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>23-36 years, two atopic</td>
<td>$R_l$ increased from 1.5 to 2.4 (p &lt; 0.01); questionable decrease in PaO2 (8 torr).</td>
<td>Von Neding et al. (1979)</td>
</tr>
<tr>
<td>186 NO2 + 786 SO2</td>
<td>120</td>
<td>60</td>
<td>20</td>
<td>23-36 years, two atopic</td>
<td>No effects</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from US EPA (1993)

Abbreviations:

Amb = Ambient air; CO = Carbon monoxide; F = Female; FEV₁ = Forced expiratory volume in 1 second; FEV₁₁₀ = Forced expiratory volume in 1 second; FVC = Forced vital capacity; H₂SO₄ = Sulfuric acid; M = Male; NaCl = Sodium chloride; (NH₄)₂SO₄ = Ammonium sulfate; NO = Nitric oxide; NS = Non-smoker; O₃ = Ozone; PaO₂ = Arterial partial pressure of oxygen; $R_l$ = Total respiratory resistance; S = Active smoker; SGaw = Specific airway conductance; SO₂ = Sulfur dioxide; W = Watts; ZnSO₄ = Zinc sulfate
Abe (1967) studied brief exposures to NO$_2$-SO$_2$ mixtures. Both gases were at 4 to 5 ppm (i.e., 7520 to 9400 µg/m$^3$ NO$_2$ and 4920 to 6150 µg/m$^3$ SO$_2$). The effects were additive, with both gases causing bronchoconstriction. Independently, the effect of SO$_2$ was immediate and short-lasting, whereas the effect of NO$_2$ was delayed and more persistent. The effect of the mixed gases was intermediate between the two independent responses. Kagawa (1983a,b) reported that the interaction of 282 µg/m$^3$ (0.15 ppm) NO$_2$ plus 393 µg/m$^3$ (0.15 ppm) SO$_2$ in normal subjects exposed for 2 h with light intermittent exercise caused an increase in SG$_{aw}$. However, because a large number of repeated t-tests with an alpha level of 0.05 were used, it is possible that the responses were due to chance.

The Rancho Los Amigos group (Linn et al., 1980b; Linn & Hackney, 1983; Avol et al., 1983, 1985a, 1987) conducted several studies of NO$_2$-containing ambient air mixtures. The mean NO$_2$ level in the ambient air (from the Los Angeles Air Basin) ranged from 75 to 132 µg/m$^3$ (0.04 to 0.07 ppm). Normal and asthmatic adults, adolescents and children were exposed for approximately 2 h during the summer smog seasons of 1978 to 1984. The various pulmonary function effects observed (see Table 45) were attributed to O$_3$. However, in another study, Hazucha et al. (1994) found that ozone-induced increases in airway responsiveness to methacholine were enhanced by prior (3 h earlier) exposure to 1128 µg/m$^3$ (0.60 ppm) NO$_2$. There was also a slightly greater FEV$\_1$ decrement after the NO$_2$-O$_3$ sequence.

There has been one study on the effects of HNO$_3$ vapour in combination with O$_3$ (Aris et al., 1991). Ten healthy men were exposed (with moderate exercise) to 430 µg/m$^3$ HNO$_3$ for 2 h and then, after 1 h, to 392 µg/m$^3$ (0.20 ppm) O$_3$ for 3 h. No changes were observed in FVC, FEV$\_1$, or SR$_{aw}$ after HNO$_3$ exposure. Ozone exposure caused increased SR$_{aw}$ and decreased FVC and FEV$\_1$. Prior exposure to HNO$_3$ vapour rather than air resulted in somewhat smaller changes in lung function after ozone exposure. Clearly HNO$_3$ did not potentiate responses to ozone.

### 6.5 Summary of controlled human exposure studies of oxides of nitrogen

Human responses to a variety of oxidized nitrogen compounds have been evaluated. By far, the largest database and the one most suitable for risk assessment is that available for controlled exposures to NO$_2$. The database on human responses to NO, nitric
acid vapour, nitrous acid vapour and inorganic nitrate aerosols is not as extensive. A number of sensitive or potentially sensitive subgroups have been examined, including adolescent and adult asthmatics, older adults, and patients with chronic obstructive pulmonary disease and pulmonary hypertension. Exercise increases the total uptake and alters the distribution of the inhaled material within the lung. The proportion of NO₂ deposited in the lower respiratory tract is also increased by exercise. This may increase the effects of the above compounds in people who exercise during exposure.

As is typical with human biological response to inhaled particles and gases, there is variability in the biological response to NO₂. Healthy individuals tend to be less responsive to the effects of NO₂ than individuals with lung disease. Asthmatics are clearly the most responsive group to NO₂ that has been studied to date. Individuals with chronic obstructive pulmonary disease may be more responsive than healthy individuals, but they have limited capacity to respond to NO₂ and thus quantitative differences between COPD patients and others are difficult to assess. There is not sufficient information available at present to evaluate whether age or gender should be considered in the risk evaluation.

NO₂ causes decrements in lung function, particularly increased airway resistance in resting healthy subjects at 2-h concentrations as low as 4700 μg/m³ (2.5 ppm). Available data are insufficient to determine the nature of the concentration-response relationship.

NO₂ exposure results in increased airway responsiveness to bronchoconstrictive agents in exercising healthy, non-smoking subjects exposed to concentrations as low as 2800 μg/m³ (1.5 ppm) for exposure durations of 1 h or longer.

Exposure of asthmatics to NO₂ causes, in some subjects, increased airway responsiveness to a variety of provocative mediators, including cholinergic and histaminergic chemicals, SO₂ and cold air. The presence of these responses appears to be influenced by the exposure protocol, particularly whether or not the exposure includes exercise. These responses may begin at concentrations as low as 380 μg/m³ (0.20 ppm). A meta analysis suggests that effects may occur at even lower concentrations. However, no concentration-response relationship is observed between 350 and 1150 μg/m³ (0.2 and 0.6 ppm).
Modest increases in airway resistance may occur in patients with COPD from brief exposure (15-60 min) to concentrations of NO\textsubscript{2} as low as 2800 µg/m\textsuperscript{3} (~1.5 ppm) and decrements in spirometric measures of lung function (3 to 8%) change in FE\textsubscript{V\textsubscript{1}} may also be observed with longer exposures (3 h) to concentrations as low as 600 µg/m\textsuperscript{3} (~0.3 ppm).

Exposure to NO\textsubscript{2} at levels above 2800 µg/m\textsuperscript{3} (~1.5 ppm) may alter numbers and types of inflammatory cells in the distal airways or alveoli. NO\textsubscript{2} may alter the function of cells within the lung and production of mediators that may be important in lung host defences. The constellation of changes in host defences, alterations in lung cells and their activities, and changes in biochemical mediators is consistent with the epidemiological findings of increased host susceptibility associated with NO\textsubscript{2} exposure.

In studies of mixtures of NO\textsubscript{2} with other pollutants, NO\textsubscript{2} has not been observed to increase responses to other co-occurring pollutant(s) beyond what would be observed for the other pollutant(s) alone. A notable exception is the observation that pre-exposure to NO\textsubscript{2} enhances the ozone-induced change in airway-responsiveness in healthy, exercising subjects during a subsequent ozone exposure. This observation suggests the possibility of delayed or persistent responses to NO\textsubscript{2}.

Within an NO\textsubscript{2} concentration range that may be of interest with regard to risk evaluation (i.e., 100-600 µg/m\textsuperscript{3}), the characteristics of the concentration-response relationship for acute changes in lung function, airway responsiveness to bronchoconstricting agents, or symptoms cannot be determined from the available data.

NO is acknowledged as an important endogenous second messenger within several organ systems. Inhaled NO concentrations above 6000 µg/m\textsuperscript{3} (~5 ppm) can cause vasodilation in the pulmonary circulation without affecting the systemic circulation. The lowest effective concentration is not established. Information on pulmonary function and lung host defences consequent to NO exposure are too limited for any conclusions to be drawn at this time. Relatively high concentrations (> 40 000 µg/m\textsuperscript{3}) have been used in clinical applications for brief periods (<1 h) without reported adverse reactions.

Nitric acid levels in the range of 250-500 µg/m\textsuperscript{3} (100-200 ppb) may cause some pulmonary function responses in adolescent asthmatics, but not in healthy adults.
Limited information on nitrous acid suggests that it may cause eye inflammation at 760 µg/m³ (0.40 ppm). There are currently no published data on human pulmonary responses to nitrous acid.

Limited data on inorganic nitrates suggest that there are no lung function effects of nitrate aerosols with concentrations of 7000 µg/m³ or less.
7. EPIDEMIOLOGICAL STUDIES OF NITROGEN OXIDES

7.1 Introduction

This chapter discusses epidemiological evidence regarding effects of NO₃ on human health. Primary emphasis is placed on assessment of the effects of NO₂ because it is the oxide of nitrogen measured in most epidemiological studies and the one of greatest concern from a public health perspective. Human health effects associated with exposure to NO₂ have been the subject of several literature reviews since 1970 (National Research Council, 1971, 1977; US EPA, 1982a, 1993; Samet et al., 1987, 1988). Oxides of nitrogen have also been reviewed previously by the World Health Organization (WHO, 1977), which presented a comprehensive review of studies conducted up to 1977. This chapter focuses on studies conducted since 1977, while also using some key information from earlier literature, as reviewed in more detail by US EPA (1993).

The studies discussed in this chapter are those that provide useful quantitative information on exposure-effect relationships for health effects associated with levels of NO₂ likely to be encountered in the ambient air. In addition, some studies that do not provide quantitative information are briefly discussed in the text in order to help elucidate particular points concerning the health effects of NO₂.

7.2 Methodological considerations

Key epidemiological studies on NO₂ health effects are evaluated below for several factors of importance for interpreting their results (US EPA, 1982a,c). Such factors include: (1) exposure measurement error; (2) misclassification of the health outcome; (3) adjustment for covariates; (4) selection bias; (5) internal consistency; and (6) plausibility of the effect based on other evidence.

7.2.1 Measurement error

Measurement error regarding exposure may be a major problem in epidemiological studies of NO₂. Ideally, personal monitors should be placed on all subjects for the entire period of a study, but this is often not feasible. Moreover, personal monitoring may not overcome measurement error altogether. For
example, the monitors that are presently available do not accurately measure short-term peaks or long-term chronic exposures. Other means of estimating NO\textsubscript{2} exposure include source description, in-home monitors and fixed-site outdoor monitors. These approaches are generally cheaper than personal monitors but may be subject to greater measurement error, both random (non-systematic) and systematic.

In general, a measurement error in estimation of exposure that is independent of the health outcome will result in underestimation of associations between exposure and dichotomous health outcomes (Samet & Utell, 1990). Whittlemore & Keller (1988) examined the data of Melia et al. (1980) and showed that a 20% misclassification rate of the exposure category could result in an underestimate of the logistic regression coefficient by as much as 50%. Even when exposure measurement error is not independent of the outcome, measures of association are biased towards the null, unless the probability of the health outcome is very close to 0 or 1 (Stefanski & Carroll, 1985).

At present, there is little information on the relative importance of peak and average NO\textsubscript{2} levels as causes of respiratory effects in humans. In most homes and outdoor settings, peak values may be related to average values, and reduction of peaks may lower time-weighted averages. However, if health effects are largely associated with the peak levels of NO\textsubscript{2}, then the use of averages as the sole guide to exposures will increase measurement error.

NO\textsubscript{2} may act as a precursor for other biologically active substances (such as nitrous acid). If these agents are responsible for some or all of the observed respiratory effects, then measurement of NO\textsubscript{2} will provide an imprecise estimate of the effective dose.

### 7.2.2 Misclassification of the health outcome

Misclassification of the health outcome can occur whether the outcome is continuous, (such as a measure of pulmonary function) or dichotomous (such as the presence or absence of respiratory symptoms). Lung function is typically measured with spirometry, a well-standardized technique (Ferris, 1978). The measurement errors of the instruments collecting the data have also been carefully estimated, and random errors will simply add to the error variance. On the other hand, respiratory symptoms and health
status are usually measured by a questionnaire. Responses to symptom questions will be correlated and will depend on the interpretation of the respondent. As noted below, a specific respiratory disease is likely to be reflected by a constellation of symptoms. Therefore, it is appropriate to consider aggregate, as well as single, specific symptom reports. Obviously, questionnaire measurements involving recent recall are better than those based on recall of events occurring several years earlier. Questionnaires for cough and phlegm production have been standardized, e.g., the British Medical Research Council (BMRC) questionnaire (American Thoracic Society, 1969) and revisions of that questionnaire (Ferris, 1978; Samet, 1978). These questionnaires and modifications of them have been used extensively.

7.2.3 Adjustment for covariates

It is common when analysing a data set to discover that one or more key covariates for the analysis were not measured. Schenker et al. (1983) discussed socioeconomic status, passive smoking and gender as important covariates in childhood respiratory disease studies. Other covariates often of importance are age, humidity and other co-occurring pollutants (e.g., particulate matter). The concern is that, had missing covariates been measured, the estimate of the regression coefficient of a variable of interest would have been significantly different. Although the problem is faced by most investigators, literature on the subject is sparse. For example, Kupper (1984) showed that high correlations between the variables just described will result in "unreliable parameter estimates with large variances". Gail (1986) considered the special case of omitting a balanced covariate from the analysis of a cohort study and concluded that: "In principle, the bias may be either toward or away from zero, though in more important examples — the bias is toward zero. In important applications with additive or multiplicative regression, there is no bias". Neither report provided information on how to attempt to correct for the bias or on approaches for investigating the possible bias in a given situation.

Most studies of respiratory disease and NO₂ exposure discussed here measured important covariates such as age, socioeconomic level of the parents, gender and parental smoking habits. The estimated effect (regression coefficient of disease on NO₂ exposure) will be overestimated if a missing covariate is positively or negatively correlated with both exposure and health outcome. The estimated effect will be underestimated if positively
correlated with exposure or outcome and negatively correlated with the other. Ware et al. (1984) found that parents with some college education were more likely to report respiratory symptoms and less likely to use a gas stove, leading to an underestimate of the health effect, if education were omitted from the analysis.

7.2.4 Selection bias

The possibility of selection bias, although a concern of every study, seems very low for NO₂ epidemiological studies. Selection bias would require selection of participants based on exposure (e.g., use of gas stove) and also health outcome. Because most epidemiological studies of these exposures are population based, there is little possibility of selection based on health end-points. Nevertheless, the loss of subjects by attrition associated with both exposure and health studies must be considered.

7.2.5 Internal consistency

Internal consistency is also a useful check on the validity of a study, but authors often do not report sufficient detail to check for such consistency. For example, in the case of known risk factors for respiratory effects, a study should find the anticipated associations (e.g., passive smoking with increased respiratory illness or with more wheeze in asthmatic children), and certain patterns of age or gender effects should be observed. Consistency between studies also provides an indication of the overall strength of the database.

7.2.6 Plausibility of the effect

Health outcomes should be ones for which there are plausible bases to suspect that NO₂ exposure could contribute to such effects. Two health outcome measures have been most extensively considered in the epidemiological studies: lung function measurements and respiratory illness occurrence. Human clinical and animal toxicological studies have not indicated a demonstrated effect on lung function at ambient levels in normal subjects. On the other hand, human clinical and animal toxicological studies have shown that NO₂ exposure can impair components of the respiratory host defense system, resulting in increased susceptibility of the host to respiratory infection. Thus, reported increases in respiratory symptoms and disease among children in epidemiological studies of NO₂ exposure can be plausibly hypothesized to reflect an increase in respiratory infection.
Each study is subsequently reviewed with special attention given to the above factors. Those studies that address these factors most appropriately provide a stronger basis for the conclusions that they draw. Consistency between studies indicates the level of the strength of the whole database.

7.3 Studies of respiratory illness

Respiratory illness and factors determining its occurrence and severity are important public health concerns. The possible association of NO$_2$ exposure with respiratory illness is of public health importance because both the potential for exposure to NO$_2$ and childhood respiratory illness are common (Samet et al., 1983; Samet & Utell, 1990). This takes on added importance because recurrent childhood respiratory illness (independent of NO$_2$) may be a risk factor for later susceptibility to lung damage (Samet et al., 1983; Glezen, 1989; Gold et al., 1989). The epidemiological studies relating NO$_2$ exposure to respiratory illness are discussed in sections 7.3.1 and 7.3.2.

7.3.1 Indoor air studies

In this section, studies that meet criteria for use in a quantitative analysis are presented. Firstly, studies conducted by Melia and colleagues in the United Kingdom are discussed. This is followed by an evaluation of two large studies conducted in six cities in the USA. Several other quantitative studies conducted by different authors in various countries and cities are then presented. These are followed by discussion of some additional recent large-scale studies that yield useful quantitative information, e.g., a study of NO$_2$ relationship to respiratory disease in young children in Albuquerque, New Mexico, USA. Lastly, other studies that provide information concerning respiratory illness are also discussed.

7.3.1.1 St Thomas’ Hospital Medical School Studies (United Kingdom)

Results of several British studies have been reported by Melia et al. (1977, 1978, 1979, 1980, 1982a, b, 1983, 1988), Goldstein et al. (1979, 1981), and Florey et al. (1979, 1982). Parts of these studies were reviewed previously (US EPA, 1982a), but their importance requires a more complete discussion of them.

The initial study (Melia et al., 1977) was based on a survey of 5658 children (excluding asthmatics, thus 100 less than the number
reported), aged 6 to 11 years, with sufficient questionnaire information in 28 randomly selected areas of England and Scotland. A self-administered questionnaire was completed by a parent to obtain information on the presence of morning cough, day or night cough, colds going to chest, chest sounds of wheezing or whistling, and attacks of bronchitis. The questionnaire, distributed in 1973, asked about symptoms during the previous 12 months. Colds going to the chest accounted for the majority of symptoms reported. Information about cooking fuel (gas or electric), age, gender and social class (manual versus non-manual labour) was obtained, but there were no questions about parental smoking. Melia et al. (1977) noted that although they could not include family smoking habits in the analysis, the known relation between smoking and social class (Tobacco Research Council, 1976) allowed them to avoid at least some of the potential bias from this source. It seemed unlikely that, within the social class groups studied, there was a higher prevalence of smoking in homes where gas was used for cooking. No measurements of NO\textsubscript{2}, either indoors or outdoors, were given.

The authors presented their results in the form of a contingency table for non-asthmatics with complete covariate information. Table 46 is a summary of that data for non-asthmatic children. The authors indicated that there was a trend for increased symptoms in homes with gas stoves, but the increase was only significant for girls in urban areas. The authors gave no measures of increased risk. The data in Table 46 have been reanalysed using a multiple logistic model as shown in Table 47. Because it had been suggested that gender had an effect on the relationship with “gas cooker”, interaction terms for gender were included in the original model. None of these proved to be significant, and they were subsequently dropped from the model. When separate terms for each gender were used for the effect of “gas cooker”, an estimated odds ratio of 1.25 was obtained for boys and an odds ratio of 1.39 was obtained for girls. The combined odds ratio for both genders was 1.31 (95% confidence limits of 1.16 and 1.48) and was statistically significant (p < 0.0001). The other main effects of gender, SES and age were all statistically significant. This reanalysis suggests that gas stove use was associated with an estimated 31% increase in the odds of children having respiratory illness symptoms.

Melia et al. (1979) reported further results of a national survey covering a new cohort of 4827 boys and girls, aged 5 to 10 years, from 27 randomly selected areas that were examined in 1977. The
### Table 46. Symptom rates of United Kingdom children by age, gender, social class and type of cooker

<table>
<thead>
<tr>
<th>Social classes I-IIa (non-manual)</th>
<th>Social classes IIIb-V (manual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric</td>
<td>Gas</td>
</tr>
</tbody>
</table>

#### Age < 8 years

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
</tr>
<tr>
<td>Boys</td>
<td>25.6%</td>
<td>22.2%</td>
<td>29.9%</td>
<td>31.8%</td>
</tr>
<tr>
<td></td>
<td>(203)</td>
<td>(171)</td>
<td>(375)</td>
<td>(393)</td>
</tr>
<tr>
<td>Girls</td>
<td>26.1%</td>
<td>30.4%</td>
<td>37.5%</td>
<td>33.5%</td>
</tr>
<tr>
<td></td>
<td>(88)</td>
<td>(112)</td>
<td>(309)</td>
<td>(337)</td>
</tr>
</tbody>
</table>

#### Age 8 to 11 years

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
<td>(&lt;8 years)</td>
</tr>
<tr>
<td>Boys</td>
<td>20.8%</td>
<td>18.1%</td>
<td>29.0%</td>
<td>27.8%</td>
</tr>
<tr>
<td></td>
<td>(365)</td>
<td>(303)</td>
<td>(675)</td>
<td>(674)</td>
</tr>
<tr>
<td>Girls</td>
<td>23.3%</td>
<td>19.2%</td>
<td>17.8%</td>
<td>29.0%</td>
</tr>
<tr>
<td></td>
<td>(189)</td>
<td>(167)</td>
<td>(654)</td>
<td>(623)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to number of subjects; source: Melia et al. (1977)

### Table 47. Multiple logistic analysis of data from the study of Melia et al. (1977)

<table>
<thead>
<tr>
<th>Factor*</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SES and age by gender interactions (2 d.f.)</td>
<td>1.31</td>
<td>1.16-1.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gas by gender interaction (1 d.f.)</td>
<td>1.06</td>
<td>0.96-1.15</td>
<td>0.3953</td>
</tr>
<tr>
<td>Gas cooker</td>
<td>1.31</td>
<td>1.14-1.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.86</td>
<td>0.76-0.97</td>
<td>0.0121</td>
</tr>
<tr>
<td>SES (manual)</td>
<td>1.31</td>
<td>1.14-1.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age (&lt; 8 years)</td>
<td>1.47</td>
<td>1.30-1.66</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* SES = Socioeconomic status; d.f. = Degrees of freedom
study collected information on the number of smokers in the home. In the 1977 cross-sectional study, only prevalence of day or night cough in boys (p = 0.02) and colds going to the chest in girls (p < 0.05) were found to be significantly higher in children from homes where gas was used for cooking compared with children from homes where electricity was used. As shown in Table 48, grouping responses according to the six respiratory questions into (1) none or (2) one or more symptoms or diseases yielded a prevalence higher in children from homes where gas was used for cooking than in those from homes where electricity was used (p = 0.01 in boys, p = 0.07 in girls). The effects of gender, social class, use of pilot lights and number of smokers in the house were examined.

Table 48. Unadjusted rates of one or more symptoms among United Kingdom children by age, gender, social class and type of cooker

<table>
<thead>
<tr>
<th>Social classes I-IIIa (non-manual)</th>
<th>Social classes IIIb-V (manual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric</td>
<td>Gas</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age &lt; 8 years</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>27.4%</td>
<td>31.7%</td>
<td>32.8%</td>
<td>36.7%</td>
</tr>
<tr>
<td></td>
<td>(277)</td>
<td>(145)</td>
<td>(485)</td>
<td>(313)</td>
</tr>
<tr>
<td>Girls</td>
<td>24.4%</td>
<td>27.6%</td>
<td>27.8%</td>
<td>36.3%</td>
</tr>
<tr>
<td></td>
<td>(291)</td>
<td>(134)</td>
<td>(497)</td>
<td>(336)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age 8 to 11 years</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>19.2%</td>
<td>28.3%</td>
<td>23.6%</td>
<td>26.9%</td>
</tr>
<tr>
<td></td>
<td>(286)</td>
<td>(113)</td>
<td>(501)</td>
<td>(338)</td>
</tr>
<tr>
<td>Girls</td>
<td>14.8%</td>
<td>18.6%</td>
<td>21.5%</td>
<td>18.5%</td>
</tr>
<tr>
<td></td>
<td>(243)</td>
<td>(116)</td>
<td>(437)</td>
<td>(313)</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to number of subjects; source: Melia et al. (1979)

The reanalysis of the data in Table 48, applying a multiple logistic model, is given in Table 49. This model contained the same terms as the analysis in Table 47. As in the previous analysis, none
Table 49. Multiple logistic analysis of data from the study of Melia et al. (1979)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SES and age by gender</td>
<td>0.5749</td>
<td></td>
<td></td>
</tr>
<tr>
<td>interactions (2 d.f.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas by gender interaction (1 d.f.)</td>
<td>0.5566</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas cooker</td>
<td>1.24</td>
<td>1.09-1.42</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.82</td>
<td>0.72-0.94</td>
<td>0.0030</td>
</tr>
<tr>
<td>SES (manual)</td>
<td>1.25</td>
<td>1.08-1.45</td>
<td>0.0034</td>
</tr>
<tr>
<td>Age (&lt; 8 years)</td>
<td>1.69</td>
<td>1.48-1.93</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

SES = Socioeconomic status; d.f. = Degrees of freedom

of the interaction terms proved to be significant, and they were subsequently dropped from the model. When separate terms for each gender were used for the effect of "gas cooker", an estimated odds ratio of 1.29 was obtained for boys and an odds ratio of 1.19 was obtained for girls. The combined odds ratio for both genders was 1.24 (95% confidence limits of 1.09 and 1.42). This effect was statistically significant (p < 0.0002). The other main effects of gender, SES and age were all statistically significant. This reanalysis suggests that gas stove use in this study is associated with an estimated 24% increase in the odds of having symptoms.

In 1978, 808 schoolchildren (Melia et al., 1980), aged 6 to 7 years, were studied in Middlesborough, an urban area of northern England. Respiratory illness was defined as in the previous study. Weekly indoor NO₂ measurements were collected from 66% of the homes, the remaining 34% refusing to participate. NO₂ was measured weekly by triethanolamine diffusion tubes (Palmes tubes) attached to walls in the kitchen area and in the children's bedrooms. In homes with gas stoves, weekly levels of NO₂ in kitchens ranged from 10 to 596 μg/m³ (0.005 to 0.317 ppm) with a mean of 211 μg/m³ (0.112 ppm) and levels in bedrooms ranged from 8 to 318 μg/m³ (0.004 to 0.169 ppm) with a mean of 56 μg/m³ (0.031 ppm). In homes with electric stoves, weekly levels of NO₂ in kitchens ranged from 11 to 353 μg/m³ (0.006 to
0.188 ppm) with a mean of 34 μg/m³ (0.018 ppm), and levels in bedrooms ranged from 6 to 70 μg/m³ (0.003 to 0.037 ppm) with a mean of 26 μg/m³ (0.014 ppm). Outdoor levels of NO₂ were determined using diffusion tubes systematically located throughout the area; the weekly average ranged from 26 to 45 μg/m³ (0.014 to 0.024 ppm). One analysis by the authors was restricted to those 103 children in homes where gas stoves were present and where bedroom NO₂ exposure was measured; the data are shown in Table 50. A linear regression model was fit to the logistic transformation of the rates. Cooking fuel was found to be associated with respiratory illness, independent of social class, age, gender or presence of a smoker in the house (p = 0.06). However, when social class was excluded from the regression, the association was weaker (p = 0.11). For the 6- and 7-year-old children living in homes with gas stoves, there appeared to be an increase in respiratory illness with increasing levels of NO₂ in their bedrooms (p = 0.10), but no significant relationship was found between respiratory symptoms in those children, their siblings or parents and levels of NO₂ in kitchens.

<table>
<thead>
<tr>
<th>Bedroom levels of NO₂ (ppm)</th>
<th>&lt; 0.020</th>
<th>0.020-0.039</th>
<th>&gt; 0.039</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>43.5% (23)</td>
<td>57.9% (19)</td>
<td>69.2% (13)</td>
<td>54.5% (55)</td>
</tr>
<tr>
<td>Girls</td>
<td>44.0% (25)</td>
<td>60.0% (15)</td>
<td>75.0% (8)</td>
<td>54.2% (48)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>43.7% (48)</td>
<td>58.8% (34)</td>
<td>71.4% (21)</td>
<td>54.4% (103)</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to number of subjects (from: Mela et al., 1980)

Because no concentration-response estimates were given by the authors, a multiple logistic model was fitted to the data in
Table 50 with a linear slope for NO$_2$ and separate intercepts for boys and girls. NO$_2$ levels for the groups were estimated by fitting a log-normal distribution to the grouped NO$_2$ data, and the average exposures within each interval were estimated (see Hasselblad et al., 1980). The estimated logistic regression coefficient for NO$_2$ (in $\mu$g/m$^3$) was 0.015 with a standard error of 0.007. The likelihood ratio test for NO$_2$ gave a chi-square of 4.94 with one degree of freedom, with a corresponding p value of 0.03.

The study was repeated in January to March of 1980 by Melia et al. (1982a,b). This time, children aged 5 to 6 years were sampled from the same neighbourhood as the previous study, but only families with gas stoves were recruited. Environmental measurements were made and covariate data were collected in a manner similar to the previous study (Melia et al., 1980). Measurements of NO$_2$ were available for 54% of the homes. The unadjusted rates of one or more symptoms by gender and exposure level are shown in Table 51. The authors concluded that "... no relation was found between the prevalence of respiratory illness and levels of NO$_2$". A reanalysis by Hasselblad et al. (1992) of the data in Table 51 was made using a multiple logistic model similar to the one used for the previous study (Melia et al., 1980). The model included a linear slope for NO$_2$ and separate intercepts for boys and girls. Nitrogen dioxide levels for the groups were estimated by fitting a log-normal distribution to the grouped bedroom NO$_2$ data. The estimated logistic regression coefficient for NO$_2$ (in $\mu$g/m$^3$) was 0.0037 with a standard error of 0.0052. The likelihood ratio test for the effect of NO$_2$ gave a chi-square of 0.51 with one degree of freedom ($p = 0.48$).

Melia et al. (1983) investigated the association between gas cooking in the home and respiratory illness in a study of 390 infants born between 1973 and 1978. When the child reached 1 year of age, the mother was interviewed by a trained field worker to complete a questionnaire. The mother was asked whether the child usually experienced morning cough, day or night cough, wheeze or colds going to the chest, and whether the child had experienced bronchitis, asthma or pneumonia during the past 12 months. No relation was found between type of fuel used for cooking at home and the prevalence of respiratory symptoms and diseases recalled by the mother after allowing for the effects of gender, social class and parental smoking. The authors gave prevalence rates of children having at least one symptom, according to gas stove use and gender. The combined odds ratio for presence of symptoms according to gas stove use was 0.63 with 95% confidence interval of 0.36 to 1.10.
Table 51. Unadjusted rates of one or more symptoms among United Kingdom boys and girls according to bedroom levels of nitrogen dioxide*

<table>
<thead>
<tr>
<th>Bedroom levels of NO$_2$ (ppm)</th>
<th>&lt; 0.020</th>
<th>0.020-0.039</th>
<th>&gt; 0.039</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>56.4%</td>
<td>67.6%</td>
<td>72.0%</td>
<td>64.4%</td>
</tr>
<tr>
<td></td>
<td>(39)</td>
<td>(37)</td>
<td>(25)</td>
<td>(101)</td>
</tr>
<tr>
<td>Girls</td>
<td>60.0%</td>
<td>41.0%</td>
<td>52.2%</td>
<td>49.4%</td>
</tr>
<tr>
<td></td>
<td>(25)</td>
<td>(39)</td>
<td>(23)</td>
<td>(87)</td>
</tr>
<tr>
<td>Total</td>
<td>57.8%</td>
<td>53.9%</td>
<td>62.5%</td>
<td>57.5%</td>
</tr>
<tr>
<td></td>
<td>(64)</td>
<td>(76)</td>
<td>(48)</td>
<td>(188)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to number of subjects; source: Melia et al. (1982a,b)

Melia et al. (1988) studied factors affecting respiratory morbidity in 1964 primary school children living in 20 inner city areas of England in 1983 as part of a national study of health and growth. Data on age, gender, respiratory illness, cooking fuels, mother’s education and size of family were obtained by questionnaire. Smoking was not studied. The same respiratory questions were asked as in previous studies. Melia et al. (1990) reported indoor levels of NO$_2$ associated with gas stoves in inner city areas of England in 1987. The mean weekly NO$_2$ level measured in 22 bedrooms of homes with gas stoves was 45 ± 25 µg/m$^3$ (24.1 ± 13.2 ppb). The mean weekly NO$_2$ level measured in four bedrooms of homes without gas stoves was 40 ± 22 µg/m$^3$ (20.7 ± 11.8 ppb). Melia et al. (1988) reported a relative risk of 1.06 (95% confidence interval of 0.94 to 1.17) for one or more respiratory conditions associated with exposure to gas or kerosene fuel used in the home after adjustment for ethnic group, gender, age group, mother’s education, family size and single parent family status.

7.3.1.2 Harvard University - Six Cities Studies (USA)

Several authors (Spengler et al., 1979, 1986; Speizer et al., 1980; Ferris et al., 1983; Ware et al., 1984; Berkey et al., 1986; Quackenboss et al., 1986; Dockery et al., 1989a; Neas et al., 1990, 1991) have reported on two cohorts of children studied in six
different cities in the USA. The six cities were selected to represent a range of air quality based on their historic levels of outdoor pollution. They included: Watertown, Massachusetts; Kingston and Harriman, Tennessee; southeast St. Louis, Missouri; Steubenville, Ohio; Portage, Wisconsin; and Topeka, Kansas. In each community during 1974-1977, approximately 1000 first- and second-grade schoolchildren were enrolled in the first year and an additional 500 first-graders were enrolled in the next year (Ferris et al., 1979). Families reported the number of people living in the home and their smoking habits, parental occupation and educational background, and fuels used for cooking and heating. Outdoor pollution was measured at fixed sites in the communities as well as at selected households. Indoor pollution including NO₂ was measured in several rooms of selected households.

Speizer et al. (1980) reported results from the six cities studies based on 8120 children, aged 6 to 10 years, who had been followed for 1 to 3 years. Health end-points were measured by a standard respiratory questionnaire completed by the parents of the children. The authors used log-linear models to estimate the effect of current use of gas stoves versus electric stoves on the rates of serious respiratory illness before age 2, yielding an odds ratio of 1.12 (95% confidence limits of 1.00 and 1.26) for gas stove use. The results were adjusted for presence of adult smokers, presence of air conditioning, and family SES.

Ware et al. (1984) reported results for a larger cohort of 10 160 white children, aged 6 to 9 years, in the same six cities over a longer period (1974-1979). Directly standardized rates of reported illnesses and symptoms did not show any consistent pattern of increased risk for children from homes with gas stoves. Logistic regression analyses controlling for age, gender, city and maternal smoking level gave estimated odds ratios for the effect of gas stoves ranging from 0.93 to 1.07 for bronchitis, chronic cough, persistent wheeze, lower respiratory illness index, and illness for the last year. The lower respiratory illness index indicated the presence of bronchitis, restriction of activity due to lower respiratory illness, or chronic cough during the past year. The 95% confidence bounds around all of these symptom-specific odds ratios included 1. Only two odds ratios approached statistical significance: (1) history of bronchitis (odds ratio = 0.86, 95% confidence interval 0.74 to 1.00) and (2) respiratory illness before age 2 (odds ratio = 1.13, 95% confidence interval 0.99 to 1.28). When the odds ratio for respiratory illness before age 2 was adjusted for parental education, the odds ratio was 1.11 with 95%
confidence limits of 0.97 and 1.27 (p = 0.14). Thus, the study suggests an increase of about 11% in respiratory illness before the age of 2 years, which is about the same as that reported by Speizer et al. (1980), although the increase was not statistically significant at the 0.05 level. The end-point in the Ware et al. (1984) study most similar to that of the Melia studies was the lower respiratory illness index. The authors gave the unadjusted prevalence, and from those data, an estimated odds ratio of 1.08 with 95% confidence limits of 0.97 and 1.19 was calculated. Although this odds ratio was not adjusted for other covariates, such adjustments minimally affected other end-points in this study. Analyses by Ware et al. (1984) on the other end-points found that effects of adjustment for covariates was minimal.

During the period from 1983 to 1986, a new cohort of about 1000 second- to fifth-grade schoolchildren in each community was enrolled and given an initial symptom questionnaire (Dockery et al., 1989a). The authors studied reported respiratory symptoms on a subsequent symptom questionnaire (second annual) for 5338 white children aged 7 to 11 years at the time of enrolment. The end-points of chronic cough, bronchitis, restriction of activity due to chest illness, and persistent wheeze were not associated with gas stove use in the home, but the health end-point of doctor-diagnosed respiratory illness prior to age 2 yielded an odds ratio of 1.15 with 95% confidence limits of 0.96 to 1.37. The odds ratio for chronic cough was 1.15 with 95% confidence limits of 0.89 and 1.91. The odds ratio was adjusted for age, sex, parental education, city of residence, and use of unvented kerosene heaters.

Neas et al. (1990, 1991) studied the effects of measured NO₂ among a stratified one-third random sample of the children that were part of the Dockery et al. (1989a) analysis. The sample was restricted to 1286 white children 7 to 11 years of age at enrolment with complete covariate information and at least one valid indoor measurement of both NO₂ and respirable particles. Methods for measuring indoor pollutants were described by Spengler et al. (1986). Indoor pollutants were measured in each child's home for 2 weeks during the heating season and 2 weeks during the cooling season. The two 2-week measurements were averaged to estimate each child's annual average NO₂ exposure. NO₂ was measured by Palmes passive diffusion tubes at three locations: kitchen, activity room and the child's bedroom. The three locations were averaged to create a household annual average NO₂ exposure.

346
The analysis of the Neas et al. (1990, 1991) study was based on the final symptom questionnaire (third annual), completed by parents following the indoor measurements. The questionnaire reported symptoms during the previous year, including attacks of shortness of breath with wheeze, persistent wheeze, chronic cough, chronic phlegm and bronchitis. The authors used a multiple logistic model with separate city intercepts, indicator variables for gender and age, parental history of chronic obstructive pulmonary disease, parental history of asthma, parental education and single parent family status. Increases in symptoms were estimated for an additional NO$_2$ exposure of 28.3 µg/m$^3$ (0.015 ppm). Table 52 shows the odds ratios for the five separate symptoms associated with the increase in NO$_2$ exposure.

Table 52. Odds ratios and 95% confidence intervals for the effect of an additional load of 0.015 ppm NO$_2$ on the symptom prevalence (from: Neas et al., 1991)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortness of breath</td>
<td>1.23</td>
<td>0.93 to 1.61</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>1.16</td>
<td>0.89 to 1.52</td>
</tr>
<tr>
<td>Chronic cough</td>
<td>1.18</td>
<td>0.87 to 1.60</td>
</tr>
<tr>
<td>Chronic phlegm</td>
<td>1.25</td>
<td>0.94 to 1.66</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>1.05</td>
<td>0.75 to 1.47</td>
</tr>
</tbody>
</table>

Neas et al. (1990, 1991) defined a combined symptom as the presence of any of the symptoms just reported. A multiple logistic regression of this combined lower respiratory symptom, equivalent to the single response regression, gave an estimated odds ratio of 1.40 with a 95% confidence interval of 1.14 to 1.72. The odds ratio for the combined symptom score was slightly higher than in other studies, but was not inconsistent with those results. The reference category for each of the symptom-specific odds ratios included some children with the other lower respiratory symptoms, whereas the children in the reference category for combined lower respiratory symptoms were free of any of these symptoms. When
split by gender, the odds ratio was higher in girls, a result similar
to the gender modification reported by Melia et al. (1979). When
separate logistic analyses were performed for each community, the
adjusted odds ratios ranged from 1.26 for Topeka, Kansas, to 1.86
for Portage, Wisconsin. When the cohort was restricted to the 495
children in homes with a gas stove, the adjusted odds ratio was
1.37 with a 95% confidence interval of 1.02 to 1.84. Table 53
provides the adjusted odds ratios for combined lower respiratory
symptoms across ordered NO\textsubscript{2} exposure categories. The association
is statistically significant for the upper exposure category and the
overall results are consistent with a linear dose-response
relationship between NO\textsubscript{2} and lower respiratory symptoms in
children.

Table 53. Odds ratios and 95% confidence intervals for the effect of
ordered NO\textsubscript{2} exposures on the prevalence of lower respiratory
symptoms (from: Neas et al., 1991)

<table>
<thead>
<tr>
<th>NO\textsubscript{2} level (ppm)</th>
<th>Number of children</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 0.0049</td>
<td>0.0037</td>
<td>263</td>
<td>1.00</td>
</tr>
<tr>
<td>0.005 to 0.0099</td>
<td>0.0073</td>
<td>360</td>
<td>1.06</td>
</tr>
<tr>
<td>0.010 to 0.0199</td>
<td>0.0144</td>
<td>317</td>
<td>1.36</td>
</tr>
<tr>
<td>0.020 to 0.0782</td>
<td>0.0310</td>
<td>346</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Neas et al. (1992) reported that the estimated effect of an
additional load of 28.3 \(\mu\)g NO\textsubscript{2}/m\textsuperscript{3} (0.015 ppm) on lower
respiratory symptoms was consistent across the seasons and
sampling locations. Table 54 provides the odds ratios and 95%
confidence intervals for this association by season and sampler
location. The NO\textsubscript{2} levels measured by the activity room and
bedroom sampler were more strongly associated with lower respir-
atory symptoms than those in the kitchen. The NO\textsubscript{2} measurements
in the kitchen were influenced more by transient peak levels
associated with meal preparation on gas stoves, whereas the other
sampling locations were more reflective of the child's long-term
average exposures to NO\textsubscript{2} in the home. Spengler et al. (1992)
Table 54. Odds ratios and 95% confidence intervals for the effect of an additional 0.015 ppm NO\textsubscript{2} on the prevalence of lower respiratory symptoms according to sampling location and season (from: Neas et al., 1992)

<table>
<thead>
<tr>
<th>Sampler location and season</th>
<th>Mean difference gas vs. electric (ppm)</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household annual average</td>
<td>0.016</td>
<td>1.40</td>
<td>1.14 to 1.72</td>
</tr>
<tr>
<td>Household winter average</td>
<td>0.018</td>
<td>1.16</td>
<td>1.04 to 1.29</td>
</tr>
<tr>
<td>Household summer average</td>
<td>0.014</td>
<td>1.46</td>
<td>1.13 to 1.85</td>
</tr>
<tr>
<td>Kitchen annual average</td>
<td>0.022</td>
<td>1.23</td>
<td>1.05 to 1.44</td>
</tr>
<tr>
<td>Activity room annual average</td>
<td>0.014</td>
<td>1.50</td>
<td>1.20 to 1.87</td>
</tr>
<tr>
<td>Bedroom annual average</td>
<td>0.013</td>
<td>1.47</td>
<td>1.17 to 1.85</td>
</tr>
</tbody>
</table>

suggested that children spend relatively little time (0.5 h per day) in the kitchen when the range is operating.

7.3.1.3 University of Iowa Study (USA)

Ekwo et al. (1983) surveyed 1355 children 6 to 12 years of age for respiratory symptoms and lung function in the Iowa City School District. Parents of the children completed a questionnaire that was a modification of one developed by the American Thoracic Society. The children were a random sample from those families whose parents had completed the questionnaire. Eight measures of respiratory illness were reported by the authors, but only two were similar to the endpoints used in the United Kingdom studies (section 7.3.1.1) and the Harvard Six City studies (section 7.3.1.2). Parental smoking was also measured and used as a covariate in the analyses. Results of the analyses, based on 1138 children, are presented in Table 55. No measurements of NO\textsubscript{2} exposure, either inside or outside the homes, were reported.

7.3.1.4 Agricultural University of Wageningen (The Netherlands)

Houthuijs et al. (1987), Brunekreef et al. (1987), and Dijkstra et al. (1990) studied the effect of indoor factors on respiratory health in 6- to 9-year-old children from 10 primary schools in five non-industrial communities in the southeast region of the
Table 55. Analysis of Iowa city school children respiratory symptoms according to gas stove type and parental smoking (from: Ekwo et al., 1983)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hospitalization for chest illness before age two</th>
<th>Chest congestion and phlegm with colds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>SEa</td>
</tr>
<tr>
<td>Gas stove use</td>
<td>2.4b</td>
<td>0.584</td>
</tr>
<tr>
<td>Smoking effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father alone smokes</td>
<td>2.3b</td>
<td>0.856</td>
</tr>
<tr>
<td>Mother alone smokes</td>
<td>2.9b</td>
<td>1.239</td>
</tr>
<tr>
<td>Both smoke</td>
<td>1.6</td>
<td>0.859</td>
</tr>
</tbody>
</table>

* SE = Standard error of the odds ratio

Indicates statistical significance at the 0.05 probability level

Netherlands. Personal exposure to NO$_2$ and home concentrations were measured. An important NO$_2$ emission and exposure source in these homes are geysers, which are unvented, gas-fired, hot water sources at the water tap. Exposure to tobacco smoke was assessed by a questionnaire that also reported symptom information. The study used Palmes diffusion tubes to measure a single weekly average personal NO$_2$ exposure. In January and February 1985, NO$_2$ in the homes of 593 children who had not moved in the last 4 years was measured for 1 week. Personal exposure was also estimated from time budgets and room monitoring. Estimated and measured exposures to NO$_2$ are given in Table 56.

Three health measures were obtained from the questionnaire, a modified form of the WHO questionnaire. The different items were combined to create three categories: cough, wheeze and asthma. Asthma was defined as attacks of shortness of breath with wheezing in the past year. The presence of any of the three symptoms was used as a combination variable. The results are presented in Table 57. A logistic regression model was used to fit the combination variable. Exposure was estimated by fitting a log-normal distribution to the grouped data, and the mean exposure values for each group were estimated by a maximum likelihood
7.3.1.5 Ohio State University Study (USA)

Mitchell et al. (1975) and Keller et al. (1979a) conducted a 12-month study of respiratory illness and pulmonary function in families in Columbus, Ohio, prior to 1978. The sample included 441 families divided into two groups using either gas or electric cooking. Participating households were given diaries to record respiratory illnesses for 2-week periods. Respiratory illnesses included colds, sore throat, hoarseness, earache, phlegm, and cough. Only one incident of illness per person per 2-week period

---

Table 56. Estimated and measured personal NO$_2$ exposure (μg/m$^3$) for a single weekly average (from: Houthuijs et al., 1987)

<table>
<thead>
<tr>
<th>NO$_2$ Source</th>
<th>Number</th>
<th>Estimated Arithmetic mean</th>
<th>Estimated Standard deviation</th>
<th>Measured Arithmetic mean</th>
<th>Measured Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No geyser</td>
<td>370</td>
<td>22</td>
<td>7</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>Vented geyser</td>
<td>112</td>
<td>29</td>
<td>9</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Unvented geyser</td>
<td>111</td>
<td>40</td>
<td>9</td>
<td>42</td>
<td>11</td>
</tr>
</tbody>
</table>

The estimated logistic regression coefficient was —0.002, corresponding to an odds ratio of 0.94 for an increase of 28.3 μg/m$^3$ (0.015 ppm) in NO$_2$ with 95% confidence interval of 0.70 to 1.27. Thus, these studies did not demonstrate an increase in respiratory disease with increasing NO$_2$ exposure, but the range of uncertainty is quite large and the rates were not adjusted for covariates such as parental smoking and age of the child. One potential explanation offered by the authors for the negative findings with respect to NO$_2$ exposure was the smaller sample size of the measured NO$_2$ data compared to the categorical data (i.e., gas stove versus electric stove use). They could not estimate whether more precision was gained by use of measured NO$_2$ than was lost by the reduction in the sample size. Houthuijs et al. (1987) reported earlier that the presence of an unvented geyser in the kitchen is associated with a higher prevalence of respiratory symptoms and that the NO$_2$ difference between no geyser present and an unvented geyser is about 0.01 ppm.
Table 57. Frequency and prevalence of reported respiratory symptoms with respect to different categories of mean indoor NO$_2$ concentrations in a population of 775 children aged 6 to 12 old (from: Dijkstra et al., 1990)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>0-20 µg/m$^3$ (n = 336)</th>
<th>21-40 µg/m$^3$ (n = 267)</th>
<th>41-60 µg/m$^3$ (n = 93)</th>
<th>&gt; 60 µg/m$^3$ (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>15 (4.8%)</td>
<td>12 (4.5%)</td>
<td>7 (7.5%)</td>
<td>3 (3.8%)</td>
</tr>
<tr>
<td>Wheeze</td>
<td>30 (9.0%)</td>
<td>18 (6.7%)</td>
<td>3 (3.2%)</td>
<td>7 (8.9%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>22 (6.6%)</td>
<td>12 (4.5%)</td>
<td>2 (2.2%)</td>
<td>3 (3.8%)</td>
</tr>
<tr>
<td>One or more symptoms</td>
<td>36 (10.7%)</td>
<td>24 (9.0%)</td>
<td>8 (8.6%)</td>
<td>8 (10.1%)</td>
</tr>
</tbody>
</table>
was recorded. The study measured NO₂ exposure, by both the Jacobs-Hochheiser and continuous chemiluminescence methods. The electric stove users averaged 38 μg/m³ (0.02 ppm) NO₂ exposure, whereas the gas stove users averaged 94 μg/m³ (0.05 ppm). The report did not indicate which rooms were measured in order to obtain this average.

No differences were found in any of the illness rates for fathers, mothers or children. No analyses were carried out using multiple logistic regression or Poisson regression (these methods were relatively new at the time). No estimates were made that can be considered comparable to the odds ratios reported in the other studies. However, the authors did show a bar graph of all respiratory illness for children under 12. The rates were 389 (per 100 person-years) for electric stove use and 377 for gas stove use. These rates were not significantly different even after adjustment for covariates, including family size, age, gender, length of residence and father’s education. No mention was made of adjustments for smoking status or smoking exposure for the children.

In a second, related study (Keller et al., 1979b), 580 people drawn from households that participated in the earlier study were examined to confirm the reports and to determine the frequency distribution of reported symptoms among parents and children in gas or electric cooking homes. A nurse-epidemiologist examined selected subjects who reported ill and obtained throat cultures. The percentage of children having respiratory illnesses in homes with a gas stove was 85.1% (n = 87) versus 88.8% (n = 89) in homes with electric stoves. The unadjusted proportions permit the calculation of an estimated odds ratio of 0.71 with 95% confidence interval of 0.30 to 1.74. Unfortunately the adjusted rates were not reported.

Neas et al. (1991) commented that Keller’s model controls for a series of variables that specify the child’s prior illness history and that if chronic exposure to NO₂ is a risk factor for prior illnesses, controlling for the child’s illness history would substantially reduce the estimated effect of current NO₂ exposure.

7.3.1.6 University of Dundee (United Kingdom)

Ogston et al. (1985) studied infant mortality and morbidity in the Tayside region of northern Scotland. The subjects were 1565 infants born to mothers who were living in Tayside in 1980.
Episodes of respiratory illness were recorded during the first year of life. The information was supplemented by observations made by a health visitor and scrutinized by a paediatrician who checked diagnostic criteria and validity. One health end-point assessed was defined as the presence of any respiratory disease during the year. The use of gas cooking fuel was associated with increased respiratory illness (odds ratio = 1.14, 95% confidence interval 0.86 to 1.50) after adjustment for parental smoking, mother's age and type of home heating (Table 58). The study did not give measured NO\textsubscript{2} exposure values, but referenced the other studies conducted elsewhere in the United Kingdom for exposure estimates.

Table 58. Regression coefficients for multiple logistic analyses of respiratory illness in Tayside children (from: Ogston et al., 1985)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Regression coefficient</th>
<th>Odds ratio</th>
<th>95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental smoking</td>
<td>0.429</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Age of mother</td>
<td>-0.094</td>
<td>not available</td>
<td></td>
</tr>
<tr>
<td>(in 5-year groups)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of gas stove</td>
<td>0.130</td>
<td>1.14</td>
<td>0.86, 1.50</td>
</tr>
</tbody>
</table>

7.3.1.7 Harvard University - Chestnut Ridge Study (USA)

Schenker et al. (1983) reported a large respiratory disease study of 4071 children aged 5 to 14 in the Chestnut Ridge region of western Pennsylvania. The region is predominately rural, with numerous underground coal mines and four large coal-fired electricity-generating plants in the area. A standardized children's questionnaire (Ferris, 1978) was sent to parents of all children in grades 1 to 6 in targeted schools. An SES scale derived from the parent's occupation and education was divided into quintiles to provide SES strata. Important confounding factors considered in the analysis were gender, SES and maternal smoking. In the multiple logistic model, no significant association was found between gas stove use and any of the respiratory or illness variables after adjusting for SES. No odds ratios or other numerical data were reported.
Samet et al. (1993) conducted a prospective cohort study between January 1988 and June 1990 to test the hypothesis that exposure to NO₂ increases the incidence and severity of respiratory illness during the first 18 months of life. A total of 1315 infants were enrolled into the study at birth in Albuquerque, New Mexico. The subjects were healthy infants from homes without smokers and who spent less than 20 h/week in day care. Illness experience was monitored by a daily diary of symptoms completed by the mother and a telephone interview conducted every two weeks. For a sample of the ill children, a nurse practitioner made a home visit to conduct a standardized history and physical assessment. Exposure to NO₂ was estimated by a 2-week average concentration measured in the subjects' bedrooms with passive samplers. Estimates of exposure based on bedroom concentration were tightly correlated with estimates of exposures calculated as time-weighted averages of the concentrations in the kitchen, bedroom and activity room. The authors defined illness events as the occurrence on at least two consecutive days of any of the following: runny or stuffy nose, wet cough, dry cough, wheezing or trouble with breathing. Wheezing was defined as a high-pitched musical sound audible during breathing, and trouble with breathing as the parent's perception of rapid or laboured breathing. Illness events ended with two consecutive symptom-free days.

The analysis was limited to the 1205 subjects completing at least 1 month of observation; of these, 823 completed the full protocol. Multivariate methods were used to control for potential confounding factors and to test for effect modification. In analyses of determinants of incident illnesses, the outcome variable was the occurrence of illness during 2-week intervals of days at risk. The independent variables considered in the multivariate analyses included the fixed factors of birth order, gender, ethnicity, parental asthma and atopic status, household income, and maternal education. Other variables considered were the temporally varying factors of age, calendar month, day-care attendance and breast-feeding. Potential confounding and effect modification by cigarette smoking was controlled by excluding subjects from households with smokers.

Lambert et al. (1993) reported that in this prospective cohort study during the winter, bedroom concentrations in homes with gas stoves averaged 0.021 ppm (SD = 0.022 ppm). In bedrooms of
homes with electric stoves, concentrations averaged 0.007 ppm (SD = 0.006 ppm). Approximately 77% of the bedroom NO₂ observations were less than 0.02 ppm; only 5% were greater than 0.04 ppm. The 90th percentile of the weekly measured concentrations was 0.05 ppm NO₂ in bedrooms.

Samet et al. (1993) performed the analysis using the generalized estimated equations described by Zeger & Liang (1986). This takes into account the correlation structure when estimating regression coefficients and their standard errors. The multivariate models examined the effects of the unlagged NO₂ exposures, lagged NO₂ exposures and stove type (Table 59). None of the odds ratios was significantly different from unity, the value for the reference category of 0 to 0.02 ppm. Additionally, the odds ratios did not tend to increase consistently from the middle category of exposure to the highest category. Furthermore, exposure to NO₂ and the durations of the four illness categories were not associated. The authors added NO₂ exposure to the model as a continuous variable, while controlling for the same covariates included in Table 59. For each of the five illness variables, the estimated multiplier of the odds ratio per 0.001 ppm increment of NO₂ was 0.999, with confidence limits extending from approximately 0.995 to 1.002.

7.3.1.9 University of Basel Study (Switzerland)

Braun-Fahrländer et al. (1989, 1992) and Rutishauser et al. (1990a,b) studied the incidence and duration of common airway symptoms in children up to 5 years old over a 1-year period in a rural, a suburban and two urban areas of Switzerland. Parents were asked to record daily their child's respiratory symptoms (from a list) over a 6-week period. Additionally, covariates, including family size, parental education, living conditions, health status of the child, parents' respiratory health, and smoking habits of the family, were assessed by questionnaire. During the same 6-week period NO₂ was measured weekly using Palmes tubes, both inside and outside the home of the participants. Meteorological data were obtained from local monitoring stations, but additional air quality data from fixed monitoring sites were only available for the two urban study areas. NO₂ concentrations inside the home were on average lower than in the outside air (Fig. 24). Indoor levels for Basel, Zurich, Wetzikon and Rufzerfeld were 33.8, 28.4, 20.5 and 11.2 μg/m³ (0.018, 0.015, 0.011 and 0.006 ppm), respectively. The indoor NO₂ concentration depended to some extent on the concentration of the outside air.
Table 59. Odds ratios for effect of nitrogen dioxide exposure on incidence of respiratory illness (from: Samet et al., 1993)

<table>
<thead>
<tr>
<th>NO₂ exposure</th>
<th>All illnesses</th>
<th>All lower</th>
<th>Lower, with wet cough</th>
<th>Lower, with wheezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlagged</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02-0.06 ppm</td>
<td>1.04</td>
<td>0.96-1.12</td>
<td>0.98</td>
<td>0.82-1.09</td>
</tr>
<tr>
<td>&gt; 0.04 ppm</td>
<td>0.94</td>
<td>0.81-1.08</td>
<td>0.93</td>
<td>0.75-1.13</td>
</tr>
<tr>
<td>Lagged</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02-0.06 ppm</td>
<td>1.01</td>
<td>0.92-1.10</td>
<td>0.97</td>
<td>0.87-1.08</td>
</tr>
<tr>
<td>&gt; 0.04 ppm</td>
<td>0.92</td>
<td>0.77-1.10</td>
<td>0.91</td>
<td>0.72-1.15</td>
</tr>
<tr>
<td>Gas Stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.08-0.13 ppm</td>
<td>0.96</td>
<td>0.90-1.07</td>
<td>0.91</td>
<td>0.81-1.04</td>
</tr>
</tbody>
</table>

* Obtained by generalized estimating equation method. Adjusted for season, age, gender, ethnicity, birth order, day care, income, maternal education, breast feeding, parental atopy and asthma, and maternal history of respiratory symptoms.

* CI = Confidence interval

* Reference category is 0-0.02 ppm NO₂.

* Reference category is electric stove.
Fig. 24. NO₂ ambient and indoor concentrations in four Swiss regions with 95% confidence range
(from: Braun-Fahrtaender et al., 1989)
The analysis was restricted to 1063 Swiss nationals (from a total of 1225 participating families). For all four study areas, regional mean incidence rates of upper respiratory illness, cough, breathing difficulties and total respiratory illness, adjusted for individual covariates and weather data, were regressed (using Poisson regression) against regional differences in annual mean NO₂ concentrations. All the relative risks were computed for a 20-μg/m³ (0.011-ppm) increase in pollution concentration. The NO₂ concentration measured by indoor passive sampler was associated with the duration of any episode (relative duration of 1.16, 95% confidence interval of 1.12 to 1.21), upper respiratory episodes (relative duration of 1.18, 95% confidence interval of 1.01 to 1.38), and coughing episodes (relative duration of 1.15, 95% confidence interval of 1.03 to 1.29). A discussion of associations with outdoor levels is presented in section 7.3.2.

7.3.1.10 Yale University Study (USA)

Berwick et al. (1984, 1987, 1989), Leaderer et al. (1986) and Berwick (1987) reported on a 12-week study (six 2-week time periods) of lower and upper respiratory symptoms in 159 women and 121 children (aged 12 or less) living in Connecticut. Levels of NO₂ were measured in 91% of the homes, 57 of which had kerosene heaters and 62 of which did not. Ambient NO₂ levels ranged from 9 to 19 μg/m³ (0.005 to 0.01 ppm) for the six 2-week time periods. Two-week average indoor NO₂ levels in homes of monitored children were highest for homes with kerosene heaters and gas stoves (91 μg/m³, 0.05 ppm; n = 8), second highest for kerosene only (36 μg/m³, 0.02 ppm; n = 45), third highest for gas stoves only (32 μg/m³, 0.02 ppm; n = 13), and lowest for no sources (6 μg/m³, 0.003 ppm; n = 43). Indoor levels did not fluctuate greatly over time, as indicated by the 2-week averages. A comparison of personal NO₂ exposures, as measured by Palmes diffusion tubes, and NO₂ exposures measured in residences had a correlation of 0.94 for a subsample of 23 individuals. Results of this comparison show an excellent correlation between average household exposure and measured personal exposure (see section 3.6 and Fig. 13).

The study defined lower respiratory illness as the presence of at least two of the following: fever, chest pain, productive cough, wheeze, chest cold, physician-diagnosed bronchitis, physician-diagnosed pneumonia and asthma. Information on many potential covariates (e.g., SES, age, gender and exposure to environmental tobacco smoke) was obtained. The covariates having the largest
effect were age of child, family SES and history of respiratory illness, as shown by multiple logistic analysis. When controlling for SES and history of respiratory illness, children under 7 years of age exposed to 30 μg NO₂/m³ (0.016 ppm) or more were found to have a risk of lower respiratory symptoms 2.25 times higher than that of unexposed children (95% confidence limits of 1.69 and 4.79). Older children and adults showed no increased risk.

Although the Berwick study had relatively extensive information on exposure, several problems are evident. Unvented kerosene space-heaters also release volatile organic compounds and combustion particles. The 4-year age-specific relative risks for lower respiratory disease are very variable, and it is not clear why these 3-year strata were collapsed into 2 strata at 7 years of age. The analyses may be sensitive to the adjustment for SES, which can be correlated with exposure. This is less of a problem in studies with larger sample sizes (e.g., Melia et al. 1977, 1979), but may be critical in the Berwick study. Furthermore, Neas et al. (1991) noted that the Berwick study controlled for prior illnesses, as did the Keller study, which would reduce the estimated effect of current NO₂ exposure.

7.3.1.11 Freiburg University Study (Germany)

Kuehr et al. (1991) conducted a cross-sectional study on the prevalence of asthma in childhood in relation to NO₂ levels in the city of Freiburg and two Black Forest communities. A study group of 704 children (with 41 asthmatic) aged 7 to 16 years took part in a standardized interview and medical examination. Indoor and outdoor exposure information was taken into account. Passive smoking exposures were assessed. Stoves used as heating devices carried a 4.8-fold relative risk for asthma compared to other types of heating (95% CI 1.95-11.8).

7.3.1.12 McGill University Study (Canada)

In a case-control study carried out in Montreal, Quebec, Canada, between 1988 and 1990, NO₂ levels measured by passive NO₂ monitoring badge were studied in relation to the incidence of asthma among 3- and 4-year-old children (Infante-Rivard, 1993). Multivariate unconditional logistic regression was carried out for the 140 subjects who had NO₂ measurements; the analysis included NO₂ and the variables retained in the final conditional model that includes SES and parental smoking. The author reported an increase in asthma incidence associated with NO₂ exposure levels.
However, the Task Group noted the exceptionally large effect estimates given the exposure levels.

7.3.1.13 Health and Welfare Canada Study (Canada)

Dekker et al. (1991) studied asthma and wheezing syndromes as part of a questionnaire-based study of 17,962 Canadian school children. The questionnaire was developed from the 1978 American Thoracic Society questionnaire, which was the same as that used in the Harvard Six Cities Study. For analysis, the sample was restricted to children aged 5 to 8 years and excluded those children with cystic fibrosis as well as those living in mobile homes, tents, vans, trailers and boats. The authors calculated odds ratios adjusted for age, race, gender, parental education, gender of the respondent, region of residence, crowding, dampness and environmental tobacco smoke. The adjusted odds ratio of asthma as a function of gas cooking was 1.95 with 95% confidence limits of 1.41 and 2.68. The adjusted odds ratio of wheezing as a function of gas cooking was 1.04 with 95% confidence limits of 0.77 and 1.42. The authors noted that this finding needed to be treated with caution, however, because of the few subjects with asthma in the study who were exposed to gas cooking (n = 60).

7.3.1.14 University of North Carolina Study (USA)

Margolis et al. (1992) studied the prevalence of persistent respiratory symptoms in 393 infants of different SES by analysing data from a community-based cohort study of respiratory illness in the first year of life in central North Carolina between 1986 and 1988. Infants were limited to those weighing more than 2000 g and who did not require neonatal care outside the normal newborn nursery. Of those eligible, 47% were enrolled and, of these, 77% completed the study and were included in the analysis. Compared with the 1241 infants from families refusing enrolment, the 1091 eligible study infants were more likely to be of high SES and were more often black. Study infants were less likely to have mothers who smoked.

The presence of persistent respiratory symptoms was measured at the 12-month home interview using an American Thoracic Society children questionnaire (modified for infants) for studies of respiratory illness. Infants who were reported to "usually cough" or "occasionally wheeze" were classified as having persistent respiratory symptoms.
Of the 393 infants that Margolis et al. (1992) included in their study, approximately 41 lived in homes with gas cooking. The relative risk of persistent respiratory symptoms among infants exposed to gas cooking unadjusted for any covariates was 1.12 (95% confidence interval of 0.63 to 2.04).

### 7.3.1.15 University of Tucson Study (USA)

The study by Dodge (1982) was based on a cohort of 676 children in the third and fourth grades (about 90% aged 8-10 years) of schools in three Arizona communities. Gas cooking stoves were associated with increased symptoms: asthma odds ratio = 1.47, wheeze odds ratio = 1.24, sputum odds ratio = 2.28, and cough odds ratio = 2.21. However, only 79 children (19%) had electric heat, so the numbers were small and only cough was significant at the 0.05 level. After controlling for height and age, gas stoves were not associated with a decline in the growth of FEV1.

### 7.3.1.16 Hong Kong Anti-Cancer Society Study (Hong Kong)

In 1985, 362 primary school children (age 7-13 years) were included in a study of NO2 exposure and respiratory illness in Hong Kong (Koo et al., 1990). Exposures to NO2 were estimated by use of personal badge monitors, worn for a single period of 24 h, and supplemented by monitors placed in classrooms. NO2 exposures were estimated in the same manner for the mothers of the study children. Mothers and children completed respiratory illness questionnaires. No association was found between respiratory symptoms and NO2 exposures for children (mean 19 ppb). Among the mothers (mean exposure 19 ppb) allergic rhinitis and chronic cough were associated with NO2.

### 7.3.1.17 Recent studies

This section includes studies that have reported preliminary results only or have appeared recently in the scientific literature.

Spengler et al. (1993) reported results for evaluation of more than 15,000 schoolchildren in various sites in the USA and Canada, but found no statistically significant increases in respiratory symptoms to be associated with use of gas heaters or cookers.
Goren et al. (1993) reported no association between gas heating and respiratory health effects among 8000 schoolchildren in Israel.

Preliminary results reported by Peat et al. (1990) indicated no relationship between relatively high NO$_2$ in Australian homes with gas use in Sydney and respiratory symptoms or bronchial hyperresponsiveness.

Pilotto (1994) reported a prospective study of health effects of unflued gas heater emissions on 425 Australian schoolchildren aged 6-11 years. Short-term indoor monitoring by means of passive diffusion badge monitors placed in classrooms or worn at home was carried out to determine daily 6-h averages. Children exposed to a level of 0.08 ppm or more, compared with a background level of 0.02 ppm, had increased rates of respiratory illnesses and school absences.

### 7.3.2 Outdoor studies

Several studies have examined the relationship of estimated ambient NO$_2$ levels to respiratory health outcome measures, including various respiratory symptomatologies. Those that provide a quantitative estimate of effect are indicated in Table 60.

#### 7.3.2.1 Harvard University - Six City Studies (USA)

As part of the US Six City Studies, Dockery et al. (1989b) obtained respiratory illness and symptom data from questionnaires distributed from September 1980 to April 1981. Indoor air aspects of this study (Dockery et al., 1989a) were described in the section on indoor studies. The questionnaires obtained information on bronchitis, cough, chest illness, wheeze and asthma. A centrally located air monitoring station was established in 1974 where ambient sulfur dioxide, NO$_2$, ozone, total suspended particulate matter and meteorological variables were measured. The authors used multiple logistic regression analysis in order to adjust for covariates of gender, age, maternal smoking, gas stove use and separate intercepts for each city. Although the strongest associations were found between respiratory symptoms and particulate matter, there were increased odds ratios of respiratory symptoms with ambient NO$_2$. These were not statistically significant, but the direction for bronchitis, chronic cough and chest illness was consistent with the studies of indoor exposure. The odds ratios for various health end-points for an increase in NO$_2$ from the lowest-exposure city to the highest-exposure city 12 to 43 µg/m$^3$ (0.0065 to 0.0226 ppm) are shown in Table 60.
Table 60. Effects of outdoor NO2 exposure on respiratory disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Health end-point</th>
<th>NO2 levels (ppm)/period</th>
<th>Odds ratio or estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dockery et al. (1989b)</td>
<td>Bronchitis</td>
<td>0.007-0.023 annual average</td>
<td>1.7</td>
<td>0.5 to 5.5</td>
</tr>
<tr>
<td></td>
<td>Chronic cough</td>
<td></td>
<td>1.6</td>
<td>0.3 to 10.5</td>
</tr>
<tr>
<td></td>
<td>Chest illness</td>
<td></td>
<td>1.2</td>
<td>0.3 to 4.8</td>
</tr>
<tr>
<td></td>
<td>Wheeze</td>
<td></td>
<td>0.8</td>
<td>0.4 to 1.6</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td></td>
<td>0.6</td>
<td>0.3 to 0.9</td>
</tr>
<tr>
<td>Braun-Fahrtaender et al. (1992)</td>
<td>Duration of respiratory</td>
<td>Change of 0.011 6-week change of 0.011 6-week average</td>
<td>1.11</td>
<td>1.07 to 1.16</td>
</tr>
<tr>
<td>Schwartz et al. (1991)</td>
<td>Croup</td>
<td>0.005-0.037 daily</td>
<td>1.28</td>
<td>1.07 to 1.54</td>
</tr>
<tr>
<td>Jaakkola et al. (1991)</td>
<td>Upper respiratory infection</td>
<td>Contrasted polluted versus less polluted areas by comparison of annual levels</td>
<td>1.5</td>
<td>1.1 to 2.1</td>
</tr>
</tbody>
</table>
Braun-Fahrländer et al. (1992) studied the incidence and duration of common airway symptoms in children up to 5 years old. This study, also discussed in section 7.3.1.9, was conducted over a 1-year period in a rural, a suburban and two urban areas of Switzerland. Parents were asked to record their child's respiratory symptoms (from a list) daily over a 6-week period. Additionally, covariates including family size, parental education, living conditions, health status of the child, parents' respiratory health and smoking habits of the family were assessed by questionnaire. Weekly NO₂ measurements were made during the same 6-week period using Palmes tubes, both inside and outside the home of the participants. Meteorological data were obtained from local monitoring stations, but additional air quality data from fixed monitoring sites were only available for the two urban study areas. The analysis was restricted to 1063 Swiss nationals (from a total of 1225 participating families). For all four study areas, regional mean incidence rates of upper respiratory illness, cough, breathing difficulties and total respiratory illness, adjusted for individual covariates and weather data, were regressed (using Poisson regression) against regional differences in annual mean NO₂ concentrations. There was no association between long-term differences in NO₂ levels by region and mean annual rates of respiratory incidence.

The adjusted annual mean symptom duration by region and the corresponding NO₂ levels (measured by passive samplers) are shown in Table 61. A second-stage regression of the adjusted natural logarithm of regional mean duration on NO₂ levels yields significant associations between outdoor NO₂ levels and the average duration of any respiratory episode (relative duration of 1.11, 95% confidence interval of 1.07 to 1.16) and upper respiratory episodes (relative duration of 1.14, 95% confidence interval of 1.03 to 1.25). A positive trend for the duration of coughing episodes was also seen (relative duration of 1.09, 95% confidence interval of 0.97 to 1.22). No association was seen with the duration of breathing difficulties. All the relative risks are computed for a 20-μg/m³ (0.011-ppm) increase in pollution concentration. In the suburban and rural areas, NO₂ was the only air pollutant measured. Correlation between outdoor passive NO₂ sampler and total suspended particulate (TSP) measurements in the two urban study areas was quite high (0.52). The high correlation between NO₂ and TSP suggests that this NO₂ association may reflect confounding with TSP. The lack of TSP data for the other
Table 61. Adjusted annual symptom duration (days) and NO\textsubscript{2} levels in four regions of Switzerland
(from: Braun-Fahrländer et al., 1992)

<table>
<thead>
<tr>
<th>Region</th>
<th>Any symptom duration</th>
<th>URI duration\textsuperscript{*}</th>
<th>Cough duration</th>
<th>Breathing difficulty duration</th>
<th>Indoor NO\textsubscript{2} concentration (ppm)</th>
<th>Outdoor NO\textsubscript{2} concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basel</td>
<td>4.50</td>
<td>1.99</td>
<td>2.32</td>
<td>1.55</td>
<td>0.0166</td>
<td>0.0272</td>
</tr>
<tr>
<td>Zurich</td>
<td>4.21</td>
<td>1.85</td>
<td>2.01</td>
<td>1.72</td>
<td>0.0118</td>
<td>0.0248</td>
</tr>
<tr>
<td>Wetzikon</td>
<td>4.00</td>
<td>1.62</td>
<td>2.10</td>
<td>3.47</td>
<td>0.0103</td>
<td>0.0173</td>
</tr>
<tr>
<td>Retschfeld</td>
<td>3.96</td>
<td>1.72</td>
<td>2.02</td>
<td>1.25</td>
<td>0.0059</td>
<td>0.0133</td>
</tr>
</tbody>
</table>

\textsuperscript{*} URI = Upper respiratory illness
two regions precludes eliminating TSP as a possible confounder in this analysis. But the consistency of the NO$_2$ findings are evident and, although the association with symptom duration in Zurich and Basel may well be due to confounding with TSP, the cross-sectional association across the four regions supports a possible NO$_2$ role.

7.3.2.3 University of Wuppertal Studies (Germany)

Schwartz et al. (1991) evaluated respiratory illness in five German communities. Children's hospitals, paediatric departments of general hospitals, and paediatricians reported daily the numbers of cases of croup. A diagnosis of croup was based on symptoms of hoarseness and barking cough, inspiratory stridor, dyspnoea, and a sudden onset. The counts were modelled using Poisson regression with adjustments for weather, season, temperature, humidity and autoregressive errors. Statistically significant effects of both ambient particulate matter and NO$_2$ were found on the counts of respiratory illnesses. A relationship between short-term fluctuations in air pollution and short-term fluctuations in medical visits for croup symptoms was found in this study. The estimated relative risk was 1.28 with 95% confidence limits of 1.07 and 1.54 for an increase from 10 to 70 pg NO$_2$/m$^3$ (0.005 to 0.037 ppm).

7.3.2.4 University of Tubingen (Germany)

Rebmann et al. (1991) studied 875 cases of croup in Baden-Württemberg in relation to ambient NO$_2$ levels over a 2-year period. Monthly NO$_2$ means varied from 23 to 78 pg/m$^3$. Statistical regression methods indicated weak but statistically significant influences of the daily ambient NO$_2$ mean on the occurrence of croup.

7.3.2.5 Harvard University - Chestnut Ridge Study (USA)

In the autumn of 1980, Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge study. Parents and children were instructed at the beginning of the school year in completing daily diaries of respiratory symptoms. Lower respiratory illness was defined as wheeze, pain on breathing, or phlegm production. Of the 351 subjects selected for the 8 month of follow-up, 128 participated in the completion of diaries. Three subgroups were established: one without respiratory symptoms, one with symptoms of persistent wheeze, and one with cough or phlegm production but without persistent
wheeze. Maximum hourly NO\textsubscript{2} levels, measured at a single monitoring site in the study region, for each 24-h period were used to reflect the daily pollutant level. During September 1980 to April 1981, the mean NO\textsubscript{2} maximum daily level was 40.5 µg/m\textsuperscript{3} (0.021 ppm) with a range of 12 to 79 µg/m\textsuperscript{3} (0.006 to 0.042 ppm). Regression models could not be fit for asymptomatic subjects; thus 55 subjects were included in the analysis of lower respiratory illness, but NO\textsubscript{2} levels were not predictive of any symptom outcome.

7.3.2.6 University of Helsinki Studies (Finland)

Jaakkola et al. (1991) studied the effects of low-level air pollution in three cities by comparing the frequency of upper respiratory infections over a 12-month period in 1982 as reported by parents of children aged 14 to 18 months (n = 679) and 6 years (n = 759). Pollutants studied included ambient levels of NO\textsubscript{2}, the annual mean of which was 15 µg/m\textsuperscript{3} (0.008 ppm). Other pollutants monitored were sulfur dioxide, hydrogen sulfide and particles. Passive smoking and SES were taken into account. The authors reported a significant association between the occurrence of upper respiratory infections and living in an air-polluted area for both age groups studied, both between and within cities. The adjusted odds ratio was 1.6 (95% confidence interval of 1.1 to 2.1) in the 6-year-old age group. The authors concluded that the combined effect of sulfur dioxide, particulates, NO\textsubscript{2}, hydrogen sulfide and other pollutants may be a contributing factor in the study results.

7.3.2.7 Helsinki City Health Department Study (Finland)

Pönkä (1991) studied effects of ambient air pollution and minimum temperature on the number of patients admitted to hospital for asthma attacks in Helsinki from 1987 to 1989. During the 3-year period, 4209 hospitalizations for asthma occurred. The temperature ranged from -37 to +26 °C, with a 3-year mean of 5 °C, and the number of admissions increased during cold weather. After standardization for minimum temperature, the multiple-regression analysis indicated that NO\textsubscript{2} and carbon monoxide levels were significantly related to asthma admission. The NO\textsubscript{2} levels averaged 38.6 µg/m\textsuperscript{3} (0.02 ppm) for the 3-year period, ranging from 4.0 to 169.6 µg/m\textsuperscript{3} (0.002 to 0.09 ppm). During the period of high NO\textsubscript{2} (mean 45.8 µg/m\textsuperscript{3}, 0.024 ppm) levels, the mean number of all admissions was 29% greater than during the lower pollution period (28.1 µg/m\textsuperscript{3}, 0.015 ppm). Indoor NO\textsubscript{2} levels and cooking fuel use were not reported.
Epidemiological Studies of Nitrogen Oxides

7.3.2.8 Oulu University Study (Finland)

The number of daily attendances for asthma at the emergency room of the Oulu University Central Hospital, Finland, was recorded for one year, along with daily measures of air pollutants at four points around the city (Rossi et al., 1993). Daily mean levels of NO\textsubscript{2} ranged up to 69 \(\mu\text{g/m}^3\) (maxima 0-154 \(\mu\text{g/m}^3\)). Asthma visits were reported to be significantly associated with NO\textsubscript{2}, SO\textsubscript{2}, H\textsubscript{2}S and TSP levels. After adjustment for daily temperature, only NO\textsubscript{2} was significantly correlated with attendances. The association of NO\textsubscript{2} and asthma attacks was stronger in winter months than during the summer.

7.3.2.9 Seth GS Medical College Study (India)

A survey of air pollution and health was carried out in Bombay, India, in 1978 (Kamat et al., 1980). The study included 4129 adults in three urban areas and one rural area. A single monthly mean NO\textsubscript{2} level was reported for each study area - annual averages were 4 \(\mu\text{g/m}^3\) in the rural area, and 14-16 \(\mu\text{g/m}^3\) in the city. Winter levels in the city study were higher than at other times of the year (up to 40 \(\mu\text{g/m}^3\)). It was reported that chronic cough with sputum, frequent colds and exertional dyspnoea were significantly associated with NO\textsubscript{2} levels. These symptoms were also associated with atmospheric levels of SO\textsubscript{2} and suspended particulate matter, and it was not possible to identify a separate influence of NO\textsubscript{2} alone.

7.4 Pulmonary function studies

Pulmonary function studies are part of any comprehensive investigation of the possible effects of any air pollutant. Measurements can be made in the field, they are non-invasive, and their reproducibility has been well documented. Age, height, gender and presence of respiratory symptoms are important determinants of lung function. Furthermore, changes in pulmonary function have been associated with exposure to tobacco smoke, particulate matter and other factors. The studies reviewed below evaluate pulmonary function changes in relation to indoor or outdoor NO\textsubscript{2} exposures. Several of the respiratory disease studies described earlier also included information on pulmonary function.

7.4.1 Harvard University - Six City Studies (USA)

Ware et al. (1984) described analysis of lung function values using multiple linear regression on the logarithm of the lung
function measures. Covariates included sex, height, age, weight, smoking status of each parent, and educational attainment of the parents. Exposure to gas stoves was associated with reductions of 0.7% in mean FEV1 (forced expiratory volume in 1 second) and 0.6% in mean forced vital capacity (FVC) at the first examination \((p < 0.01)\), and reductions of 0.3% at the second examination (not significant). The estimated effect of exposure to gas stoves was reduced by approximately 30% after adjustment for parental education. The authors stated that the adjustment for parental education may be an over-adjustment, and may partially represent gas stove use because of association between parental education and type of stove.

Berkey et al. (1986) used the data from children seen at two to five annual visits to study factors affecting pulmonary function growth. Children whose mothers smoked one pack of cigarettes per day had FEV1 growth rates approximately 0.17% per year lower \((p = 0.05)\). The same data provided no evidence for an effect of gas stove exposure on growth rate.

Dockery et al. (1989b) obtained pulmonary function data during the 1980 and 1981 school year. Only TSP concentration was consistently associated with estimated lower levels of pulmonary function. There was little evidence for an association between lower pulmonary function levels and the annual mean concentration of NO2 or any other pollutant.

Neas et al. (1991) also reported that indoor NO2 levels were not significantly associated with a deficit in children's pulmonary function levels in either of two examinations (FEV1 and FVC).

### 7.4.2 National Health and Nutrition Examination Survey Study (USA)

Schwartz (1989) studied air pollution effects on lung function in children and youths aged 6 to 24 years. FVC, FEV1, and peak flow measurements taken as part of the National Health and Nutrition Examination Survey II (NHANES II) were examined after controlling for age, height, race, gender, body mass, cigarette smoking and respiratory symptoms. Air pollution measurements were taken from all population-oriented monitors in the US EPA database. Each person was assigned the average value of each air pollutant from the nearest monitor for the 365 days preceding the spirogram. Highly significant negative regression coefficients were found for three pollutants (TSP, NO2 and O3) with the three lung function measurements. For an increase of NO2 exposure of
28.3 µg/m³ (0.015 ppm), an estimated decrease of about 0.045 litres was seen in both FVC and FEV₁.

7.4.3 Harvard University - Chestnut Ridge Study (USA)

Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge cross-sectional study of elementary school-aged children (mean age = 9.5 years). Peak expiratory flow (PEF) was measured daily in 144 children for 9 consecutive weeks and was regressed against daily maximum hourly ambient concentrations of NO₂, SO₂ and coefficient of haze. No air pollutant was strongly associated with PEF. All pollutant levels were relatively low; NO₂ levels ranged from 12 to 79 µg/m³ (0.006 to 0.042 ppm). No indoor measurements were made, nor were any surrogates for indoor pollution included in the analysis.

7.4.4 Other pulmonary function studies

Ekwo et al. (1983) obtained pulmonary function measurements from 89 children whose parents did not smoke and 94 children whose parents smoked, and reported no differences in lung function associated with gas stove use in a cohort of children 6 to 12 years of age.

Dijkstra et al. (1990) examined pulmonary function in Dutch children; lung function was measured at the schools. There was a weak negative association between FEF₂₅-₇₅% (25 and 75% of FVC) and exposure to NO₂. FEV₁, PEF and FEF₂₅-₇₅% were all negatively associated with exposure to tobacco smoke. The authors concluded that the study failed to document clear associations between indoor exposure to NO₂ and lung function changes in 6- to 12-year-old Dutch children.

Lebowitz et al. (1985) studied a cluster sample of 117 middle-class households in Tucson, Arizona, USA. Symptom diaries and peak flows were obtained over a 2-year period. Outdoor sampling of O₃, TSP, CO and NO₂ was done in or near the clusters. Indoor sampling of O₃, TSP, respirable suspended particles and CO was done in a subsample of the homes. Information such as the presence of a gas stove or smoking was also obtained. The presence of a gas stove was used as a surrogate for indoor NO₂ exposure. Children's peak flow was associated with gas stove use (p = 0.066) for an analysis excluding TSP. In adult asthmatics, gas stove use was significantly associated with peak flow decrements (p < 0.001).
This was true across smoking groups, but the difference was greatest for smokers.

Lung function studies were conducted in a prospective survey undertaken by Kamat et al. (1980) on 4129 subjects in three urban areas of Bombay and a rural control area during February to July, 1977. The survey revealed that the population in low polluted areas had higher lung function for FEF_{75%} and PEF. Thus, there was suggestive evidence that the higher values obtained from lung function tests in rural subjects as compared to urban subjects could be due to increased levels of NO_{2}.

### 7.5 Other exposure settings

Certain recreational settings have been shown to result in NO_{2} exposures that greatly exceed the chronic, low-level exposures described in the previous epidemiological studies.

#### 7.5.1 Skating rink exposures

Hedberg et al. (1989) reported cough, shortness of breath, and other symptoms among players and spectators of two high school hockey games played at an indoor ice arena in Minnesota, USA. These symptoms were related to emissions from a malfunctioning engine of the ice resurfacer. Although the exact levels of NO_{2} were not known at the time of the hockey game, levels of 7500 µg/m³ (4 ppm) were detected 2 days later with the ventilation system working, suggesting that levels during the games were higher. Hedberg et al. (1989, 1990) reported that pulmonary function testing performed on members of one hockey team with a single exposure demonstrated no decrease in lung function parameters at either 10 days or 2 months after exposure. Dewailly et al. (1988) reported another incident at a skating rink in Quebec, Canada, in 1988 involving referees and employees with respiratory symptoms such as coughing, dyspnoea and a suffocating feeling. Five days after the incident, NO_{2} levels had come down to 5600 µg/m³ (3 ppm), suggesting much higher levels during the incident.

In another skating rink study, Smith et al. (1992) reported the outcome of a questionnaire administered to all students from two high schools on 25 February, 1992. 3 days after 11 students participating in a Wisconsin indoor ice hockey tournament had been treated in emergency rooms for acute respiratory symptoms (i.e., cough, haemoptysis, chest pain and dyspnoea). The game had
been attended by 131 students, 57 of whom reported symptoms. A simulation test on 24 February yielded NO$_2$ levels of 2800 µg/m$^3$ (1.5 ppm) in the air over the rink after use of the ice resurfacing machine. Higher levels may have been reached on the night of the game.

Brauer & Spengler (1994) measured indoor air NO$_2$ concentrations at 20 skating rinks (most of all the operating ones) in the New England area of the USA. Palmes tubes were used to measure NO$_2$ over a 7-day sampling period at each rink, the samplers being placed on the main resurfacer used in the rink, at the score keepers' bench around a breathing height, at the opposite side of the rink from the scorekeeper bench, and outdoors nearby the rink away from parking lots or other vehicular traffic. In contrast to the outdoor NO$_2$ concentrations observed (geometric mean = 0.018 ppm, range 0.001-0.193 ppm), those found indoors averaged about 10-fold higher (geometric means = 0.128, 0.169, 0.168 ppm for resurfacer, bench area, and second sampler opposite to bench, respectively). These NO$_2$ levels may be fairly typical for the approximately 2000 operating rinks in North America, with some differences being found depending on whether the resurfacer was propane- or gasoline-powered or used a catalytic converter.

7.6 Occupational exposures

Certain occupational exposure studies have shown that NO$_2$ exposures in occupational settings greatly exceed the chronic, low-level exposure described in general population epidemiological studies. Occupational exposure studies generally refer to a highly selected group of adult workers, usually male. The probability of a healthy worker effect needs to be considered when evaluating the significance of such studies.

Gamble et al. (1994) studied 232 workers in four diesel bus garages for the effects of NO$_2$ on acute respiratory illness and pulmonary function. Response was assessed by an acute respiratory questionnaire and before- and after-shift spirometry. Measurements over the shift of NO$_2$ (using passive Palmes tube samplers) were made on each worker and collected on the same day as the pulmonary function tests and questionnaires. Other irritant gases were measured and were well below federal standards. Mean NO$_2$ levels over the shift ranged from 0.56 (SD = 0.38) ppm NO$_2$ in the highest garage to 0.13 (SD = 0.06) ppm NO$_2$ in the lowest garage. Short-term NO$_2$ measurements indicated levels above 1 ppm as
being common. The authors reported that the prevalence of acute respiratory symptoms were elevated above expected in the high-exposure (> 0.3 ppm) group only. No reduction in pulmonary function was associated with exposure.

Gamble et al. (1983) examined chronic respiratory effects in 259 sodium chloride miners for whom diesel emissions were the principal NO₂ source. The Medical Research Council respiratory symptom questionnaire containing smoking history was administered by trained interviewers. A chest X-ray and spirometry were also conducted. Personal samples of NO₂ and respirable particles for jobs in each mine were used to estimate cumulative exposure. Mean exposure ranged from a low of 0.2 (SD = 0.1) ppm NO₂ to a high of 2.5 (SD = 1.3) ppm NO₂. The author reported that although cough was associated with age and smoking, and dyspnoea was associated with age, neither symptom was associated with exposure (i.e., years worked, estimated cumulative NO₂ or respiratory particle exposure). Reduced pulmonary function showed no association with NO₂ exposure.

Robertson et al. (1984) reported on a 4-year study of lung function in 560 British coal miners for whom the NO source consisted of diesel emissions and blasting. Overall average NO₂ levels at nine coal mine sites ranged from 38 to 113 µg/m² (0.02 to 0.06 ppm), and nitric oxide (NO) levels ranged from 0.13 to 1.19 ppm. No relationship was found between exposure and decline in FEV₁ or respiratory symptoms. Jacobsen et al. (1988) conducted a more extensive investigation on nearly 20 000 miners at the same nine British coal mines to examine whether long-term exposure to low concentration of NO₂ and NO was associated with increased susceptibility to respiratory infections. Shift median levels were 0.2 ppm NO and 0.03 ppm NO₂. This complete and intensive study had problems with misclassification of exposure and outcome that are not uncommon when existing data are used for purposes that were not foreseen when the data were collected. The authors concluded that the long-term exposure to the above-mentioned levels do not detectably increase the chance that miners will absent themselves from work because of a chest infection.

Douglas et al. (1989) reported data obtained between 1955 and 1987 on 17 patients examined at the Mayo Clinic for silo-filler's disease shortly after exposure to silo gas (NO₂ levels from 200 to 2000 ppm). Health outcomes ranged from hypoxaemia and transient airway obstruction to death. Epler (1989) noted that prevention is essential for elimination of silo-filler's disease.
Meulenbelt & Sangster (1990) indicated that, after a symptom-free period immediately following exposure to NO\(_2\), severe respiratory failure can develop several hours later. Other studies also examined high exposures (Lowry & Schuman, 1956; Grayson, 1956; Gregory et al., 1969; Yockey et al., 1980).

### 7.7 Synthesis of the evidence for school-age children

The weight of the evidence does not indicate that NO\(_2\) exposures at levels reported in studies evaluated here have any consistent effect on pulmonary function of a biologically significant magnitude. Many of the indoor studies, however, suggest an increase in respiratory morbidity in children from exposure to NO\(_2\), although the effects in the majority of the studies do not reach statistical significance (p < 0.05). The consistency of the results across the indoor studies is examined and the evidence from some of the studies is combined in a quantitative analysis presented below. Indoor NO\(_2\) epidemiological studies not included in combined analysis are listed in Table 62.

#### 7.7.1 Health outcome measures

The studies in the quantitative analysis that follows use health outcome measures that provide an indication of the state of respiratory health of the various samples of children aged up to 12 years. The NO\(_2\) studies utilized standard questionnaires to evaluate lower respiratory health in children. Diagnoses of specific respiratory diseases such as bronchiolitis or asthma were not made. The factor of importance here is that an attempt was made to measure some aspect of lower respiratory morbidity. Table 63 lists the health outcome measures for each study considered. Whereas specific measures such as colds going to the chest (Melia et al., 1977), chest congestion, and phlegm with colds (Ekwo et al., 1983) are used to provide measures of lower respiratory morbidity, other measures use indexes, grouped responses or combined indicators of lower respiratory morbidity, some of which include measures such as colds going to chest.

Childhood lower respiratory morbidity is characterized by a grouping of similar symptoms and diseases that reflect changes located anatomically in the lower respiratory tract. This characterization represents an indication of severity of the respiratory morbidity status of the children and is a multifaceted approach to respiratory health in a population living under natural conditions. Lower respiratory morbidity is the combination of
Table 62. Indoor NO\textsubscript{2} epidemiological studies not included in combined analysis - school age children (≥ 5 years)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Published result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas stoves</td>
<td>Increased prevalence of cough</td>
<td>Dodge (1982)</td>
</tr>
<tr>
<td>Gas stoves</td>
<td>No significant association with respiratory illness</td>
<td>Schenker et al. (1983)</td>
</tr>
<tr>
<td>Gas stoves</td>
<td>No association with respiratory illness</td>
<td>Melia et al. (1988)</td>
</tr>
<tr>
<td>Gas stoves</td>
<td>Increased lower respiratory symptoms in children &lt; 7 years; no increase in children aged ≥ 7 years</td>
<td>Berwick et al. (1989)</td>
</tr>
<tr>
<td>Gas stoves</td>
<td>Increased prevalence of asthma</td>
<td>Kuehr et al. (1991)</td>
</tr>
<tr>
<td>Gas cookers</td>
<td>Increased odds ratio for asthma; non-significant increase in wheeze</td>
<td>Dekker et al. (1991)</td>
</tr>
<tr>
<td>Gas heaters and cookers</td>
<td>No statistically significant increase in overall respiratory illness in 24 cities in the USA</td>
<td>Spengler et al. (1993)</td>
</tr>
<tr>
<td>Gas heaters</td>
<td>No association with respiratory illness</td>
<td>Goren et al. (1993)</td>
</tr>
<tr>
<td>NO\textsubscript{2} levels</td>
<td>No significant association with bronchitis, asthma, frequent coughs, allergy</td>
<td>Hoek et al. (1984)</td>
</tr>
<tr>
<td>NO\textsubscript{2} levels</td>
<td>No association with respiratory symptoms or bronchial hyper-responsiveness</td>
<td>Peat et al. (1990)</td>
</tr>
<tr>
<td>NO\textsubscript{2} levels</td>
<td>No association with lower respiratory symptoms</td>
<td>Koo et al. (1990)</td>
</tr>
<tr>
<td>NO\textsubscript{2} level</td>
<td>Increased odds ratio for asthma</td>
<td>Infante-Rivard (1993)</td>
</tr>
<tr>
<td>NO\textsubscript{2} level</td>
<td>Increased respiratory symptoms and absences from school</td>
<td>Pilotto (1994)</td>
</tr>
</tbody>
</table>
Table 63. Health outcome measures in selected NO₂ epidemiological studies

<table>
<thead>
<tr>
<th>Location (date of study)</th>
<th>Health outcome used in meta-analysis</th>
<th>Method*</th>
<th>NO₂ exposure measure used in analysis</th>
<th>Age (years)</th>
<th>Sample size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands (1986)</td>
<td>Respiratory illness combination variable of presence of one or more of cough, wheeze or asthma.</td>
<td>Questionnaire completed by parent (WHO).</td>
<td>NO₂ measured with Palmes tubes. Gas and electric appliances.</td>
<td>6-12</td>
<td>775</td>
<td>Brunekreef et al. (1987); Dijkstra et al. (1990)</td>
</tr>
<tr>
<td>28 areas of England and Scotland (1973)</td>
<td>Colds going to chest showed a prevalence of 26.8-19.8%.</td>
<td>Respiratory symptoms questionnaire completed by parent of child for the last 12 months</td>
<td>Gas stove vs. electric stove</td>
<td>6-11</td>
<td>5658</td>
<td>Melia et al. (1977)</td>
</tr>
<tr>
<td>27 areas of England and Scotland (1977)</td>
<td>Group response to respiratory questions into none or one or more symptoms or diseases. Colds going to chest (26.4-19.6%) showed the highest prevalence, followed by wheeze (11.1-6.2%), cough and episodes of asthma or bronchitis in last year.</td>
<td>As above</td>
<td>Gas stove vs. electric stove</td>
<td>5-10</td>
<td>4827</td>
<td>Melia et al. (1979)</td>
</tr>
<tr>
<td>Location (date of study)</td>
<td>Health outcome used in meta-analysis</td>
<td>Method</td>
<td>NO₂ exposure measure used in analysis</td>
<td>Age (years)</td>
<td>Sample size</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td>Middlesborough, England (1978)</td>
<td>Group response to respiratory questions as above.</td>
<td>As above</td>
<td>NO₂ measured with Palmes tubes. Gas stove homes only.</td>
<td>6-7</td>
<td>103</td>
<td>Florey et al. (1979); Goldstein et al. (1979); Melia et al. (1980, 1982a,b)</td>
</tr>
<tr>
<td>Middlesborough, England (1980)</td>
<td>As above</td>
<td>As above</td>
<td>NO₂ measured with Palmes tubes. Gas stove homes only.</td>
<td>5-6</td>
<td>186</td>
<td>Melia et al. (1982a,b)</td>
</tr>
<tr>
<td>6 USA cities (1974-1979)</td>
<td>Lower respiratory illness index (index of respiratory health) indicating during the past year the presence of either bronchitis, respiratory illness that kept the child home 3 days or more, or persistent cough for 3 months of the year.</td>
<td>Questionnaire (Ferris, 1978) completed by parent for symptoms during previous 12 months</td>
<td>Gas vs. electric</td>
<td>6-10</td>
<td>8240</td>
<td>Ware et al. (1984)</td>
</tr>
</tbody>
</table>
Table 63 (contd).

<table>
<thead>
<tr>
<th>Study</th>
<th>City</th>
<th>Symptoms Described</th>
<th>Questionnaire Method</th>
<th>NO$_2$ Measured Method</th>
<th>Study Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 USA cities (1983-1996)</td>
<td></td>
<td>Combined indicator of one or more lower respiratory symptoms as defined. The highest prevalences were for chronic phlegm and wheeze. The other symptoms in the index are shortness of breath, chronic cough and bronchitis. Chest illness reflects a restriction of the child's activities for 3 or more days.</td>
<td>Symptom questionnaire completed by parent for the year during which measurements of NO$_2$ were taken.</td>
<td>NO$_2$ measured with Palmes tubes, Gas and electric stoves.</td>
<td>Neas et al. (1990, 1991)</td>
</tr>
<tr>
<td>Iowa City, Iowa, USA</td>
<td></td>
<td>Chest congestion and phlegm with colds.</td>
<td>Questionnaire completed by parent (ATS).</td>
<td>Gas stove vs. electric stove.</td>
<td>Elwo et al. (1993)</td>
</tr>
<tr>
<td>Columbus, Ohio, USA (1978)</td>
<td></td>
<td>Lower respiratory illness syndrome characterized by cough, wheezing, bringing up phlegm and like symptoms considered as &quot;chest colds&quot;.</td>
<td>Telephone interview by nurse epidemiologist.</td>
<td>Gas stove vs. electric stove.</td>
<td>Keller et al. (1979a,b)</td>
</tr>
</tbody>
</table>

*ATS = American Thoracic Society*
different respiratory effects that have in common an evaluation of the morbidity status of the lower respiratory tract. The measure of effect on the lower respiratory tract varied among the studies; the indicators, however, are conventional symptom and illness outcomes. The symptoms are tabulated from similar standardized questionnaires (Ferris, 1978) and are directed at eliciting the same basic data—an indication of the presence of illness or infection in the lower respiratory tract.

Although the use of identical health outcome measures would be most desirable, the level of similarity and the common elements between the outcome measures in the NO₂ studies provide some confidence in their use in the quantitative analysis. However, the symptoms and illnesses combined are to some extent different and could indeed reflect different underlying processes. Thus caution is necessary in interpreting the analysis. This concern is addressed further later in this section as part of the statistical aspects of the random effects model.

7.7.2 Biologically plausible hypothesis

The human clinical and animal toxicological studies that examined NO₂ effects on aspects of the respiratory host defence system provide a biologically plausible hypothesis compatible with the relationship seen between respiratory symptoms and morbidity and NO₂ exposure in epidemiological studies. However, research gaps in both animal toxicological and clinical studies exist, indicating the need for further research efforts. A brief discussion is presented here.

The evidence from animal toxicological and human clinical studies of host defence provides a rationale for investigating the relation between exposure to NO₂ and an increase in frequency and severity of respiratory symptoms and/or infections in humans. When microorganisms attack a lung that has been exposed to NO₂, host defence mechanisms altered by the NO₂ exposure may result in increased severity or rate of respiratory illness. Although the host defence system reacts both very specifically and generally to the challenge, the overall response in humans is expressed as a generalized demonstration of signs and symptoms that may be associated with a site such as the lower respiratory tract. It may also be reported or objectively discerned as a general outcome, such as a chest cold, a cough or an incident of asthma or bronchitis.
Publication bias, also known as the "file drawer problem" (Rosenthal, 1979), is the result of the increased likelihood of publication of studies that have positive results, leading to a bias in the literature reviewed towards positive results. There are two factors that make this bias less likely for epidemiological studies of NO₂. Firstly, the studies are expensive, well publicized, and the results are usually published in order to give credit to the researchers involved. Secondly, many of the studies included in this section did not produce statistically significant findings, indicating that there was not a substantial barrier in publishing negative studies. However, some studies are necessarily excluded because they provide insufficient information. Although, this can lead to bias, there is little that can be done to correct for this problem. This problem is not normally referred to as a publication bias, but it is a similar problem.

Selection of studies

An attempt has been made to include as many studies as possible in the quantitative analysis. The requirements for inclusion were: (1) the health end-point measured must be reasonably close to the standard end-point; (2) significant exposure differences between subjects must exist and some estimate of exposure must be available; and (3) an odds ratio for a specified exposure gradient must have been calculated, or data presented so that an odds ratio can be calculated. The standard end-point chosen was the presence of lower respiratory symptoms and illness in children aged 5 to 12 years. The subsequent analysis is based on the assumption that the relative risk of developing lower respiratory symptoms is similar across this age range and across the range of study settings as a function of NO₂ exposure, even though the baseline rates may differ by age and study setting. After a careful review of the published literature, nine studies that met these criteria were selected for inclusion in the quantitative analysis.

The NO₂ exposure gradient for the quantitative analysis of relative risks was selected as 28.3 μg/m³ (0.015 ppm). This is comparable to the reported long-term exposure difference between homes with gas stoves and homes with electric stoves. In the USA, Neas et al. (1991) reported a household annual average difference of 32.5 μg/m³ (0.0173 ppm) between homes with electric stoves and homes with gas stoves with pilot lights.
United Kingdom, Melia et al. (1980, 1982a,b) reported a difference of 31.1 µg/m³ (0.0165 ppm) in bedroom levels between homes with electric stoves and homes with gas stoves. In four studies, chronic NO₂ exposures were estimated from direct measurements using 1- to 2-week integrated indoor samples by Palmes passive diffusion tubes.

For five studies that characterized NO₂ exposure according to differences between gas stove and electric stoves, the exposure gradients were estimated from the two previously sited studies with direct NO₂ measurements. Appropriate exposure estimates ideally should be country-specific, current with the studies in location and time, and derived from a representative sample that appropriately characterizes the exposure. For three studies conducted in the USA (Keller et al., 1979a,b; Ekwo et al., 1983; Ware et al., 1984), exposure gradients were based on the studies of Neas et al. (1991). For the studies of Melia et al. (1977, 1979), exposure gradients were based on Melia et al. (1980, 1982a,b). The effects of exposure measurement error related to the use of surrogate exposure estimates were discussed earlier.

7.7.4.1 Brief description of selected studies

Melia et al. (1977) studied children aged 6 to 11 years and developed an indicator of the presence of at least one of a group of symptoms including cough, colds going to the chest, and bronchitis. The symptom reported most of the time was a cold going to the chest, which was used as an indicator of lower respiratory morbidity. This study did not measure NO₂ exposure, and so the assumption was made that the increase in NO₂ exposure from gas stove use in England was reasonably similar to that in the other British studies that measured NO₂ (31.1 µg/m³, 0.0165 ppm). The estimated odds ratio was 1.31, with 95% confidence limits of 1.16 and 1.48. After adjusting to a standard increase of 28.3 µg/m³ (0.015 ppm), the odds ratio became 1.28 with 95% confidence limits of 1.14 and 1.43. No adjustment was made for parental smoking in this study.

The cross-sectional data reported by Melia et al. (1979) on children aged 5 to 10 years were also employed to estimate an odds ratio, although no exposure estimates were made. The presence or absence of a gas stove was used as a surrogate as in the Melia et al. (1977) study. The estimated odds ratio was 1.24, with 95% confidence limits of 1.09 and 1.42. After adjusting to a standard increase of 28.3 µg/m³ (0.015 ppm), the odds ratio became 1.22 with 95% confidence limits of 1.08 and 1.37.
Melia et al. (1980) studied children aged 6 to 7 years and measured bedroom NO₂ levels for the exposure estimate. This study applied the same combined health end-point as the previous study. The estimated odds ratio for an increase of 28.3 µg/m³ (0.015 ppm) was 1.49 with 95% confidence limits of 1.04 and 2.14. Melia et al. (1982a,b) studied children aged 5 to 6 years and also measured NO₂ exposure in the bedroom and applied the same combined health end-point. The estimated odds ratio for an increase of 0.015 ppm was 1.11, with 95% confidence limits of 0.84 and 1.46. The 10th and the 90th percentiles of the weekly measured concentrations were 0.009 and 0.065 ppm NO₂, respectively, in bedrooms (Melia et al., 1982b).

In the first Harvard Six Cities study cohort, Ware et al. (1984) reported an index of respiratory illness. Exposure to NO₂ was based on the presence or absence of a gas stove (32.5 µg/m³, 0.0173 ppm). The estimated odds ratio was 1.08 with 95% confidence limits of 0.97 and 1.19. After adjusting to a standard increase of 28.3 µg/m³ (0.015 ppm), the odds ratios became 1.07 with 95% confidence limits of 0.98 and 1.17.

A second cohort of subjects in the Harvard Six Cities study was initially reported by Dockery et al. (1989a). This cohort of children aged 7 to 11 years was then reinterviewed after indoor NO₂ measurements were made, and the results of this analysis were reported by Neas et al. (1990, 1991). The 10th and 90th percentiles of the weekly measured concentrations were 0.008 and 0.033 ppm NO₂, respectively, in bedrooms (Neas et al., 1991). The estimated odds ratio for an increase in the presence of any respiratory symptom resulting from an increase in exposure of 28.3 µg/m³ (0.015 ppm) was 1.40, with 95% confidence limits of 1.14 and 1.72.

Ekwo et al. (1983) studied several respiratory illness end-points from children surveyed at ages 6 to 12 years. No exposure measurements were obtained, and the exposure was based on the presence or absence of a gas stove (32.5 µg/m³, 0.0173 ppm). None of the end-points matched the end-point of interest closely. The two most similar end-points were hospitalization for chest illness before age 2 and chest congestion and phlegm with colds. The estimated odds ratio for hospitalization was 2.40. The estimated confidence limits for cough and phlegm with colds was 1.09, with 95% confidence limits of 0.82 and 1.45. This last symptom appears to be most similar to the end-point of interest, and so it was included in the synthesis.
The data presented by Dijkstra et al. (1990) on the study in the Netherlands were analysed and gave an estimated odds ratio of 0.94 for an increase of 28.3 µg/m³ (0.015 ppm) in NO₂ exposure. The 95% confidence limits were 0.70 and 1.27. The study had measured NO₂ exposure data, but the meta-analysis did not adjust for covariates because the covariates were not included in the tables that included NO₂ exposure.

Keller et al. (1979b) did not find any statistically significant changes in respiratory disease associated with gas stove use, but the unadjusted estimated odds ratio for lower respiratory illness was 1.10, with 95% confidence limits of 0.74 and 1.54. Assuming that the exposure increase was 32.5 µg/m³ (0.0173 ppm), the odds ratio was adjusted to an exposure of 28.3 µg/m³ (0.015 ppm). This resulted in an odds ratio of 1.09 with 95% confidence limits of 0.82 and 1.46.

7.7.4.2 Studies not selected for quantitative analysis

Five studies with sufficient information for analysis were excluded from the synthesis. Two studies (Melia et al., 1983; Ogston et al., 1985) were on children under 1 year of age, whereas the others were on children of elementary school age. Furthermore, the end-point of wheeze is more predominant in children less than 1 year old as opposed to older children, and the outcome measure in Ogston et al. (1985) included upper respiratory illness, making it dissimilar to the others. The Berwick et al. (1989) analysis has been criticized for its lack of consistency across age groups, which may have resulted from the very small sample sizes. The Swiss study (Braun-Fahrländer et al., 1989, 1992) examined end-points that might not be considered similar to those of the other studies, such as upper respiratory disease, breathing difficulties and duration of various respiratory measures. The Melia et al. (1988, 1990) study did not demonstrate significant exposure differences between the two groups contrasted (6.4 µg/m³, 0.0034 ppm). These differences in exposure were much smaller than those seen for any other study of gas stove exposure. If the relative risk were adjusted for an increase of 28.3 µg/m³ (0.015 ppm), the relative risk would be about 1.29, which is comparable to the odds ratios seen in the other studies. Because the difference in exposure groups was so small, requiring a very large adjustment, it was decided not to combine this study with the other studies. For these reasons, the above-mentioned studies were not included in the synthesis. These studies, however, support qualitatively the results of the synthesis.

384
Combining evidence, often referred to as meta-analysis, is not new, having been used as early as 1904 (Pearson, 1904). Such analyses are being used more frequently as indicated by Mann (1990). The National Research Council (1986) combined evidence on the effect of environmental tobacco smoke on lung cancer using Peto's method as described by Yusuf et al. (1985). Several methods for combining clinical trials were discussed by Laird & Mosteller (1990). The evidence to be combined in this section comes from observational studies. As a result, some of the methods used for clinical trials are not appropriate here, and the findings must be treated cautiously in light of the assumptions made when combining non-experimental studies.

Two basic models are employed in order to combine evidence (Hasselblad et al., 1992). The first model assumes that each study estimates the same fixed, but unknown, parameter. Most methods of combining evidence make this assumption. One of the earliest attempts to combine data using a fixed-effects model was given by Birge (1932). His method weights the estimates inversely by their variances and produces combined estimate and associated confidence limits. Other methods include the Mantel-Haenszel method (Mantel & Haenszel, 1959), which is used to combine contingency tables. Recently, Bayesian methods have been used to combine evidence, and methods particularly appropriate to these kinds of studies were described by Eddy (1989) and Eddy et al. (1990a,b). Bayesian analyses require the choice of a prior distribution for the parameter of interest, which is often a non-informative prior. A non-informative prior is one that, prior to seeing the evidence, favours no value of the parameter over any other. The interesting fact about use of these methods is that, for the data sets considered in Table 63, the results of the computations were nearly identical. This is because the (marginal) likelihood for the odds ratio is closely approximated by a log-normal curve.

The second basic model assumes that the parameter of interest is not fixed, but is itself a random variable from a distribution. The value of this random variable is different for each study, but each study gives some information about the mean of the distribution. These models go by several names, including random-effects models, mixed models, two-stage models and hierarchical models. The purpose of a random-effects model is to relax the assumption that each study is estimating exactly the same parameter. This idea is not new, having been discussed by Cochran (1937). A discussion of the interpretation of random-effects
models in clinical trials and several methods of estimating the parameters of these models was provided by DerSimonian & Laird (1986). If the studies being combined tend to estimate the same parameter, then the results using a random-effects model will approach the results using a fixed-effects model. On the other hand, if the studies are estimating very different parameters, then the confidence limits will tend to be much broader than those obtained from a fixed-effects model.

The nine studies described earlier (Tables 63 and 64) were combined using both kinds of models. Graphs of the odds ratio from each study are depicted in Fig. 25. Each curve can be given one of three interpretations: (1) the normal approximation to the marginal likelihood of the logarithm of the odds ratio, (2) a distribution such that the 2.5 percentile and the 97.5 percentile points of the distribution are the 95% confidence limits of the estimated odds ratio, and (3) the posterior for the odds ratio for a particular study given a flat prior on the log odds ratio. The results using a fixed-effects model are labelled “fixed”, and results of the random effects model are labelled “random” (see Fig. 25). Methods for estimating the parameters of a random-effects model were described by DerSimonian & Laird (1986) and Eddy et al. (1992). The results of the analyses are provided in Table 65 (US EPA, 1993).

Table 64. Summary of odds ratios from studies on the effects of NO₂ increased by 0.015 ppm (from US EPA, 1993)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Estimated odds ratio</th>
<th>2.5 and 97.5 Percentiles (confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia et al. (1977)</td>
<td>1.28</td>
<td>1.14 to 1.43</td>
</tr>
<tr>
<td>Melia et al. (1979)</td>
<td>1.22</td>
<td>1.06 to 1.37</td>
</tr>
<tr>
<td>Melia et al. (1980)</td>
<td>1.49</td>
<td>1.04 to 2.14</td>
</tr>
<tr>
<td>Melia et al. (1982a,b)</td>
<td>1.11</td>
<td>0.84 to 1.46</td>
</tr>
<tr>
<td>Ware et al. (1984)</td>
<td>1.07</td>
<td>0.96 to 1.17</td>
</tr>
<tr>
<td>Neas et al. (1991)</td>
<td>1.40</td>
<td>1.14 to 1.72</td>
</tr>
<tr>
<td>Ekwo et al. (1993)</td>
<td>1.09</td>
<td>0.82 to 1.45</td>
</tr>
<tr>
<td>Dijkstra et al. (1990)</td>
<td>0.94</td>
<td>0.70 to 1.27</td>
</tr>
<tr>
<td>Keller et al. (1979b)</td>
<td>1.09</td>
<td>0.82 to 1.46</td>
</tr>
</tbody>
</table>
Fig. 25. Meta-analysis of epidemiological studies on effects of nitrogen dioxide exposure on respiratory disease in children. Each curve can be treated as a likelihood function or posterior probability distribution. If treated as a likelihood function, the 95% confidence limits for the odds ratio can be calculated as those two points on the horizontal axis for which 95% of the area under the curve is contained between the two points. If treated as a posterior probability distribution, then the area under the curve between any two points is the probability that the odds ratio lies between those two points (From: US EPA, 1993)

The first line of Table 65 shows the results of combining all nine studies using a fixed model. The estimated odds ratio is 1.17 and the 95% confidence limits are 1.11 and 1.23. The analysis was made assuming that the parameters were homogeneous, and this can be tested. The chi-square test for homogeneity for the nine studies was 12.32 for 8 degrees of freedom, which has a p value of 0.1375. Thus, there is some evidence that the parameters from each study are not identical. The estimates for the random-effects
Table 65. Combined analyses of studies on respiratory illness effects of nitrogen dioxide increased by 0.015 ppm  
(from: US EPA, 1993)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of studies</th>
<th>Fixed-effects model</th>
<th>Random-effects model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds ratio</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>All</td>
<td>9</td>
<td>1.17</td>
<td>1.11 to 1.23</td>
</tr>
<tr>
<td>USA</td>
<td>4</td>
<td>1.11</td>
<td>1.03 to 1.20</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>4</td>
<td>1.25</td>
<td>1.15 to 1.35</td>
</tr>
<tr>
<td>Measured NO₂</td>
<td>4</td>
<td>1.23</td>
<td>1.08 to 1.41</td>
</tr>
<tr>
<td>Gas stove surrogate</td>
<td>5</td>
<td>1.15</td>
<td>1.05 to 1.22</td>
</tr>
<tr>
<td>SES adjusted</td>
<td>3</td>
<td>1.27</td>
<td>1.17 to 1.37</td>
</tr>
<tr>
<td>SES not adjusted</td>
<td>6</td>
<td>1.08</td>
<td>1.00 to 1.16</td>
</tr>
<tr>
<td>Smoking adjusted</td>
<td>2</td>
<td>1.26</td>
<td>1.09 to 1.52</td>
</tr>
<tr>
<td>Smoking not adjusted</td>
<td>7</td>
<td>1.15</td>
<td>1.09 to 1.22</td>
</tr>
<tr>
<td>Gender adjusted</td>
<td>5</td>
<td>1.26</td>
<td>1.18 to 1.36</td>
</tr>
<tr>
<td>Gender not adjusted</td>
<td>4</td>
<td>1.06</td>
<td>0.98 to 1.15</td>
</tr>
</tbody>
</table>
Epidemiological Studies of Nitrogen Oxides

model are similar to the estimates for the fixed-effects model, but the confidence limits are slightly broader. The conclusion from both models is the same, namely that the odds ratio is estimated to be about 1.2, with 95% confidence intervals ranging from about 1.1 to 1.3 (Hasselblad et al., 1992). Many researchers have suggested that the random-effects model is the more appropriate one, because it does not assume that all studies estimate the same parameter.

These studies include results from North America and Europe. Meta-analyses of studies from different countries are common. For example, Canner (1987), Littenberg (1988), and Jaeschke et al. (1990) all combined some studies in both North America and Europe and did not adjust for geographic differences. The indoor NO₂ studies were compared by country.

The studies were compared by similarity of subjects. Four of them were conducted in the United Kingdom (Melia et al., 1977, 1979), and four in the USA (Keller et al., 1979a, b; Ware et al., 1984; Neas et al., 1990, 1991; Ekwo et al., 1993). The United Kingdom studies provide a higher estimated odds ratio (1.25) than the USA studies (1.11).

Four of the nine studies used measured NO₂ values, whereas the other five did not. The use of a surrogate for exposure should tend to reduce the estimate of the effect (Samet & Utell, 1990). The measured NO₂ studies gave an estimated odds ratio of 1.23, whereas the others gave an estimate of 1.15, which is consistent with a measurement error effect. The chi-square tests for homogeneity were not significant at the 0.1 level for either group of studies.

Table 66 lists the important covariates considered in these nine studies and shows if the covariate was used in the study and the meta-analysis (US EPA, 1993). Study design and exposure measurement source are also presented. The effect of having adjusted for various covariates can be seen in Table 64. In general, those studies that adjusted for a particular covariate found larger odds ratios than those that did not.

Although there may be reasons to weight certain studies or groups of studies more heavily than others, the results indicate that there is an increase in the odds of respiratory disease of children exposed to NO₂, especially those of elementary school age. The estimates are generally centered around an odds ratio of
<table>
<thead>
<tr>
<th>Reference</th>
<th>SES</th>
<th>Covariates*</th>
<th>Gender</th>
<th>Design</th>
<th>Exposure measurement source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia et al. (1977)</td>
<td>A</td>
<td>NM</td>
<td>A</td>
<td>Cross-sectional</td>
<td>Gas stove vs. electric stove</td>
</tr>
<tr>
<td>Melia et al. (1979)</td>
<td>A</td>
<td>M</td>
<td>A</td>
<td>Cross-sectional</td>
<td>Gas stove vs. electric stove</td>
</tr>
<tr>
<td>Melia et al. (1980)</td>
<td>M</td>
<td>M</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO\textsubscript{2} measured with Palmes tubes.</td>
</tr>
<tr>
<td>Melia et al. (1982a,b)</td>
<td>M</td>
<td>M</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO\textsubscript{2} measured with Palmes tubes.</td>
</tr>
<tr>
<td>Ware et al. (1984)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>Cross-sectional</td>
<td>Gas stove vs. electric stove</td>
</tr>
<tr>
<td>Neas et al. (1991)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO\textsubscript{2} measured with Palmes tubes.</td>
</tr>
<tr>
<td>Ekwe et al. (1983)</td>
<td>NM</td>
<td>A</td>
<td>M</td>
<td>Cross-sectional</td>
<td>Gas stove vs. electric stove</td>
</tr>
<tr>
<td>Dijkstra et al. (1990)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>Cross-sectional</td>
<td>NO\textsubscript{2} measured with Palmes tubes.</td>
</tr>
<tr>
<td>Keller et al. (1979b)</td>
<td>M</td>
<td>NM</td>
<td>M</td>
<td>Prospective</td>
<td>NO\textsubscript{2} emissions sources in homes.</td>
</tr>
</tbody>
</table>

* SES = Socioeconomic status; A = Covariate included in study and meta-analysis; NM = Not measured in study; M = Measured in study but data not available for meta-analysis

* Estimate of exposure derived from assumption of gas stove versus electric stove levels in bedrooms in England from data in Melia et al. (1980, 1982a,b) of approximately 0.0195 ppm.

* Estimate of exposure derived from assumption of gas stove with pilot light versus electric stove levels averaged in the home in the USA in Neas et al. (1991) of approximately 0.0173 ppm.
1.2 with 95% confidence limits of 1.1 and 1.3 (Hasselblad et al., 1992), although the studies using measured NO₂ give a slightly higher estimate of the odds ratio. The estimates are not sensitive to the assumption that each study is estimating the same parameter as indicated by the random-effects model. In fact, the finding of increased risk across a wide variety of study conditions suggests that the effects seen are not an artifact of any one particular study.

These results are not sensitive to the inclusion or exclusion of any one study. If the analysis had included the hospitalization results of Ekwo et al. (1983), the analysis of the Swiss study, or the Berwick et al. (1989) study, there would have been little change in the estimated odds ratios or their 95% confidence limits. It is also possible to delete any one study from the analysis, and still obtain nearly the same results. In fact, any two studies can be deleted from the analysis, and the estimated odds ratio will have a confidence interval that does not include 1.0.

There is always the concern that the studies described in this monograph are not the complete list of studies, but contain primarily the positive studies because these are the studies most likely to be published. Alternatively, non-significant results may not be reported with sufficient quantitative detail to permit their inclusion. Both of these effects can be considered as "publication bias" (see section 7.6.3). It is of interest to contemplate an undiscovered study with results so negative that, when combined with the other studies, produces a confidence interval for the odds ratio that includes the value 1.0. If we assume that the hypothetical study would be the size of the Ware et al. (1984) study, then its odds ratio for increased respiratory symptoms as the result of a 28.3 μg/m³ (0.015 ppm) exposure would have to be 0.77. Subject to assumptions made for the combined analysis for school-aged children, the main conclusion from the analysis was that an increased risk of about 20% for respiratory symptoms and disease corresponded to each increase of 28 μg/m³ (0.015 ppm) in estimated 2-week average NO₂ exposure, where mean weekly bedroom concentrations in studies reporting NO₂ levels were predominantly between 0.008 and 0.065 ppm NO₂ (Hasselblad et al., 1992).

7.8 Synthesis of the evidence for young children

Various researchers have conducted studies of children less than 2 years of age (see Table 67). A major difference for this group of studies is that the health outcome measures are less...
Table 67. Summary of odds ratios of the effects of nitrogen dioxide, health outcome and exposure estimates in epidemiological studies on young children (< 2 year)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Estimated odds ratio</th>
<th>2.5 and 97.5 Percentiles (confidence interval)</th>
<th>Health outcome</th>
<th>NO\textsubscript{2} exposure estimate (ppm)</th>
<th>Age</th>
<th>Location (date of study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia et al. (1983)</td>
<td>0.63</td>
<td>0.36-1.10</td>
<td>Respiratory illness incidence</td>
<td>0.0165\textsuperscript{a}</td>
<td>&lt; 1 year</td>
<td>England (1978)</td>
</tr>
<tr>
<td>Ekwo et al. (1983)</td>
<td>2.4</td>
<td>1.06-3.74</td>
<td>Hospitalization for chest illness before age 2</td>
<td>0.0173\textsuperscript{b}</td>
<td>&lt; 2 years</td>
<td>Iowa, USA</td>
</tr>
<tr>
<td>Ware et al. (1984)</td>
<td>1.11</td>
<td>0.97-1.27</td>
<td>Respiratory illness before age 2</td>
<td>0.0173\textsuperscript{b}</td>
<td>&lt; 2 years</td>
<td>Six Cities USA (1974-1979)</td>
</tr>
<tr>
<td>Ogston et al. (1985)</td>
<td>1.14</td>
<td>0.86-1.50</td>
<td>Respiratory illness incidence</td>
<td>0.0165\textsuperscript{a}</td>
<td>&lt; 1 year</td>
<td>Scotland (1980)</td>
</tr>
<tr>
<td>Dockery et al. (1989a)</td>
<td>1.15</td>
<td>0.90-1.37</td>
<td>Respiratory illness before age 2</td>
<td>0.015\textsuperscript{c}</td>
<td>&lt; 2 years</td>
<td>Six Cities USA (1983-1986)</td>
</tr>
<tr>
<td>Margolis et al. (1992)</td>
<td>1.12</td>
<td>0.63-2.04</td>
<td>Persistent lower respiratory symptoms</td>
<td>0.0105\textsuperscript{d}</td>
<td>&lt; 1 year</td>
<td>North Carolina, USA (1986-1988)</td>
</tr>
<tr>
<td>Samet et al. (1993)</td>
<td>0.90\textsuperscript{e}</td>
<td>0.94-1.04\textsuperscript{f}</td>
<td>Lower respiratory illness incidence</td>
<td>0.015\textsuperscript{d}</td>
<td>&lt; 18 months</td>
<td>Albuquerque, USA (1988-1990)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Estimate of exposure derived from assumption of gas stove versus electric stove levels in bedrooms in England from data in Melia et al. (1980, 1982a) of approximately 0.0165 ppm.

\textsuperscript{b} Estimate of exposure derived from assumption of gas stove with pilot light versus electric stove levels averaged in the home in the USA in Neas et al. (1991) of approximately 0.0173 ppm.

\textsuperscript{c} Estimate of exposure derived from assumption of gas stove versus electric stove levels averaged in the home in USA in Neas et al. (1991) of approximately 0.015 ppm.

\textsuperscript{d} Estimate of exposure derived from assumption of gas stove versus electric stove levels averaged in the home in the Albuquerque study (Samet et al., 1993) of approximately 0.0105 ppm.

\textsuperscript{e} Computed from logistic regression coefficient derived from Samet et al. (1993).

\textsuperscript{f} Exposure level used to convert logistic regression to an odds ratio.
uniform than the studies of older children. For purposes of comparability, a meta-analysis similar to the one for older children was made.

The seven studies of young children shown in Table 67 show mixed results. A test of homogeneity of the odds ratios gives a chi-squared value of 22.66 for 6 degrees of freedom, which has a p value of 0.0009, implying that the studies are not homogenous. The variation in results could be due to several factors, including different health outcome measures and other factors. Dockery et al. (1989a) noted that the associations discussed by Ware et al. (1984) and Dockery et al. (1989a) must be viewed with caution because they compared recalled respiratory events early in the child's life. Because of the heterogeneity, the studies were combined using a random-effects model. Subject to the assumptions made for the meta-analysis, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm NO₂ was 1.09 with a 95% confidence interval of 0.95 to 1.26, where mean weekly concentrations in bedrooms were predominately between 0.005 and 0.05 ppm NO₂ in studies reporting levels. The increase in risk was very small and was not reported consistently by all studies. We cannot conclude that the evidence suggests an effect in young children comparable to that seen in older children.

7.9 Summary

The evidence from individual studies of the effect of NO₂ on lower respiratory symptoms and disease in school-aged children is somewhat mixed. The consistency of these studies was examined and the evidence synthesized in a combined quantitative analysis (meta-analysis) of the subject studies. Most of the indoor studies showed increased lower respiratory morbidity in children associated with long-term exposure to NO₂. Mean weekly NO₂ concentrations in bedrooms in studies reporting NO₂ levels were predominately between 15 and 122 μg/m³ (0.008 and 0.065 ppm) (Hasselblad et al., 1992). Combining the indoor studies as if the end-points were similar gives an estimated odds ratio of 1.2 (95% confidence limits of 1.1 and 1.3) for the effect per 28.3 μg/m³ (0.015 ppm) increase of NO₂ on lower respiratory morbidity (Hasselblad et al., 1992). This suggests that, subject to assumptions made for the combined analysis, an increase of about 20% in the odds of lower respiratory symptoms and disease corresponded to each increase of 28.3 μg/m³ (0.015 ppm) in estimated 2-week average NO₂ exposure. Thus, the combined evidence is supportive for the effects of estimated exposure to
In the individual indoor studies of young children (2 years of age or younger), no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analysis of these indoor infant studies, subject to the assumptions made for the meta-analysis, the combined odds ratio for the increase in respiratory disease per increase of 28.2 µg/m³ (0.015 ppm) NO₂ was 1.09 with a 95% confidence interval of 0.95 to 1.26, where mean NO₂ weekly concentrations in bedrooms were predominately between 9.4 and 94 µg/m³ (0.005 and 0.05 ppm) in studies reporting levels. The increase in risk was very small and was not reported consistently by all studies. We cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children. The reasons for these age-related differences are not clear.

The measured NO₂ studies gave a higher estimated odds ratio than the surrogate estimates, which is consistent with a measurement error effect. The effect of having adjusted for covariates such as socioeconomic status, smoking and sex was that those studies that adjusted for a particular covariate found larger odds ratios than those that did not.

Although many of the epidemiological studies that involved measured NO₂ levels used measurements over only 1 or 2 weeks, these levels were used to characterize children’s exposures over a much longer period. The standard respiratory symptom questionnaire used by most of these studies summarizes information on health status over an entire year. The 28.2 µg/m³ (0.015 ppm) difference in NO₂ levels used in the meta-analyses relates to a difference in the household annual average exposure between gas and electric cooking stoves. Some studies measured NO₂ levels only in the winter and may have overestimated annual average exposures. This would tend to have underestimated the health effect of a 28.2 µg/m³ (0.015 ppm) difference in the annual NO₂ exposure. The study of Neas et al. (1991), which was based on household annual average exposure measured in both the winter and summer, found a stronger health effect than many of the other studies. The true biologically relevant exposure period is unknown, but these exposures extended over a lengthy period up to the entire lifetime of the child.
The association between outdoor NO\textsubscript{2} and respiratory health is not clear from current research. There is some evidence that the duration of respiratory illness may be increased at higher ambient NO\textsubscript{2} levels. A major difficulty in the analysis of outdoor studies is distinguishing possible effects of NO\textsubscript{2} from those of other associated pollutants.

Several uncertainties need to be considered in interpreting the above studies and results of the meta-analysis. Error in measuring exposure is potentially one of the most important methodological problems in epidemiological studies of NO\textsubscript{2}. Although there is evidence that symptoms are associated with indicators of NO\textsubscript{2} exposure, the quality of these exposure estimates may be inadequate to determine a quantitative relationship between exposure and symptoms. Most of the studies that measured NO\textsubscript{2} exposure did so only for periods of 1 to 2 weeks and reported the values as averages. Few of the studies attempted to relate the effects seen to the pattern of exposure, such as transient peaks. Furthermore, measured NO\textsubscript{2} concentration may not be the biologically relevant dose per se; estimating actual exposure requires knowledge of both pollutant levels and related human activity patterns. However, only very limited activity and aerometric data are available that examine such factors, and the extrapolation to possible patterns of ambient exposure is difficult. In addition, although the level of similarity and common elements between the outcome measures in the NO\textsubscript{2} studies provide some confidence in their use in the quantitative analysis, the symptoms and illnesses combined are to some extent different and could indeed reflect different underlying processes. Thus, caution is necessary in interpreting the meta-analysis results.

Other epidemiological studies have attempted to relate some measure of indoor and/or outdoor NO\textsubscript{2} exposure to changes in pulmonary function. These changes were marginally significant. Most studies did not find any effects, which is consistent with controlled human exposure study data (see Chapter 6). However, there is insufficient epidemiological evidence to draw any conclusions about the long- or short-term effects of NO\textsubscript{2} on pulmonary function.

On the basis of a background level of 15 $\mu$g/m\textsuperscript{3} (0.008 ppm) and the fact that significant health effects occur with an additional level of 28.2 $\mu$g/m\textsuperscript{3} (0.015 ppm) or more, an annual guideline value of 40 $\mu$g/m\textsuperscript{3} (0.023 ppm) is proposed. This value will avoid the most severe exposures. The fact that a no-effect level for
subchronic or chronic NO₂ exposure concentrations has not yet been determined should be emphasized.
8. EVALUATION OF HEALTH AND ENVIRONMENT RISKS ASSOCIATED WITH NITROGEN OXIDES

8.1 Sources and exposure

Combustion provides the major source of nitrogen oxides in both indoor and outdoor air, producing mostly NO, typically about 90%, with some NO2 and small quantities of other species. Some domestic combustion appliances can produce more than 10% of NO as NO2. The sum of NO and NO2 is generally referred to as NOx. In the air, NO is oxidized to NO2. This happens rapidly by reaction with ozone, and also by a slower photochemical process requiring the presence of reactive organic compounds and sunlight. Nitrogen oxides together with reactive organic compounds are precursors for ozone and photochemical smog formation. NO and NO2 may also undergo reactions to form a range of other nitrogenous species, including HNO2, HNO3, NO3, N2O5, PAN and other organic nitrates. The complete range of gas phase nitrogen oxides is often referred to as NOV. The partitioning of nitrogen among these different compounds is strongly dependent on the concentrations of other oxidants and on the meteorological history of the air.

Nitrogenous species have lifetimes in the air ranging from minutes to several days. In general, emissions of NO and NO2 are progressively oxidized to HNO3 and nitrate. Air contaminated by NOx emissions and their reaction products can be advected large distances. Exposure tends to be predominantly to NO close to a source, NO2 in the local region and HNO3 and nitrates at distances of up to several hundred kilometres or more.

Human and environmental exposure to nitrogen oxides varies greatly from indoors to outdoors, from the city to the countryside, and with the time of day and season. The concentrations of NO and NO2 typically present outdoors in a range of urban situations is relatively well established. The concentrations encountered indoors depend on the specific details of the nature of combustion appliances, chimneys and ventilation. When unvented combustion appliances are used for cooking or heating, indoor concentrations of nitrogen oxides usually greatly exceed those existing outside.

Nitrogen oxides are ultimately removed from the atmosphere mostly as nitrate by processes of dry and wet deposition.
Nitrous oxide (N$_2$O) is emitted to the atmosphere from biological and some combustion processes. It is inert in the troposphere but in the stratosphere plays a role in the chemistry of stratospheric ozone. N$_2$O is also a greenhouse active gas.

In indoor air, the concentration and composition of nitrogen oxide species is largely the result of indoor combustion sources. NO is in greater concentration than NO$_2$, usually by a factor of up to ten-fold. In some cases indoors, HNO$_2$ has been reported at concentrations that are more than 10% of those of NO$_2$. HNO$_2$ may be produced from surface reactions of NO or NO$_2$ with water.

There are several difficulties with measurements of nitrogen oxides. A straightforward interference-free method exists for measuring NO (the chemiluminescent reaction with ozone), but this is the exception for nitrogenous species. By firstly converting NO$_2$ to NO, this chemiluminescence technique is also commonly used to measure NO$_2$. Unfortunately, the catalysts usually employed for this conversion are not specific and, especially for emissions that have undergone substantial photochemical reaction, other oxidized nitrogen compounds present in the sampled air are also converted to NO and are measured as NO$_2$. For this reason, great care must be taken in interpreting the results of the common chemiluminescence analyser in terms of NO$_2$ and several other nitrogen compounds. Additional measurement difficulties arise because oxidized nitrogen in the atmosphere can also be present in both the gas phase and as particulate matter. For indoor air measurements, the Palmes tube technique of NO$_2$ measurement is frequently used. This technique is not suited for measurement of short-term peak concentrations.

8.2 Evaluation of the effects of atmospheric nitrogen species on the environment

Guidance values have been estimated for both critical levels of NO$_2$ (the air concentration threshold for effects on plants) and critical loads of total nitrogen (the deposited nitrogen load to ecosystems above which adverse effects can occur). Since deposited nitrogen acts on ecosystems by increasing the nutrient status of soils, there is no definitive threshold for effects; all additional nitrogen will result in some response.

The individual nitrogen species present in the polluted atmosphere cannot be completely separated with respect to their
effects on the environment. The relative contribution of NO and NO\textsubscript{2} to the NO\textsubscript{x} effect on plants is unclear. The vast majority of information is on effects of NO\textsubscript{2} but available information on NO suggests that NO and NO\textsubscript{2} have comparable phytotoxic effects. Total nitrogen deposition has been used to assess effects on ecosystems since it is not possible to identify the relative contribution of nitrogen species to nutrient nitrogen elevation.

Concerning organisms in the environment, information is almost exclusively restricted to plants, with minimum data on soil fauna. The evaluation and guidance values are, therefore, expressed in terms of nitrogen species effects on vegetation. However, it is expected that plants will form the most sensitive component of natural systems and that the effect on biodiversity of plant communities is a sensitive indicator of biotic effects on the whole ecosystem.

Gaseous nitrogen species reduce photosynthesis and biomass production and increase sensitivity to other stresses (such as frost, drought and insect damage) of individual plants. At the level of plant communities and ecosystems, eutrophication is more important than toxicity, nitrogen causing reduction in biodiversity in nutrient-limited habitats.

Deposited nitrogen will change the chemistry of soils; these changes are reflected biologically in total ecosystem effects. However, there is one feature that needs separate consideration; deposited nitrogen contributes to the leaching of nitrate through soil profiles and into surface and groundwater.

The atmospheric chemistry of nitrogen oxides includes the capacity for ozone generation in the troposphere, ozone depletion in the stratosphere, and direct and indirect contribution to global warming as greenhouse gas. Nitrogen oxides and ammonia contribute to soil acidification (along with sulfur oxides) and thereby to increased bioavailability of aluminium.

The phytotoxic effects of gaseous nitrogen oxides on plants have little direct relevance to crop plants when concentrations are close to the critical level (see section 8.2.1). However, the role of NO\textsubscript{2} in the generation of ozone and other phytotoxic substances leads to crop loss. Nitrogen deposited on growing crops will represent a very small increase in total available nitrogen compared to that added as fertilizer.
8.2.1 Guidance values - critical levels for air concentrations of nitrogen oxides

Critical levels are mostly derived from fumigation experiments. In the majority of studies with NO or NO₂, no significant effects on plants were found at air concentrations below 100 μg/m³. Most of the experiments were designed to evaluate mechanisms of action of nitrogen oxides rather than to quantify adverse effect thresholds, and few exposure-response relationships have been established.

Interactions between NO₂ and other atmospheric pollutants have been reported. Generally SO₂ acts synergistically with NO₂. In mixtures of NO₂ with other gases, such as NO, O₃, and CO₂, interactive effects are the exception rather than the rule.

In order to include the impact of NO, a critical level for NO is proposed instead of one for NO₂ for this purpose it has been assumed that NO and NO₂ act in an additive manner.

A strong case can be made for the provision of critical levels for short-term exposure. However, there are currently insufficient data to provide these with confidence. Current evidence suggests a critical level of about 75 μg/m³ for NO as a 24-h mean.

The critical level for NO₃ (NO and NO₂ added in ppb and expressed as NO₂ in μg/m³) is considered to be 30 μg/m³ as an annual mean (see section 4.1.8 for detailed reasoning).

At concentrations slightly above this critical level, growth stimulation is the dominant effect (possibly combined with some increase in sensitivity to biotic and abiotic stresses) if the ambient conditions allow growth and NO₃ is the only pollutant. Where biomass production is beneficial, for example in agriculture or plantation forest, the critical level is probably higher.

The critical level can be converted to deposition quantities. The annual deposition that corresponds to a NO level of 30 μg/m² is 5 to 10 kg/ha. This indicates that at a concentration near to its critical level, the contribution of NO₃ to nitrogen demand is negligible for fertilized crops but not for natural vegetation.

8.2.2 Environment-based guidance values - critical loads for total nitrogen deposition

Critical loads are derived from empirical data and steady-state soil models. Estimated critical loads for total nitrogen deposition
in a variety of natural aquatic and terrestrial ecosystems are given in Table 68 (details can be found in section 4.2). Possible differential effects of deposited nitrogen species (NO\(_x\) and NH\(_x\)) are insufficiently known to differentiate between nitrogen species for critical load estimation.

Table 68. Summary of guidelines for total nitrogen deposition (kg nitrogen per ha per year) in natural and semi-natural freshwater and terrestrial ecosystems

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Critical load</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow soft-water lakes</td>
<td>5-10(^a)</td>
<td>Decline in isoetid species</td>
</tr>
<tr>
<td>Mesotrophic fens</td>
<td>20-35(^b)</td>
<td>Increase in tall graminoids: decline in diversity</td>
</tr>
<tr>
<td>Ombrotrophic (raised) bogs</td>
<td>5-10(^c)</td>
<td>Decreased Sphagnum and subordinate species; increase in tall graminoids</td>
</tr>
<tr>
<td>Calcareous species-rich grassland</td>
<td>14-19(^d)</td>
<td>Increase in tall grasses; decline in diversity</td>
</tr>
<tr>
<td>Neutral/acid species-rich grassland</td>
<td>20-30(^b)</td>
<td>Increase in tall grasses; decline in diversity</td>
</tr>
<tr>
<td>Montane-subalpine grassland</td>
<td>10-15(^e)</td>
<td>Increase in tall graminoids; decline in diversity</td>
</tr>
<tr>
<td>Lowland dry heathland</td>
<td>15-20(^a)</td>
<td>Transition of heather to grass</td>
</tr>
<tr>
<td>Lowland wet heathland</td>
<td>17-22(^a)</td>
<td>Transition of heather to grass</td>
</tr>
<tr>
<td>Species-rich heaths/acid grassland</td>
<td>7-20(^b)</td>
<td>Decline in sensitive species</td>
</tr>
<tr>
<td>Arctic and alpine heaths</td>
<td>5-15(^e)</td>
<td>Decline in lichens, mosses and evergreen dwarf shrubs; increase in grasses and herbs</td>
</tr>
<tr>
<td>Coniferous tree health</td>
<td>11 - &gt; 50(^e)</td>
<td>Nutrient imbalance</td>
</tr>
<tr>
<td>Deciduous tree health</td>
<td>15-20(^b)</td>
<td>Nutrient imbalance, shoot-root ratio</td>
</tr>
<tr>
<td>Acidic (managed) coniferous forest</td>
<td>15-20(^b)</td>
<td>Changes in ground flora</td>
</tr>
<tr>
<td>Acidic (managed) deciduous forest</td>
<td>15-20(^b)</td>
<td>Changes in ground flora</td>
</tr>
<tr>
<td>Calcareous forests</td>
<td>15-20(^e)</td>
<td>Changes in ground flora</td>
</tr>
</tbody>
</table>

\(^a\) reliable estimate
\(^b\) reasonably reliable estimate
\(^c\) best guess

401
Atmospheric nitrogen deposition can significantly contribute to the leaching of nitrates to surface water and groundwater. There is not enough information to provide a guidance value with broad applicability for this effect, but the few studies on this subject indicate that critical loads are relatively low (see section 4.2.7).

The majority of ecosystems for which there is sufficient information to estimate critical loads have temperate climates. The few arctic and montane ecosystems included, which might be expected to be representative of higher latitudes, have the least reliable basis. There is no information on tropical ecosystems. Nutrient-poor tropical ecosystems such as rain forests and mangrove swamps are likely to be adversely affected by nitrogen deposition. The lack of both deposition data and effect thresholds make it impossible to make risk assessments for these climatic regions.

The most sensitive ecosystems for which effects thresholds can be estimated show critical loads of 5-10 kg nitrogen per ha per year based on decreased biological diversity in plant communities. A more average value for the limited range of ecosystems studied is 15-20 kg nitrogen per ha per year.

8.3 Evaluation of health risks associated with nitrogen oxides

8.3.1 Concentration-response relationships

Table 69 summarizes key health effects observed in controlled human exposure (clinical) studies with NO<sub>2</sub> exposure durations of 0.5 to 4 h. At higher exposure levels, i.e. more than 2800 µg/m<sup>3</sup> (1.5 ppm), NO<sub>2</sub> exposure results in increased airway responsiveness and increased airway resistance in healthy adults. However, some researchers have not observed any NO<sub>2</sub>-induced changes in airway resistance at NO<sub>2</sub> levels between 3800 and 7500 µg/m<sup>3</sup> (2 to 4 ppm).

The physiological endpoint that, to date, appears to be the most sensitive indicator of response is a change in airway responsiveness to bronchoconstrictors in asthmatics. This increase in airway responsiveness has been observed in some, but not all,

* For the purposes of the risk evaluation, NO<sub>2</sub> concentrations presented in µg/m<sup>3</sup> that were originally derived from concentrations expressed as ppm have been rounded off. The conversion factor is 1880 µg/m<sup>3</sup> = 1 ppm.
Table 69. Key effects of exposure to nitrogen dioxide on human health - clinical studies

| NO₂ concentration | Exposure duration | Observed effects
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>376-564 µg/m³ (0.2-0.3 ppm)</td>
<td>0.5-2.0 h</td>
<td>Trend toward increased airway responsiveness to challenges in asthmatics. However, no significant effects observed by same or other investigators at NO₂ levels up to 4 ppm. Small (4-6%) decreases in FEV₁ or FVC in adult or adolescent asthmatics, in response to NO₂ alone.</td>
</tr>
<tr>
<td>564 µg/m³ (0.3 ppm)</td>
<td>3.7 h</td>
<td>Small decreases (5-9%) in FVC and FEV₁ in COPD patients with mild exercise. No effects seen by other investigators for COPD patients at 0.5-2 ppm NO₂.</td>
</tr>
<tr>
<td>2820-3760 µg/m³ (1.5-2.0 ppm)</td>
<td>2.3 h</td>
<td>Increased airway responsiveness to bronchoconstrictors in healthy adults. However, effects not detected by other investigators at 2-4 ppm.</td>
</tr>
<tr>
<td>≥ 3760 µg/m³ (≥ 2.00 ppm)</td>
<td>1.3 h</td>
<td>Lung function changes (e.g., increased airway resistance) in healthy subjects. Effects not found by others at 2-4 ppm.</td>
</tr>
</tbody>
</table>

* FEV₁ = Forced expiratory volume in 1 second; FVC = Forced vital capacity; COPD = Chronic obstructive pulmonary disease
studies. Several individual studies found significant responses at NO₂ concentrations within the range of 380 to 560 μg/m³, with exposure periods varying from 30 to 180 min. A meta-analysis of 20 studies in asthmatics suggests that increased airway responsiveness may occur at concentrations as low as 200 μg/m³. However, no individual studies showed clearly significant effects on airway responsiveness at 190 μg/m³ (0.1 ppm) for 60 min. Additionally, small decreases in forced expiratory volume in 1 second (FEV₁) or forced vital capacity (FVC) in adult or adolescent asthmatics have been observed in response to 560 μg/m³ (0.3 ppm) NO₂ for 30 min. However, clear NO₂ concentration-response relationships are not evident for either airway responsiveness or pulmonary function changes. Other studies of asthmatics exposed to 7500 μg/m³ (4 ppm NO₂) for 75 min did not show effects on pulmonary function or airway responsiveness.

A second category of sensitive subjects comprises patients with chronic obstructive pulmonary disease (COPD). Although small decreases have been observed in FVC and FEV₁ in COPD patients exposed to 560 μg/m³ (0.3 ppm) in one study, effects were not seen in other studies at higher exposure levels. The collective evidence from epidemiological studies examining relationships between estimates of exposure to NO₂ and lower respiratory symptoms and disease is summarized in Table 70. Lower respiratory symptoms in children are generally an indicator of the incidence and severity of respiratory illnesses that are often related to viral infections.

Indoor NO₂ exposures are associated with increased lower-respiratory illness in children aged 5-12 years, but there is no consistent evidence of such an association among younger children (0-2 years). Among primary school children, the risk of lower respiratory illness increases by about 20% for an increase of 28 μg/m³ (0.015 ppm) NO₂ indoors (averaged over 1 year). There is no evidence from the epidemiological literature that the concentration-response relationship for NO₂ and respiratory illness differs from linearity. This is consistent with the concentration-response relationship reported from studies of outdoor NO₂.

The epidemiological studies mostly used estimates of NO₂ exposures that were averaged over a period of several weeks to 1 year. Moreover, the health outcomes assessed in these studies were generally accumulated over 6-12 months. This means that the risk estimates produced by most of the epidemiological studies (both indoor and outdoor) refer to long-term average exposures.
Table 70. Key effects of exposure to nitrogen dioxide on human health - epidemiological studies

<table>
<thead>
<tr>
<th>NO₂ exposure</th>
<th>Observed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015-ppm increase, where mean weekly concentrations in bedrooms in studies reporting levels were mainly between 0.006 and 0.005 ppm NO₂ (1- and 2-week integrated average NO₂ concentration)</td>
<td>A meta-analysis shows an increased risk of lower respiratory symptoms/disease in children 5 to 12 years old associated with exposure estimates of NO₂ levels. The 95% confidence interval of the odds ratio was 1.1 to 1.3. Main source of exposure contrast is homes with gas and electric stoves.</td>
</tr>
<tr>
<td>0.015-ppm increase in annual average of 2-week NO₂ levels, where mean weekly concentrations in bedrooms were mainly between 0.005 and 0.050 ppm</td>
<td>In individual indoor studies of infants ≤ 2 years of age, no consistent relationship was found between estimates of NO₂ exposure and prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm NO₂ was 1.09 with a 95% confidence interval of 0.95 to 1.26. Thus, although the overall combined estimate is positive, it contains the no-effect value of 1.0, (i.e., is not statistically significant); and so cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children.</td>
</tr>
<tr>
<td>&gt; 0.3 ppm (average exposure during work shift)</td>
<td>Elevated prevalence of acute respiratory symptoms</td>
</tr>
<tr>
<td>Episodic exposure during ice hockey game to NO₂ levels of 1.5 ppm or more</td>
<td>Occurrence of acute respiratory symptoms (cough, chest pain, dyspnoea)</td>
</tr>
<tr>
<td>25 to 100 ppm (episodic occupational exposure)</td>
<td>Bronchitis, bronchiolitis and bronchial pneumonia induced by very high NO₂ exposure.</td>
</tr>
<tr>
<td>&gt; 200 ppm (extreme episodic exposures)</td>
<td>Extreme exposure health outcomes range from hypoxaemia/transient airway obstruction to death</td>
</tr>
</tbody>
</table>
There is no evidence in the epidemiological literature to determine whether there is, or is not, a level of NO\textsubscript{2} below which health effects are not observed. However, a quantitative review of several large well-conducted studies has shown a statistically significant excess of lower respiratory symptoms in homes with gas stoves (NO\textsubscript{2} levels approximately 38-56 µg/m\textsuperscript{3} (0.02-0.03 ppm) averaged over 12 months) compared with homes with electric stoves (average NO\textsubscript{2} levels: 9-13 µg/m\textsuperscript{3} (0.005-0.007 ppm)).

Higher levels (> 560 µg/m\textsuperscript{3}, > 0.3 ppm during a shift at work) in an occupational setting were related to an elevated prevalence of acute respiratory symptoms in adults. Episodic exposures occurring over a period of 1 h or longer at levels possibly as high as 2800 µg/m\textsuperscript{3} (1.5 ppm) or more have resulted in the occurrence of acute respiratory symptoms. Exceptionally high acute occupational exposures of 47-188 mg/m\textsuperscript{3} (25 to 100 ppm) NO\textsubscript{2} have resulted in broncho-pneumonia, bronchitis or bronchiolitis; and very extreme occupational NO\textsubscript{2} exposures (> 200 ppm) have been associated with effects that range from hypoxaemia and transient obstruction of the airways to death from adult respiratory distress syndrome.

Numerous concentration-response studies have been conducted with animals using a wide range of exposure durations and endpoints. The major classes of effects observed at concentrations less than 1880 pg/m\textsuperscript{3} (1.0 ppm) include decreases in host defences, alterations in lung metabolism (e.g., increased lipid peroxidation and antioxidant metabolism), epithelial remodelling of the lower respiratory tract, thickening of the centriacinar interstitium, and a variety of extrapulmonary changes. Such findings can be qualitatively extrapolated to humans, but major uncertainties in respiratory tract dosimetry and species sensitivity currently preclude a quantitative extrapolation. Structural changes in the lung become more severe as exposures proceed from weeks to months at a given NO\textsubscript{2} concentration. Only substantially higher NO\textsubscript{2} concentrations exceeding 22 000 pg/m\textsuperscript{3} (12 ppm) have caused emphysema, as defined by criteria developed by the US National Institutes of Health.

In order to examine the relative importance of concentration (C) of NO\textsubscript{2} and duration of exposure (T) in the development of increased susceptibility to respiratory infection, the effects of different C x T products have been evaluated. Results from infectivity studies examining patterns of exposure indicate that, at the same C x T product, concentration exerts more influence than
duration of exposure in increasing susceptibility to respiratory bacterial infection in mice.

Table 71 lists quantitative findings from a few key animal studies showing the lowest concentrations that caused several types of effects. Of most importance are findings showing increased susceptibility to infection with long-term exposure to NO\textsubscript{2} levels as low as 940 \( \mu \text{g/m}^3 \) (0.5 ppm) and of other impacts on host defences with exposure to NO\textsubscript{2} levels as low as 560 to 940 \( \mu \text{g/m}^3 \) (0.3 to 0.5 ppm). These findings provide evidence supporting the biological plausibility of the association of increased respiratory illness in older children in relation to indoor NO\textsubscript{2} exposures.

### 8.3.2 Subpopulations potentially at risk

Certain groups within the population may be more susceptible to the effects of NO\textsubscript{2} exposure, including people with pre-existing respiratory disease, children and the elderly. The reasons for paying special attention to these groups is that: (i) they may be affected by lower levels of NO\textsubscript{2} than other subpopulations; or (ii) the severity of health effects at a given exposure level may be greater. Some causes of heightened susceptibility are better understood than others. Subpopulations that already have reduced ventilatory reserves (e.g., the elderly and persons with asthma, emphysema and chronic bronchitis) are likely to be affected more than other groups by similar decreases in pulmonary function. For example, a healthy young person may not notice a small change in pulmonary function, but a person whose activities are already limited by reduced lung function may not have the reserve to compensate for such a change.

Several hundred million people suffer from asthma worldwide. Asthmatic individuals appear to be the most susceptible members of the population with regard to respiratory responses to NO\textsubscript{2}. On average, asthmatic persons are much more sensitive to inhaled bronchoconstrictors such as histamine, methacholine or carbachol. The airways of asthmatics are also hyperresponsive to a variety of other inhaled materials, including pollen, cold dry air, allergens and air pollutants. The potential addition of a further NO\textsubscript{2}-induced increase in airway response to the already heightened responsiveness to other substances raises the possibility of exacerbation of this pulmonary disease by NO\textsubscript{2} in asthmatic individuals.
Table 71. Key animal toxicological effects of exposure to nitrogen dioxide

<table>
<thead>
<tr>
<th>NO2 concentration (ppm) (exposure duration)</th>
<th>Species</th>
<th>Observed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04 ppm (continuous, 9 months)</td>
<td>Rat</td>
<td>Increased lipid peroxidation (ethane in exhaled breath)</td>
</tr>
<tr>
<td>0.2 ppm (continuous base for 1 year) plus 0.8 ppm (1-h peak, 2x/day, 5 days/week)</td>
<td>Mouse</td>
<td>Increased susceptibility to respiratory infection and decreased vital capacity and respiratory system compliance, compared to control or baseline only</td>
</tr>
<tr>
<td>0.25 ppm (7 h/day, 5 days/week, 7 weeks)</td>
<td>Mouse</td>
<td>Systemic effect on cell-mediated immunity</td>
</tr>
<tr>
<td>0.3 ppm (2 h/day, 2 days)</td>
<td>Rabbit</td>
<td>Decreased phagocytosis of alveolar macrophages</td>
</tr>
<tr>
<td>0.4 ppm (continuous, 4 weeks)</td>
<td>Mouse</td>
<td>Decreased systemic humoral immunity</td>
</tr>
<tr>
<td>0.4 ppm (continuous, 9 months)</td>
<td>Rat</td>
<td>Increased antioxidants and antioxidant metabolism</td>
</tr>
<tr>
<td>0.4 ppm (continuous, up to 27 months)</td>
<td>Rat</td>
<td>Slight increase in thickness of air-blood barrier at 18 months, becoming significant by 27 months; also alterations in bronchial and alveolar epithelium by 27 months</td>
</tr>
<tr>
<td>0.5 ppm (continuous, 3 months)</td>
<td>Mouse</td>
<td>Increased susceptibility to respiratory infection</td>
</tr>
<tr>
<td>0.5-28 ppm (6 min to 1 year)</td>
<td>Mouse</td>
<td>Linear increase in susceptibility to respiratory infection with time, increased slope of curve with increased concentration, concentration more important than time</td>
</tr>
<tr>
<td>0.5 ppm (continuous base, 6 weeks) plus 1.5 ppm (1-h peak, 2x/day, 5 days/week)</td>
<td>Rat</td>
<td>Alterations in Type 2 cells and increased interstitial matrix of proximal alveolar region, no changes in terminal bronchiolar region of adults</td>
</tr>
</tbody>
</table>
Other potentially susceptible groups include patients with COPD, such as emphysema and chronic bronchitis. Several hundred million adults worldwide suffer from COPD. Some of these patients have airway hyperresponsiveness to physical and chemical stimuli. A major concern with COPD patients is the absence of an adequate ventilatory reserve, a susceptibility factor described above. In addition, the poor distribution of respiratory tract ventilation in COPD may lead to a greater delivery of NO₂ to the segment of the lung that is well ventilated, thus resulting in a greater regional tissue dose. Furthermore, NO₂ exposure may alter already impaired defence mechanisms, making patients with COPD more susceptible to respiratory infection.

On the basis of epidemiological studies, children aged 5 to 12 years constitute a subpopulation potentially susceptible to an increase in respiratory morbidity associated with NO₂ exposure. Worldwide, nearly a billion (10⁹) children fall into the age groups at increased potential risk for increased respiratory illnesses associated with NO₂ exposures. However, the fraction of the number of potentially at-risk children in various age groups that are actually exposed to NO₂ concentrations/patterns sufficient to induce respiratory morbidity has not been determined.

Another potentially susceptible subpopulation group is immunocompromised individuals, who would have an increased susceptibility for infectious pulmonary disease as well as other health effects. Such people could be potentially more susceptible to agents, such as NO₂, that further compromise pulmonary host defences. It is clear that NO₂ can affect alveolar macrophages, humoral immunity and cell-mediated immunity in otherwise normal animals. However, the animal-to-human extrapolation cannot yet be made quantitatively. Although these immunocompromised groups represent potentially susceptible populations for NO₂ effects, no human research has directly examined the effects of NO₂ exposure in these groups.

8.3.3 Derivation of health-based guidance values

Increased airway responsiveness observed in asthmatic subjects with 0.5 to 2.0 h exposures to 380–560 pg/m³ (0.2–0.3 ppm) NO₂ represents an adverse health effect of concern induced by acute, short-term human exposures to NO₂. However, some laboratories have not observed similar effects with comparable duration NO₂ exposures at levels above the 380–560 µg/m³ (0.2–0.3 ppm) range. A possible reason might be the difference in severity of asthma of
the subjects exposed. Nevertheless, increased airway responsiveness may pose a risk for asthmatic individuals (i.e. increased responsiveness to other commonly occurring stimuli such as cold air, allergens and other air pollutants).

On the basis of an effect level at 400 µg/m³ and the possibility of effects at lower levels, based on a meta-analysis, a 1-h average daily maximum NO₂ concentration not exceeding 200 µg/m³ (0.11 ppm) is recommended as a short-term guideline. This should be adequate to protect most asthmatic subjects from experiencing NO₂-induced increased airway responsiveness to stimuli that might otherwise disrupt their typical daily activities and reduce their work productivity. Similarly, adherence to such a guideline should also provide adequate protection against the occurrence of pulmonary function decreases in COPD patients or other individuals with already compromised lung function.

Epidemiological observations of associations between increased respiratory illness in school children and indoor and outdoor exposures to NO₂ are suggestive of human health effects associated with long-term NO₂ exposures. This is supported by animal toxicological findings showing increased susceptibility to respiratory infections and impairment of host defences as a result of subchronic or chronic exposures to NO₂ concentrations near to the ambient concentrations. However, no confident quantitative extrapolation can yet be made of these animal toxicological findings to determine comparable human exposures, nor can one confidently interpret the epidemiological findings as to whether the reported increased respiratory illness risk is associated with: (a) chronic low-level indoor NO₂ exposures; or (b) repeated higher short-term NO₂ excursions that also occur indoors during gas stove use (cooking, heating). However, a quantitative review of several large, well-conducted epidemiological studies has shown an excess of lower respiratory illness among children aged 5-12 years exposed to annual average indoor NO₂ concentrations of 38-56 µg/m³ (0.02 to 0.03 ppm).

On the basis of a background level of 15 µg/m³ (0.008 ppm) and the fact that significant adverse health effects occur with an additional level of 28.2 µg/m³ (0.015 ppm) or more, an annual guideline value of 40 µg/m³ (0.023 ppm) is proposed. This value will avoid the most severe exposures. The fact that a no-effect level for subchronic or chronic NO₂ exposure concentrations has not yet been determined should be emphasized.
There is uncertainty surrounding the lifetime effect because studies have not extended beyond individuals older than 12 years. There is no evidence for non-linearity in the concentration-response relationship below these levels. Long-term exposures of experimental animals to levels as low as 940 μg/m³ (0.5 ppm) with 1880 μg/m³ peaks for 5 days increased the mortality for infectious agents, indicating an impairment of the immune system. These animal data support the observations of increased respiratory infections seen in epidemiological studies. Chronic and subchronic exposures of experimental animals demonstrate biochemical, morphological and physiological changes at higher NO₂ levels. Continuing damage occurs as the exposure time increases suggesting cumulative effects from long-term NO₂ exposures. Although the long-term guidance value does not provide a margin of safety, this level will avoid the most severe concentrations to which children are commonly exposed.

With regard to possible health-based guidelines for other nitrogen oxides, insufficient information exists on which to base guidelines at this time. Before guidelines can be established for NO, HNO₃ and other oxidized nitrogen species, which may have important health impacts, much more information needs to be gathered in human clinical, epidemiological and experimental animal studies.
Nitrogen oxides can reach concentrations in ambient and indoor air that may affect human health. Short-term NO₂ exposure causes decreases in lung function and increased airway responsiveness. Other effects include decreases in host defences and alterations in lung cells and their activity. Long-term exposure to NO₂ is associated with respiratory illness. Individuals with asthma and chronic obstructive pulmonary disease are more susceptible than healthy individuals. Children aged 5 to 12 years constitute a subpopulation potentially susceptible to an increase in respiratory morbidity associated with NO₂ exposure.

On the basis of human controlled exposure studies on asthmatics and other high-risk groups, the recommended short-term guidance value is for a one-hour average daily maximum NO₂ concentration not exceeding 200 μg/m³ (0.11 ppm). The recommended long-term guidance value, based on epidemiological studies with increased risk of respiratory illness in children is 40 μg/m³ (0.023 ppm) as an annual average.

Limited information exists regarding the health effects of the other oxidized nitrogen species (e.g., NO, HNO₃, HNO₂) as well as of mixtures of nitrogenous air pollutants. Owing to the limited database, it is not possible to evaluate potential health risks of exposure to these compounds, even though they may be of significance.

Current total nitrogen deposition in some areas of the world is causing reduced biodiversity in ecosystems. To halt and/or reverse these trends, emissions of nitrogen must be reduced.

Gaseous nitrogen species can reduce photosynthesis and biomass and increase the sensitivity of individual plants to other stresses. The critical level for NO₂ is considered to be 30 μg/m³ as an annual average.

At the level of plant communities and ecosystems, eutrophication dominates over toxicity, with deposited total nitrogen acting as a nutrient and causing reduction in biodiversity in nutrient-limited habitats. Critical loads for the most sensitive ecosystems are estimated at 5–10 kg nitrogen per ha per year; a more average value for ecosystems is 15–20 kg nitrogen per ha per year.
Conclusions and Recommendations

Reversibility of ecosystem effects of deposited nitrogen can partly be achieved by managed techniques. In unmanaged systems, reversibility, where possible, can be a long-term or a very long-term process. In some cases where erosion and acidification are extreme, effects may be irreversible.

Nitrogen oxides act as greenhouse gases and thus contribute to global warming, which may have far-reaching effects on human health and the environment.
10. FURTHER RESEARCH

1. Further epidemiological research is required to resolve the issues of:

   a) the apparent age- and gender-related differences in NO$_2$-related health effects;
   b) the relative importance of chronic or subchronic low-level exposure and episodic high-level exposure to NO$_2$;
   c) the relative importance of NO$_2$ and fine particles to health effects of ambient air pollution;
   d) synergism between exposure to NO$_2$ and other airborne contaminants such as ozone, fine particles and bioaerosols;
   e) modification of the effect of NO$_2$ on the respiratory system owing to other environmental factors such as temperature, humidity and exposure to viral and other infectious pathogens;
   f) the significance of the observed health effects due to low-level NO$_2$ exposure for long-term health outcomes.

2. Further human controlled experimental studies are needed on:

   a) multi-hour, repeated exposure to NO$_2$ to simulate the episodic human exposures encountered both outdoors and indoors using bronchoalveolar lavage (BAL) and molecular biology analysis;
   b) respiratory responses to HNO$_2$ using BAL and molecular biology analysis to correlate respiratory changes with other biochemical end-points;
   c) respiratory and other physiological responses to high (~5 ppm) levels of NO to evaluate the effects of NO detected indoors;
   d) research to investigate the relative importance of concentration, exposure duration and minute ventilation to the health outcome.

3. Further animal studies are needed on:

   a) short- and long-term effects of NO at concentrations ranging about those found indoors, with emphasis on mechanisms of action so that the animal studies may be related to human end-points in epidemiology and controlled human exposure;
b) short- and long-term effects of HNO₂, with an emphasis on mechanism(s) of action and effects on the immune system, preferably using head or nose-only studies to reduce complications of characterization of the test atmosphere resulting from interaction of HNO₂ with surfaces;

c) identification of mechanism(s) of action NO₂ on those limited end-points now identified with human health effects (e.g., immune defences, airway activity, disease outcome, and lung growth);

d) effects of well-defined mixtures of nitrogenous air pollutants that will simulate those encountered indoors and in polluted outdoor air;

e) newer animal models of allergic disease and better diagnostic procedures for allergic disease in experimental animals should be applied to the study of nitrogenous air pollutants.

4. Further research on atmospheric chemistry regarding:

   a) exposure to potentially toxic nitrated organic compounds, including aromatic/organic nitrates and peroxyacyl nitrates;

   b) the formation, removal and human exposure pathways of HNO₂ and other potentially toxic compounds produced by the interaction of HNO₂ with other pollutants.

5. Further research on ecosystems is needed concerning:

   a) the relative roles of different deposited nitrogen species (NOₓ and NH₄⁺);

   b) quantitative data on the effects of NO on plants to establish the relative roles of the components of NOₓ;

   c) effects of nitrogen deposition on fauna;

   d) the study of ecosystems representative of tropical climates to develop estimates of critical loads relevant to a global assessment of the effects of nitrogen;

   e) effects of nitrogen deposition on montane and arctic ecosystems;

   f) effects of nitrogen deposition on aquatic ecosystems for both freshwater and estuarine/marine areas;

   g) effects of management regimes on grassland, heathland and plantation forest in relation to effects of nitrogen deposition.
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430


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505


RESUME

1. Oxydes d’azote et composés apparentés

Les oxydes d’azote peuvent être présents en quantités importantes dans l’air ambiant et dans l’air intérieur. La nature et la concentration des dérivés azotés dépendent largement du lieu, de l’heure et de la saison. Les émissions d’oxydes d’azote sont principalement imputables aux processus de combustion. Les centrales therмiques à combustibles fossiles, les véhicules à moteur et les appareils et ustensiles ménagers qui font appel à la combustion sont des sources d’oxydes d’azote, émis principalement sous la forme d’oxyde nitrique (NO) et, pour une moindre part (en général moins de 10%), de dioxyde d’azote (NO₂). Des réactions chimiques qui se produisent dans l’air conduisent à l’oxydation du NO en NO₂ et autres composés. Il existe également des processus biologiques qui provoquent la libération de dérivés azotés par le sol, notamment de l’oxyde nitréux (N₂O). Les émissions de N₂O peuvent nuire à la couche d’ozone stratosphérique.

Il peut y avoir atteinte à la santé humaine en présence de concentrations importantes de NO₂ ou d’autres espèces azotées comme le nitrate de peroxyacyle (PAN), l’acide nitrique (HNO₃), l’acide nitréux (HNO₂) et certains autres dérivés nitrés. En outre, les nitrates et l’acide nitrique peuvent, lorsqu’ils se déposent sur le sol, avoir des effets nocifs sur la santé et sur les écosystèmes.

On désigne généralement par NOₓ l’ensemble NO + NO₂. Une fois libérée dans l’air, NO est oxydé en NO₂ par les oxydants présents (en particulier l’oxygène, O₃). Dans certaines conditions, la réaction est rapide dans l’air extérieur; à l’intérieur, le processus est en général beaucoup plus lent. Les oxydes d’azote sont des précurseurs déterminants de la pollution atmosphérique par les oxydants photochimiques, qui débouchent sur la formation d’ozone et de smog; l’interaction entre les oxydes d’azote (sauf N₂O) et certaines espèces organiques réactives conduisent, sous l’action du rayonnement solaire, à la formation d’ozone dans la troposphère et de smog dans les zones urbaines.

NO et NO₂ peuvent également subir des réactions conduisant à la formation, dans l’air extérieur ou intérieur, d’autres oxydes et dérivés oxygénés de l’azote, notamment HNO₃, HNO₂, NO₃ (trioxyde d’azote), N₂O₅ (pentoxide de diazote), du nitrate de
peroxyacyl et d'autres nitrates organiques. L'ensemble des oxydes d'azote présents dans ce mélange gazeux complexe est désigné par NO₅. La proportion des oxydes d'azote dans ce mélange depend fortement de la concentration des autres oxydants et des antécédents météorologiques.

L'acide nitrique HNO₃ se forme par réaction de OH⁻ sur NO₂. C'est un important piège à azote actif et il entre également dans la composition des dépôts acides. Parmi les pièges physiques et chimiques potentiels à HNO₃, on peut citer les dépôts humides et secs, la photolyse, la réaction avec les radicaux OH ainsi que la réaction avec l'ammoniac gazeux qui conduit à la formation d'aérosols de nitrate d'ammonium.

Les nitrates de peroxyacyl se forment par la réaction de radicaux peroxy organiques sur NO₂. Le nitrate de peroxyacyl est le nitrate organique le plus abondant dans la troposphère et il peut servir de réservoir temporaire d'azote actif susceptible de déplacements régionaux.

Le radical NO₃⁻ une espèce de type NO₂ à courte vie, qui se forme dans la troposphère, principalement par réaction de O₃ sur NO₂, subit une photolyse rapide à la lumière du jour ou réagit sur NO. Sa concentration est appréciable pendant la nuit.

N₂O₅ est principalement un constituant nocturne de l'air ambiant qui se forme par réaction de NO₃⁻ sur NO₂. Dans l'air ambiant, N₂O₅ réagit en milieu hétérogène avec l'eau pour donner de l'acide nitrique qui se dépose à son tour.

N₂O est un composé ubiquitaire car il résulte de processus naturels qui se déroulent dans le sol. Toutefois, il n'est pas, autant qu'on sache, impliqué dans des réactions au sein de la troposphère. Dans la haute atmosphère, N₂O participe à des réactions qui contribuent à la réduction de la couche d'ozone stratosphérique et c'est également un gaz à puissant effet de serre qui intervient dans le réchauffement général du climat.

1.1 Transport atmosphérique

Le transport et la dispersion des diverses espèces azotées dans la base atmosphère dépend de paramètres chimiques et météorologiques. Des processus tels que l'advection, la diffusion et les transformations chimiques se combinent pour déterminer la durée de leur séjour dans l'atmosphère. La durée de séjour dans
l'atmosphère détermine à son tour l'ampleur du déplacement de tel ou tel composé. Les émissions de surface se dispersent verticalement et horizontalement sous l'action de processus turbulents qui dépendent largement du gradient vertical de température et de la vitesse du vent.

Par suite des processus météorologiques, les NOx émis en ville dans les premières heures de la matinée subissent une dispersion verticale caractéristique et se déplacent avec le vent au fil de la journée. Pendant les journées ensoleillées d'été, la majorité des NOx auront été transformées en HNO3 et en PAN lorsqu'arrivera le crépuscule, avec formation concomitante d'ozone. L'acide nitrique s'évacue en grande partie par dépôt lors du déplacement de la masse d'air, mais le HNO3 et le PAN entrainés avec les couches supérieures (au-dessus de et la couche d'inversion nocturne mais au-dessous de l'inversion de subsidence à altitude plus élevée) peuvent être transportés sur de grandes distances par des masses d'air chargées d'oxydants.

1.2 Dosage

On dispose d'un certain nombre de méthodes pour le dosage des dérivés azotes aéroportés. Le présent document donne un aperçu des méthodes actuelles généralement utilisées pour la surveillance in situ de leur concentration, tant dans le milieu ambiant que dans l'air intérieur. Les dérivés envisagés sont NO, NO2, NOx, l'azote réactif non usuel total (NOx), le PAN et autres nitrates organiques, HNO3, HNO2, N2O5, NO3 et N2O.

Le dosage des oxydes d'azote n'a rien d'évident. Il existe certes une méthode simple, praticable un peu partout, pour le dosage de NO (réaction de chimiluminescence avec l'ozone), mais il s'agit là d'une exception. La chimiluminescence est également la méthode la plus couramment utilisée pour NO2 (que l'on réduit préalablement en NO). Malheureusement, le catalyseur utilisé pour la réduction n'est pas spécifique et il est d'une efficacité variable selon l'oxyde d'azote à réduire. Dans ces conditions, il faut être très prudent lorsque l'on interprète les résultats d'un dosage de NO2 par cette méthode car le signal peut correspondre en fait à la superposition des signaux de nombreux autres produits. En outre, des difficultés supplémentaires peuvent survenir du fait de la répartition des oxydes d'azote entre la phase gazeuse et la phase particulaire, tant dans l'atmosphère qu'au cours du prélèvement des échantillons.
1.3 Exposition

L'exposition humaine et environnementale aux oxydes d'azote varie beaucoup selon qu'il s'agit de l'air intérieur ou extérieur, d'une zone urbaine ou rurale, ou encore en fonction de l'heure ou de la saison. On connaît relativement bien la concentration de NO et de NO₂ dans l'air extérieur qui caractérise certaines situations urbaines. À l'intérieur, la concentration de ces composés dépend de la nature exacte des appareils domestiques de chauffage ou de cuisson ou encore des cheminées et de la ventilation. En cas d'utilisation d'appareils de chauffage ou de cuisson à combustion dans des locaux non ventilés, la concentration des oxydes d'azote dans l'air intérieur se caractérise par des valeurs beaucoup plus élevées qu'à l'extérieur. Des travaux récents ont montré que dans ces conditions, la concentration de HNO₂ peut être élevée. C'est ainsi qu'il a été montré que la concentration de HNO₂ peut représenter plus de 10% de la concentration totale en oxydes d'azote (généralement indiquée en NO₂).

2. Effets des dérivés azotés présents dans l'atmosphère, et notamment des oxydes d'azote, sur la végétation

C'est dans les écosystèmes (semi-)naturels aquatiques et terrestres que la biodiversité se manifeste la plupart du temps dans sa plénitude. Dans nombre de ces écosystèmes, l'azote est un nutriment qui joue le rôle de facteur limitant pour la croissance des végétaux. La plupart des espèces végétales qui peuplent ces biotopes sont adaptées à un faible apport de nutriments et la compétition avec d'autres plantes ne peut leur être favorable que sur des sols pauvres en azote.

L'activité humaine, qu'elle soit agricole ou industrielle, a eu pour conséquence d'accroître considérablement la quantité de dérivés azotés biodisponibles, perturbant ainsi le cycle naturel de l'azote. Les polluants atmosphériques azotés existent sous diverses formes: les principales sont NO, NO₂ et l'ammoniac (NH₃) en dépôt sec; les nitrates (NO₃⁻) et les sels d'ammonium (NH₄⁺) en dépôt humide. Il peut également y avoir des dépôts occultes provenant de brouillards ou de nébuleuses diverses. En fait, les polluants atmosphériques azotés sont bien plus nombreux (par exemple, N₂O₅, le PAN, N₂O, des amines etc.) Mais nous n'en tiendrons pas compte ici, soit parce qu'ils ne contribuent, semble-t-il, que trop peu aux dépôts azotés, soit parce que leur concentration est probablement très inférieure au seuil d'apparition des effets.
Les polluants atmosphériques azotes peuvent nuire à la végétation, soit indirectement par l'intermédiaire de produits de réaction photochimiques, soit directement par dépôt sur les plantes, le sol ou l'eau. La voie de contamination indirecte n'est guère abordée dans le présent document, encore que les processus qui y sont à l'œuvre soient tout à fait intéressants et méritent d'être pris en considération lors de l'évaluation de l'impact global des polluants atmosphériques azotés: le NO$_2$ est un précurseur de l'ozone troposphérique qui agit à la fois comme phytotoxine et comme gaz à effet de serre.

L'impact d'un dépôt accru de dérivés azotés sur les systèmes biologiques peut résulter, soit d'une fixation directe de ces produits par le feuillage, soit d'un captage au niveau du sol. Pour ce qui est de la plante elle-même, les effets les plus significatifs sont des lésions tissulaires, une modification de la biomasse et une sensibilité accrue aux facteurs secondaires de stress. En ce qui concerne la végétation dans son ensemble, l'azote ainsi déposé joue le rôle d'un nutriment; il en résulte une modification des conditions de compétition entre les différentes espèces et une diminution de la biodiversité. La valeur critique de la charge azotée dépend i) de la nature de l'écosystème; ii) de l'exploitation et de l'aménagement passés et présents des sols; et iii) des conditions abiotiques du lieu (en particulier celles qui influent sur la capacité de nitrification et le taux d'immobilisation dans le sol).

L'adsorption de dérivés azotés à la surface de la feuille peut endommager la couche cireuse de la cuticule, mais on n'a pas encore la preuve que cela soit quantitativement important sur le terrain. L'existence d'un gradient de concentration entre l'atmosphère et le mesophylle favorise la fixation des NO$_2$ et de l'ammoniac. Cette fixation est généralement, mais pas systématiquement, directement liée à la conductance des stomates et dépend donc des facteurs qui en conditionnent l'ouverture. On est de plus en plus fondé à penser que la fixation de l'azote par les feuilles réduit sa fixation par les racines. La fixation et l'échange d'ions à la surface de la feuille sont des processus relativement lents et qui ne peuvent donc prendre de l'ampleur que si la surface foliaire reste humide suffisamment longtemps.

NO n'est que légèremment soluble dans l'eau, mais la présence d'autres substances peut en modifier la solubilité. NO$_2$ est davantage soluble et NH$_3$ beaucoup plus. NO$_2$ (principal produit de réaction des NO), NH$_3$ et NH$_4^+$ sont tous très phytotoxiques et peuvent très bien être à l'origine des effets nocifs provoqués par
Résumé

les polluants atmosphériques azotés. Le radical libre •N=O peut jouer un rôle dans la phytotoxicité de NO.

Des effets dépassant la simple additivité (synergie) ont été observés dans presque toutes les études relatives à SO₂ en présence de NO₂. Dans le cas des autres mélanges contenant NO₂, par exemple en présence de NO, O₃ et CO₂, les effets interactifs sont l'exception plutôt que la règle.

Lorsque conditions climatiques et apport d'autres nutriments permettent la production de biomasse, les NO₃ et NH₄ ont pour effet de stimuler la croissance à faible concentration et de la réduire à concentration élevée. Toutefois la concentration à partir de laquelle la stimulation se change en inhibition est beaucoup plus faible dans le cas de NO₃ que dans celui de NH₄.

On a pu constater que les plantes sont plus sensibles lorsque l'intensité lumineuse est faible (par exemple la nuit ou en hiver) et la température basse (juste au-dessus de 0 °C). NO₃ et NH₄ peuvent accroître la sensibilité des végétaux au gel, à la sécheresse, au vent et aux ravageurs.

Il existe une corrélation entre la chimie du sol et la sensibilité de la végétation aux dépôts de composés azotés; cette dernière dépend en effet du pH et de la disponibilité de l'azote.

La contribution relative de NO et de NO₂ aux effets des NOₓ sur les plantes n'est pas connue avec certitude. La très grande majorité des données dont on dispose concerne les effets de NO₂, mais ce que l'on sait de NO incite à penser que NO et NO₂ ont une action phytotoxique comparable.

Les valeurs-guides pour la qualité de l'air sont basées sur la notion de seuil d'apparition d'effets indésirables. On distingue deux types de seuils: les niveaux critiques (CLE) et les charges critiques (CLO). Par niveau critique, on entend la concentration d'un polluant atmosphérique à partir de laquelle des effets indésirables directs peuvent, selon nos connaissances actuelles, se produire sur certains récepteurs, qu'il s'agisse de plantes, d'écosystèmes ou de matériaux. Par charge critique, on entend la valeur estimative de l'exposition (dépôt) à un ou plusieurs polluants au-dessous de laquelle il ne se produit pas, autant qu'on sache, d'effets délétères sur les éléments sensibles de l'environnement.
Dans la pratique, on obtient les niveaux critiques en déterminant, par une méthode graphique, la concentration la plus faible qui provoque un effet indésirable sur les fonctions physiologiques ou la croissance des végétaux (en excluant les effets biochimiques).

Pour tenir compte des effets dus à NO, on a proposé un niveau critique pour NO₂ plutôt que pour NO₂ à cette fin on a posé que, par hypothèse, les effets de NO et ceux de NO₂ ne sont pas additifs. L'établissement de niveaux critiques pour une exposition de breve durée est tout à fait défendable, mais on ne possède pas actuellement de données en nombre suffisant pour proposer des valeurs de bonne fiabilité. Les résultats dont on dispose conduisent à proposer un niveau critique pour NO, d'environ 75 μg/m³ en moyenne sur 24 h.

On estime à 30 μg/m³ en moyenne annuelle le niveau critique pour NO₂ (NO et NO₂ en parties par milliard exprimés sous forme de NO₂ en μg/m³).

Les données relatives aux biotes présents dans l'environnement concernent presque exclusivement les plantes, avec un minimum de renseignements sur la faune terricole. C'est pourquoi les valeurs-guides qui sont proposées ici se rapportent aux effets des divers dérivés azotés sur la végétation. On pense toutefois que la végétation est l'élément le plus fragile des écosystèmes naturels et que l'effet sur la biodiversité végétale est un indicateur sensible des effets exercés sur l'ensemble des écosystèmes.

Les charges critiques s'obtiennent à partir de données expérimentales et de modèles pédologiques stationnaires. On trouvera dans la présente évaluation la valeur estimative de la charge critique pour les dépôts azotés sur divers écosystèmes terrestres et aquatiques. On ne connait pas suffisamment bien les effets imputables aux différentes spécies chimiques (NO₂ et NH₃) pour pouvoir différencier ces différents composés par rapport à leur charge critique.

Les écosystèmes sur lesquels on possède suffisamment de données pour établir des charges critiques sont en grande majorité situés dans la zone tempérée.

Les quelques écosystèmes arctiques ou montagnards qui figurent dans ce groupe et dont on pourrait attendre qu'ils soient représentatifs de la situation aux latitudes élevées, constituent en
Résumé

fait la base de données la moins fiable. On ne sait rien des écosystèmes tropicaux et pas grand chose des écosystèmes marins ou estuariens, quelle que soit la zone climatique où ils se situent. Les écosystèmes tropicaux pauvres en nutriments, comme la forêt ombrophile et les mangroves auraient probablement à souffrir des dépôts de dérives azotés. En l'absence de données sur ces dépôts et sur les seuils d'apparition des effets, il est impossible de se livrer à une évaluation du risque dans ces zones climatiques.

Dans les écosystèmes les plus fragiles (marais ombrotrophiques, lacs aux eaux douces et peu profondes, hautesurs arctiques et alpines) où l'on a pu évaluer la charge critique, on a obtenu des valeurs de l'ordre de 5-10 kg N. ha⁻¹.anneé. Ces évaluations sont basées sur la diminution de la diversité biologique de la végétation. On a obtenu la valeur plus moyenne de 15-20 kg N. ha⁻¹.anneé pour les quelques écosystèmes étudiés, valeur qui s'applique aux arbres des forêts.

La chimie atmosphérique des oxydes d'azote concerne leur action sur la capacité de régénération de l'ozone troposphérique et sur la réduction de la couche d'ozone stratosphérique ainsi que leur contribution au réchauffement général de la planète par effet de serre. Avec l'ammoniac et les oxydes de soufre, ils contribuent à l'acidification des sols et augmentent par conséquent la biodisponibilité de l'aluminium.

Lorsque leur concentration ne dépasse que marginalement le niveau critique, les oxydes d'azote n'exercent sur les récoltes que des effets phytotoxiques négligeables. Il n'empêche que par leur action sur la formation d'ozone et d'autres substances phytotoxiques dans la troposphère, comme les nitrates organiques par exemple, les NO₅ peuvent causer des dommages aux récoltes. Les dérivés azotés déposés sur les plantes en culture ne représentent qu'une partie infime de l'azote disponible total, comparativement à l'apport d'azote par les engrais.

3. Effets sanitaires de l'exposition aux oxydes d'azote

On a effectué de nombreuses études dans le but d'évaluer les effets des NO₅ sur la santé. L'un de ces composés, NO₂, a été extrêmement étudié. Dans ce qui suit, on s'attache principalement à NO₅, NO, HNO₃ et HNO₅, sans trop s'attarder sur les nitrates.
3.1 Études sur les effets des dérivés azotés chez les animaux de laboratoire

L'extrapolation à l'homme des résultats obtenus chez l'animal de laboratoire comporte des aspects qualitatifs et des aspects quantitatifs. Comme on l'explique succinctement dans ce qui suit, NO₂ exerce toute une gamme d'effets chez plusieurs espèces animales, en particulier, il affecte les défenses de l'hôte contre les pneumopathies infectieuses et peut modifier la biochimie et le métabolisme pulmonaires ainsi que la structure et la fonction de l'appareil respiratoire. Du fait de l'existence d'analogies structurales et métaboliques chez tous les mammifères, qu'il s'agisse de l'homme ou des animaux de laboratoire, le fait de retrouver chez plusieurs espèces animales a peu près les mêmes résultats conduit à conclure que, selon toute vraisemblance, NO₂ produit les mêmes effets chez l'homme. Toutefois, en raison des différences qui existent malgré tout entre les espèces mammaliennes, on ne peut pas dire avec certitude quel effet telle ou telle exposition produirait sur l'homme. C'est là le domaine de l'extrapolation quantitative. Les quelques recherches qui ont été consacrées à la modélisation dosimétrique de l'extrapolation quantitative (c'est-à-dire la détermination de la dose au tissu ou à la cellule cibles qui produit effectivement un effet toxique), incitent à penser que de NO₂ se repartit de façon similaire dans les voies respiratoires de l'homme et des animaux, sans toutefois que l'on puisse en tirer des valeurs extrapolables de l'animal à l'homme. On ne dispose malheureusement que de très peu de données sur un autre aspect fondamental de l'extrapolation, à savoir la sensibilité selon l'espèce (c'est-à-dire la réaction des tissus à une dose donnée chez les différentes espèces). Ainsi, nous savons, grâce à ces études sur l'animal, quels effets NO₂ est susceptible de produire chez l'homme, mais nous ne sommes pas pour autant en mesure de déterminer de manière fiable quels effets telle ou telle dose inhalée de NO₂ produit effectivement.

Compte tenu de ce qui vient d'être dit, on trouvera ci-dessous une récapitulation de la base de données toxicologiques relative à NO₂ par centres d'intérêt et par principaux types d'effets. Il est certain que les effets de NO₂ ne sont pas strictement localisés aux poumons, mais l'interprétation de ces effets généraux eu égard au risque qu'ils représentent pour l'homme, demeure incertaine. Ils ne sont donc pas évoqués dans ce qui suit, mais abordés dans les chapitres suivants. Les interactions qui peuvent se produire entre NO₂ et d'autres polluants comme l'ozone ou l'acide sulfurique (H₂SO₄) sont d'une grande importance, en particulier en cas de...
synergie, mais au stade actuel, la base de données ne permet pas
de tirer des conclusions qui conduiraient à évaluer la possibilité de
telles interactions en situation réelle.

3.1.1 Mode d'action des oxydes d'azote au niveau cellulaire et biochimique

NO₂ se comporte comme un puissant oxydant. Il oxyde facilement les lipides insaturés en donnant principalement naissance à des peroxydes. L'acide ascorbique (vitamine C) et l'α-tocophérol (vitamine E) inhibent tous deux la peroxydation des lipides insaturés. Lorsque l'acide ascorbique est emprisonné dans une double couche liposomique, il est rapidement oxydé par le NO₂. L'effet protecteur de l'α-tocophérol et de l'acide ascorbique chez l'homme et l'animal est dû à l'inhibition de l'oxydation par le NO₂. NO₂ oxyde également les protéines membranaires. L'oxydation des lipides ou des protéines membranaires conduit à la disparition du mécanisme de régulation de la perméabilité cellulaire. Dans la lumière pulmonaire des sujets humains et des animaux de laboratoire exposés au NO₂, on constate la présence d'une plus grande quantité de protéines. Ces phénomènes sont à l'origine du recrutement de cellules inflammatoires et des altérations qui se produisent au niveau pulmonaire.

Les propriétés oxydantes de NO₂ mettent en action différentes voies de detoxication: la voie de la glutathion-peroxydase, celle de la glutathion-réductase et celle de la glucose-6-phosphate déshydrogénase. Après exposition au NO₂, la montée de la voie de detoxication peroxydique suit une relation de type dose-réponse.

Le mode d'action du NO n'est pas aussi clair. Il y a d'abord oxydation en NO₂ avant que n'intervienne la peroxydation. En cas d'exposition à NO, il y a toujours une certaine exposition à NO₂ qui se produit simultanément de sorte qu'il est difficile de démêler les effets imputables à chacun des composés. NO se comporte comme un second messager intracellulaire qui module toutes sortes d'enzymes essentielles et qui, par rétroaction negative, inhibe sa propre production. NO active la guanilate-cyclase qui accroît à son tour la concentration intracellulaire de cGMP. Quant aux nitrates, il est possible qu'ils agissent en libérant l'histamine présente dans les granules des mastocytes. Les polluants atmosphériques acides constitués de dérivés azotes, en particulier HNO₃, pourraient agir en modifiant le pH intracellulaire.

Le PAN se décompose dans l'eau en donnant de l'eau oxygénée (peroxyde d'hydrogène). On sait très peu de chose sur son mode
d'action, mais il est probable qu'il agit, comme ses congénères, en provoquant un stress oxydatif.

Il se pourrait, comme on l'a d'ailleurs indiqué plus haut, que l'action des nitrates inorganiques consiste à modifier le pH intracellulaire. L'ion nitrate est transporté dans les cellules alvéolaires de type 2 dont il provoque l'acidification. Il mobilise également l'histamine des mastocytes. HNO₂ pourrait également modifier le pH intracellulaire, mais son mode d'action n'est pas encore vraiment élucidé.

Le mode d'action des autres oxydes d'azote n'est pas connu.

Une exposition aiguë à NO₂ à la concentration de 750 μg/m², soit 0,4 ppm, peut provoquer la peroxydation des lipides. NO₂ peut oxyder les lipides insaturés qui entrent dans la composition de la membrane cellulaire ainsi que les groupes fonctionnels de protéines, par exemple, de protéines solubles présentes à l'intérieur de la cellule, comme les enzymes, ou encore de protéines de structure, comme les protéines membranaires. Ces réactions d'oxydation (qui s'effectuent par l'intermédiaire de radicaux libres) sont le mécanisme par lequel NO₂ exerce son action toxique sur les cellules pulmonaires. A l'appui de l'existence de ce mode d'action, on peut citer des études sur animaux de laboratoire qui montrent l'importance des défenses antioxydantes du poumon, qu'elles soient endogènes (par exemple, maintien d'un taux suffisant de glutathion intrapulmonaire) ou exogènes (par exemple, apport alimentaire de vitamines C et E), dans la protection contre les effets de NO₂. Selon de nombreuses études, les diverses enzymes pulmonaires, et notamment la glutathion-peroxydase, la superoxyde-dismutase et la catalase, pourraient également avoir pour rôle de protéger le poumon contre les attaques oxydantes.

3.1.2 Effets sur les défenses de l'hôte

Bien que la fonction essentielle de l'arbre respiratoire soit d'assurer des échanges gazeux efficaces, cet organe constitue également la première ligne de défense de l'organisme contre les agents aéroportés, viables ou non, qu'inhalé le sujet. Une abondante base de données montre que l'exposition à NO₂ peut entraîner la perturbation de ces défenses et, par voie de conséquence, une plus grande sensibilité aux affections respiratoires d'origine infectieuse. Parmi les éléments de ces défenses qui peuvent être affectés par NO₂, figurent notamment l'activité biochimique et fonctionnelle de certaines cellules.
Résumé

pulmonaires, les macrophages alvéolaires, l'immunocompétence, la sensibilité aux infections respiratoires expérimentales et la vitesse d'élimination par l'ascenseur mucociliaire.

NO₂ s'attaque aux macrophages alvéolaires. Ces cellules ont pour fonction de maintenir la stérilité de la région pulmonaire en en éliminant les particules étrangères et en assurant également des fonctions immunologiques. Parmi les altérations fonctionnelles qui ont été relevées on peut citer les suivantes: suppression de la capacité de phagocytose et stimulation de la clairance pulmonaire à la dose de 560 μg/m³ (0,3 ppm) 2 h par jour pendant 13 jours; diminution de l'activité bactéricide à la dose de 4320 μg/m³ (2,3 ppm) pendant 17 h; affaiblissement de la réponse au facteur d'inhibition de la migration à la dose de 3760 μg/m³ (2,0 ppm) 8 h par jour, 5 jours par semaine, pendant 6 mois. L'exposition prolongée des macrophages à NO₂ provoque une modification morphologique de ces cellules.

L'importance des défenses de l'hôte saute aux yeux lorsqu'on observe des animaux de laboratoire porteurs d'infections respiratoires expérimentales. La mortalité des animaux exposés à NO₂ et qui succombent à l'infection bactérienne ou virale, dépend de la dose. La mortalité augmente également à mesure qu'augmente la concentration de NO₂ ou la durée de l'exposition. En cas d'exposition aiguë, on observe des effets dès la dose de 3760 μg/m³ (2 ppm). Sur modèle d'infectiosité, on constate des effets dans les 6 mois suivant l'exposition à une dose ne dépassant pas 940 μg/m³ (0,5 ppm).

L'exposition à NO₂ affecte les défenses humorales comme les défenses à médiation cellulaire. Dans les cas où l'on a étudié le comportement du système immunitaire, on a pu observer des effets après une exposition de courte durée à des concentrations supérieures ou égales à 9400 μg/m³ (5 ppm). Les effets sont complexes car le sens de la modification (augmentation ou diminution) dépend de la concentration de NO₂ et de la durée de l'exposition.

3.1.3 Effets d'une exposition prolongée sur l'apparition d'une pneumopathie chronique

L'homme est exposé en permanence au NO₂. C'est pourquoi ce type d'exposition a été assez largement étudié chez l'animal en ayant recours à des méthodes morphologiques ou morphométriques. En règle générale, ce genre de travaux montre que
diverses modifications de structure, avec leurs corrélats fonctionnels, se produisent au niveau pulmonaire. Certaines de ces modifications peuvent se révéler reversibles lorsque cesse l'exposition.

Chez l'animal de laboratoire, une exposition chronique au dioxyde d'azote peut entraîner une altération de la fonction respiratoire. Après exposition à du dioxyde d'azote pendant 4 mois à la dose de 7520 µg/m³, soit 4,0 ppm, on a observé une détérioration des échanges gazeux qui se traduisait par une réduction de la pression partielle d'oxygène dans le sang artériel, une diminution de la condition physique et une augmentation du métabolisme anaérobie.

Il est certain que le dioxyde d'azote provoque des modifications morphologiques au niveau des voies respiratoires, mais il peut arriver que la base de données soit un peu trompeuse sur ce point en raison des variations qualitatives et quantitatives qui se manifestent dans la sensibilité des différentes espèces, voire à l'intérieur d'une même espèce. Le rat, qui est l'animal le plus fréquemment utilisé pour l'évaluation de l'exposition sur la base des modifications morphologiques, se révèle relativement résistant au NO₂. Une exposition de brève durée à des concentrations de 9400 µg/m³ (5,0 ppm) ou moins, n'a généralement guère d'effets sur le rat, alors que dans les mêmes conditions le cobaye présente des lésions de l'épithélium centroacinaire.

Une exposition de plus longue durée peut, chez certaines espèces, provoquer des lésions à des doses ne dépassant pas 560 à 940 µg/m³ (0,3 à 0,5 ppm). Elles se caractérisent par un remodelage de l'épithélium similaire à celui qui a été décrit plus haut, mais avec extension aux voies aériennes proximales et épaissement de tissu interstitiel. Toutefois, nombre de ces altérations finissent par disparaître, même si l'exposition se poursuit, et il faut que celle-ci se situe au moins à 3760 µg/m³ (2,0 ppm) pour que des dommages plus étendus et plus persistants se produisent au niveau des poumons. Certains effets sont relativement persistants, (par exemple, la bronchiolite) alors que d'autres manifestent une tendance à la reversibilité et sont limités, même si l'exposition se poursuit. De toute façon, il semble que la réponse soit davantage liée à la dose qu'à la durée - brève ou longue - de l'exposition. On a de bonnes raisons de penser qu'une exposition de longue durée à de fortes concentrations de NO₂ provoque, chez plusieurs espèces animales, des lésions affectant la morphologie pulmonaire. La destruction de la paroi alvéolaire, qui
constitue un critère supplémentaire essentiel d'émphyème chez l'homme, a été constatée quelquefois à l'occasion d'études tout à fait dignes de foi effectuées sur l'animal. Ces résultats ne permettent toutefois pas de déterminer quelle est la concentration de NO₂ la plus faible à partir de laquelle apparaissent des lésions pulmonaires emphysémateuses.

3.1.4 Effets cancérogènes ou co-cancérogènes potentiels

On a montré que NO₂ était mutagène pour les salmonelles, mais une étude indique qu'il ne l'est pas pour des cellules mammaliennes en culture. D'autres travaux sur cultures cellulaires ont montré l'existence d'échanges entre chromatides sœurs ainsi que des ruptures au niveau d'un des brins de l'ADN. Aucun effet génotoxique n'a été mis en évidence in vivo dans les lymphocytes, les spermatocytes ou les cellules de la moelle osseuse, mais deux études au cours desquelles on a fait inhaler pendant 3 h ou 6 h (aux doses respectives de 50 760 et 56 400 µg/m³, soit 27 et 30 ppm) le produit à des animaux, ont révélé la présence de tels effets dans les poumons.

Les études bibliographiques qui ont été effectuées sur ce sujet n'ont pas révélé l'existence de travaux comportant une étude toxicologique classique sur l'animal avec exposition de longue durée, dans le but d'étudier le pouvoir cancérigène du NO₂. Les études effectuées sur des souris présentant un taux élevé de tumeurs spontanées, n'ont fourni que des résultats équivoques. Dans une étude, on a observé qu'à la concentration de 18 800 µg/m³ (10 ppm) le NO₂ augmentait légèrement l'incidence des adénomes pulmonaires chez une souche de souris sensibles (A/J). On a bien effectué un certain nombre d'études de co-cancérigénicité, mais des problèmes de méthodologie et d'interprétation empêchent d'en tirer des conclusions. Quant à savoir si l'exposition au NO₂ rend les tumeurs pulmonaires plus aptes à métastasier, les études qui ont été consacrées à ce problème ne permettent guère de conclure. Dans d'autres études, on s'est attaché à rechercher si l'exposition au NO₂ pouvait entraîner la formation de nitrates ou de nitrites susceptibles de donner naissance à des nitrosamines par réaction sur les amines présentes dans l'organisme. Certains résultats donnent à penser que des nitrosamines se forment chez les animaux exposés au NO₂ auxquels on administre des amines à haute dose, mais d'autres travaux montrent en revanche que la formation de nitrosamines est improbable.
3.1.5 Sensibilité en fonction de l'âge

Les travaux consacrés à cette question sont insuffisants et les résultats obtenus jusqu'ici sont équivoques.

3.1.6 Influence des modalités de l'exposition

Un certain nombre d'études toxicologiques ont permis d'expliciter les relations entre la concentration C et la durée T de l'exposition. Ces relations se révèlent complexes. La plupart des travaux utilisent le modèle d'infectiosité. Les premières études consacrées à la relation Effet = \( f(C,T) \), ont montré que la concentration avait davantage d'influence sur la mortalité que la durée de l'exposition. Les relations Effet = \( f(C,T) \) ne permettent pas d'évaluer la toxicité de NO\(_2\).

3.2 Exposition contrôlée aux oxydes d'azote: études sur l'homme

On a étudié les réactions humaines à divers dérivés oxygénés de l'azote. La base de données la plus abondante et la mieux adaptée à l'évaluation du risque est celle qui a été établie à partir des résultats d'expositions contrôlées au NO\(_2\). La base de données sur les réactions de l'organisme humain à une exposition à NO, HNO\(_3\) et HNO\(_2\) en phase vapeur et à divers nitrates inorganiques sous forme d'aérosols, n'est pas aussi fournie. On a examiné un certain nombre de sous-groupes sensibles ou potentiellement sensibles, notamment des adolescents et des adultes asthmatiques, ainsi que des adultes d'âge mûr atteints d'une pneumopathie obstructive chronique et d'hypertension pulmonaire. On a constaté que lorsque l'exposition à ces composés s'accompagne d'un exercice physique, il y a accroissement de leur absorption et modification de leur répartition à l'intérieur du poumon. La proportion relative de NO\(_2\) déposé dans les voies respiratoires inférieures est également augmentée par l'exercice physique. Chez les personnes qui s'adonnent à une activité physique tout en étant exposées à des dérivés oxygénés de l'azote, les effets de ces composés peuvent donc se trouver accrus.

Comme chaque fois que l'organisme humain est exposé par la voie respiratoire à des gaz ou à des particules, sa réponse biologique au NO\(_2\) se caractérise par une certaine variabilité. Les sujets en bonne santé ont tendance à moins réagir aux effets du NO\(_2\) que les individus atteints d'une pneumopathie. Il est certain que les asthmatiques constituent le groupe le plus sensible au NO\(_2\) qui ait été étudié jusqu'ici. Les sujets atteints d'une pneumopathie...
obstructive chronique pourraient être plus sensibles que les sujets sains, mais comme leur capacité de réaction au NO\textsubscript{2} est limitée, il est difficile de procéder à une évaluation quantitative. On ne possède pas suffisamment de données pour déterminer si l'âge et le sexe jouent un rôle dans la réaction au NO\textsubscript{2}.

Un sujet normal peut déceler l'odeur du NO\textsubscript{2}, quelquefois à une concentration inférieure à 188 µg/m\textsuperscript{3} (0,1 ppm). D'une façon générale, l'exposition au NO\textsubscript{2} n'a provoqué aucune augmentation des symptômes respiratoires chez les sujets étudiés.

Le NO\textsubscript{2} entraîne une réduction de la fonction pulmonaire et en particulier, une augmentation de la résistance des voies aériennes chez le sujet sain au repos exposé pendant 2 h à une concentration de 4700 µg/m\textsuperscript{3} (~ 2,5 ppm). Les données disponibles sont insuffisantes pour permettre d'expliciter la relation concentration-réponse.

L'exposition pendant 1 h ou plus à une concentration de NO\textsubscript{2} ne dépassant pas 2800 µg par m\textsuperscript{3}, c'est-à-dire ~ 1,5 ppm, rend les voies aériennes plus sensibles aux agents bronchoconstricteurs chez les sujets sains non fumeurs pratiquant une activité physique.

Chez les asthmatiques exposés au NO\textsubscript{2}, on observe, du moins chez certains d'entre eux, une augmentation de la sensibilité des voies aériennes à divers agents, en particulier des substances cholinergiques et des antihistaminiques, ou encore au SO\textsubscript{2} ou à l'air froid. Les réactions de ce type semblent dépendre du protocole expérimental, et notamment de la présence ou de l'absence d'une activité physique pendant l'exposition. Elles peuvent se produire à des concentrations ne dépassant pas 380 µg/m\textsuperscript{3} (0,2 ppm). Lorsqu'on soumet ces résultats à une méta-analyse, on est amené à penser que les réactions précitées peuvent se produire à des concentrations encore plus faibles. On a cependant constaté l'existence d'une relation concentration-réponse indiscutable entre 350 et 1150 µg/m\textsuperscript{3} (~ 0,2 à 0,6 ppm).

On ne voit pas très bien ce que signifie cette tendance générale, mais une sensibilité accrue des voies aériennes pourrait entraîner une exacerbation des réactions aux allergènes ou l'aggravation temporaire d'un asthme, avec pour conséquences une augmentation de la consommation de médicaments, voire même des hospitalisations.
Chez les malades porteurs d'une pneumopathie obstructive chronique, on peut observer une augmentation modérée de la résistance des voies aériennes après une breve exposition (15-60 min) à des concentrations de NO₂ ne dépassant pas 2800 µg/m³ (~1.5 ppm) et une diminution des valeurs spirométriques peut également s'observer dès que la concentration atteint 600 µg/m³ (~0.3 ppm) sur 3 h : le volume maximal expire en une seconde (VEMS) est en baisse de 3 à 8%.

L'exposition à des concentrations de NO₂ dépassant 2800 µg/m³ (~1.5 ppm) peut modifier le nombre et le type des cellules inflammatoires présentes dans la partie distale des voies aériennes et des alvéoles. Ce gaz peut également perturber le fonctionnement des cellules intrapulmonaires ainsi que la production de médiateurs susceptibles de jouer un rôle important dans les défenses pulmonaires. Cet ensemble de perturbations intéressant les défenses de l'hôte, la modification des cellules pulmonaires et l'altération de leurs fonctions, de même que les anomalies affectant la production de certains médiateurs biochimiques, correspondent bien aux résultats des études épidémiologiques, à savoir que l'exposition au NO₂ accroît la sensibilité des voies respiratoires du sujet.

D'après des études portant sur des mélanges de polluants contenant du NO₂, il ne semble pas que la présence de NO₂ accroisse les réactions aux autres polluants au-delà de ce qui serait observé en présence de ces polluants seuls. Il y a toutefois une exception notable, à savoir le fait qu'une exposition préalable à ce gaz rend les voies aériennes encore plus sensibles à l'ozone, comme on a pu le constater chez des sujets sains exerçant une activité physique en présence de NO₂, puis exposés à de l'ozone. Cette observation incite à penser que la réponse au NO₂ peut être retardée ou persistante.

Si l'on considère l'intervalle de concentration pour lequel il serait intéressant d'évaluer le risque que représente une exposition au NO₂ (c'est-à-dire 100-600 µg/m³), on constate que les données disponibles ne permettent pas d'établir une relation concentration-réponse concernant divers symptômes et notamment les effets aigus sur la fonction pulmonaire ou sur la sensibilité des voies aériennes aux agents bronchoconstricteurs.

En se basant sur l'effet constaté à 400 µg/m³ et la possibilité d'effets à concentration plus faible telle qu'elle ressort d'une meta-analyse des données, on recommande de prendre comme valeur-
guide de la concentration moyenne maximale journalière de NO₂ sur 1 h, le chiffre de 200 µg/m³ (~0,11 ppm).

Il est admis que le NO joue un rôle important comme deuxième messager au sein de divers organes. Lorsqu’il est inhalé à une concentration supérieure à 6000 µg/m³ (~5 ppm), il peut provoquer une vasodilatation des vaisseaux pulmonaires qui ne s’étend pas à la circulation générale. On n’a pas établi quelle est la concentration minimale capable de produire cet effet. Pour le moment, les données dont on dispose sur les effets qu’une exposition au NO serait susceptible d’avoir sur la fonction et les défenses pulmonaires sont trop limitées pour qu’on puisse en tirer la moindre conclusion. Des concentrations relativement élevées ont été utilisées en clinique (> 40 000 µg/m³) pendant de courtes périodes (< 1 h) sans que l’on n’observe d’effets indésirables.

Dans l’intervalle de concentration de 250-500 µg/m³ (97-194 parties par milliard), l’acide nitrique peut avoir des effets indésirables sur la fonction pulmonaire chez l’asthmatique adolescent mais pas chez l’adulte en bonne santé.

Les données limitées dont dispose sur HNO₂ incitent à penser que cet acide peut provoquer une inflammation oculaire à la concentration de 760 µg/m³ (0,40 ppm). Rien n’a été publié jusqu’ici sur la manière dont le poumon humain réagit à une exposition à HNO₂.

Les données relatives aux nitrates organiques sont également limitées et indiquent que sous la forme d’aérosols, ces composés n’ont pas d’effets sur la fonction pulmonaire à des concentrations inférieures ou égales à 7000 µg/m³.

3.3 Études épidémiologiques sur le dioxyde d’azote

Les études épidémiologiques consacrées aux effets des oxydes d’azote portent essentiellement sur le NO₂. Nombre d’entre elles ont été menées en extérieur ou en intérieur afin de déterminer la nature des effets de ce composé sur la santé humaine. Deux types d’effets sanitaires sont généralement pris en considération pour l’étude de l’exposition au NO₂, à savoir le retentissement sur la fonction pulmonaire et les affections ou symptômes respiratoires. Les études effectuées sur des écoliers au sujet des effets (symptômes et maladies) que le NO₂ exerce au niveau de voies respiratoires inférieures ont donné des résultats quelque peu contrastés. Ces travaux ont fait l’objet d’un examen visant à en
verifier la cohérence et une synthèse en a été élaborée sous la forme d'une analyse quantitative (méta-analyse). La plupart des études effectuées en intérieur font ressortir une augmentation de la morbidité affectant les voies respiratoires inférieures chez les enfants durablement exposés au NO₂. Les concentrations hebdomadaires moyennes relevées dans les chambres à coucher se situent essentiellement entre 15 et 122 μg/m³ (0,008 et 0,065 ppm). Une synthèse des résultats obtenus en intérieur en supposant des points d'aboutissement toxicologique communs donne, pour les effets sur les voies respiratoires inférieures, un odds ratio de 1,2 (limites de confiance à 95%: 1,1 et 1,3) par incrément de 28,3 μg/m³ (0,015 ppm) de l'exposition moyenne au NO₂ calculée sur 2 semaines. On est donc amené à penser, compte tenu des hypothèses sur lesquelles repose cette analyse globale, que chaque fois que l'exposition moyenne sur deux semaines augmente de 28,3 μg/m³ (0,015 ppm) les chances de symptômes ou de maladie affectant les voies respiratoires inférieures augmentent de 20%.

Cet ensemble de résultats milite donc en faveur de l'hypothèse selon laquelle une exposition au NO₂ provoque, chez les enfants de 5 à 12 ans, des effets au niveau de voies respiratoires inférieures.

Des études également menées en intérieur, mais cette fois au niveau individuel, chez des enfants de 2 ans au plus, n'ont pas permis de dégager une relation systématique entre les estimations de l'exposition au NO₂ et la prévalence des symptômes ou des maladies affectant les voies respiratoires inférieures. En se basant sur une meta-analyse de ces données et compte tenu des hypothèses formulées à cette fin, on a trouvé que l'odds ratio combiné pour une augmentation égale à 28,2 μg/m³ (0,015 ppm) de l'exposition au NO₂, était de 1,09, avec un intervalle de confiance à 95% de 0,95-1,26, lorsque la concentration hebdomadaire moyenne du NO₂ dans les chambres à coucher se situait entre 9,4 et 94 μg/m³ (0,005 et 0,050 ppm). L'accroissement du risque était très faible et n'a d'ailleurs pas été mentionné systématiquement dans toutes les études. Finalement, on ne peut pas conclure que ces résultats indiquent l'existence, chez les enfants en bas âge, d'effets analogues à ceux qui ont été constatés chez les enfants plus âgés. Les raisons de cette différence due à l'âge restent obscures.

Les études dans lesquelles l'exposition au NO₂ avait effectivement été mesurée ont donné un odds ratio systématiquement plus élevé que celles dans lesquelles ces estimations avaient été obtenues de façon indirecte, ce qui s'explique par les erreurs
de mesure. Les corrections apportées pour tenir compte de covariables aléatoires comme la situation socio-économique, le tabagisme et le sexe ont eu pour conséquence que les études dans lesquelles des corrections de ce type avaient été faites, ont donné un odds ratio plus élevé que celles où elles ne l'avaient pas été.

Bien que nombre des études épidémiologiques basées sur des mesures effectives de l'exposition au NO₃ n'aient utilisé que des données obtenues sur 1 à 2 semaines tout au plus, on en a tout de même déduit l'exposition des enfants sur une période beaucoup plus longue. Le questionnaire standard utilisé dans la plupart des cas pour enregistrer les symptômes respiratoires récapitule des informations sur l'état de santé des sujets qui s'étendent sur toute une année. Le chiffre de 28,2 µg/m³ (0,015 ppm) utilisé dans les méta-analyses correspond à la différence d'exposition annuelle moyenne au NO₃, selon que le ménage utilisait une cuisinière à gaz ou une cuisinière électrique. Dans certaines études, on n'a mesuré la concentration de NO₃ que pendant l'hiver, d'où une possible surestimation de l'exposition annuelle moyenne. Dans ces conditions, il y aurait eu sous-estimation de l'effet sanitaire d'une différence de 28,2 µg/m³ (0,015 ppm) dans l'exposition annuelle au NO₂. Dans une étude basée sur l'exposition annuelle moyenne dans les ménages, mesurée en hiver et en été, l'effet observé a été plus important que dans beaucoup des autres études. On ignore quelle est la période qui serait vraiment significative sur le plan biologique, mais il est à noter que l'exposition prise en considération dans ces travaux s'est poursuivie pendant de longues périodes, voire pendant toute la vie.

Les travaux actuels ne mettent pas en évidence d'association claire entre la concentration de NO₂ à l'extérieur et l'intégrité de la fonction respiratoire. Un certain nombre de résultats indiquent que les affections respiratoires pourraient se prolonger lorsque l'air est fortement chargé en NO₂. L'analyse des études portant sur l'air extérieur se heurte à une difficulté majeure: distinguer les effets imputables au NO₂ de ceux qui sont dus à d'autres polluants.

L'interprétation des résultats des études précitées et de la méta-analyse doit prendre en considération plusieurs incertitudes qui subsistent. L'erreur de mesure sur l'exposition pourrait être l'un des problèmes méthodologiques les plus importants qui se posent dans les études épidémiologiques sur le NO₂. Les résultats expérimentaux incitent à admettre l'existence d'une association entre certains symptômes et les indicateurs de l'exposition au NO₂, mais ces estimations de l'exposition ne seraient pas suffisamment
fiables pour permettre d'établir une relation quantitative entre exposition et symptômes. Dans la plupart des études au cours desquelles il a été procédé à des mesures de l'exposition, ces mesures ne portaient que sur une durée de 1 à 2 semaines et ont été rapportées sous la forme de valeurs moyennes. On a rarement cherché à établir une relation entre les effets observés et les modalités de l'exposition, par exemple l'existence de pics transitoires de concentration. En outre, il est possible que la concentration de NO₂ mesurée n'ait pas été égale à la dose biologiquement significative. D'ailleurs, l'estimation de l'exposition effective suppose la connaissance de l'espèce chimique en cause, de sa concentration et du type d'activité humaine qui lui a donné naissance. On ne dispose toutefois que d'un nombre limité de données sur l'activité humaine et les conditions météorologiques en rapport avec ces facteurs. L'extrapolation à d'autres modalités d'exposition reste un exercice difficile. En outre, même si, du fait des analogies et des éléments communs qui existent entre les variables mesurées dans ces études, on peut avoir une certaine confiance dans leur utilisation en vue d'une analyse quantitative, les symptômes et les maladies constatées sont quand même différents, jusqu'à un certain point, et peuvent parfaitement correspondre à des processus sous-jacents d'une autre nature. Dans ces conditions, la prudence s'impose dans l'interprétation des résultats de la méta-analyse.

Dans d'autres études épidémiologiques, on s'est efforcé d'établir une relation entre certaines mesures de l'exposition au NO₂ à l'intérieur ou à l'extérieur et l'altération de la fonction pulmonaire. Il s'agissait en fait d'anomalies respiratoires d'importance marginale. La plupart des études ne sont pas parvenues à déceler le moindre effet, résultat qui cadre avec ceux des études contrôlées sur l'homme. Quoiqu'il en soit, les données épidémiologiques sont insuffisantes pour que l'on puisse tirer des conclusions sur les effets qu'une exposition de courte ou de longue durée au NO₂ pourrait avoir au niveau pulmonaire.

En se basant sur un niveau de fond de 15 μg/m³ (0,008 ppm) et le fait que des effets indésirables significatifs apparaissent lorsque l'exposition augmente d'au moins 28,2 μg/m³, c'est-à-dire 0,015 ppm, on peut proposer une valeur-guide de 40 μg/m³ (0,023 ppm) en moyenne annuelle. Cette valeur permettra d'éviter les expositions les plus graves. Il reste cependant à souligner qu'il n'a pas encore été possible de déterminer la valeur de la concentration correspondant à l'absence d'effet en cas d'exposition chronique ou subchronique au NO₂.
3.4 Valeurs-guides à visée sanitaire pour le dioxyde d'azote

Le résultats des études contrôlées sur l'homme conduisent à adopter, en cas d'exposition à court terme, une valeur-guide de 200 µg/m³ (0,11 ppm) pour la concentration journalière maximale de NO₂ calculée en moyenne sur 1 h. Dans le cas d'une exposition à long terme, on recommande, en se basant sur les études épidémiologiques attestant un risque accru d'affections respiratoires chez l'enfant, une valeur-guide de 40 µg/m³ (0,023 ppm) en moyenne annuelle.
RESUMEN

1. Óxidos de nitrógeno y compuestos afines

Los óxidos de nitrógeno pueden alcanzar concentraciones considerables en el aire del medio ambiente y de espacios cerrados. Los tipos y concentraciones de los compuestos de nitrógeno presentes pueden variar notablemente de unos lugares a otros, con la hora del día y con la estación. Las fuentes principales de emisión de óxidos de nitrógeno son los procesos de combustión. Las centrales eléctricas que funcionan con combustibles fósiles, los vehículos de motor y los aparatos de combustión domésticos emiten óxidos de nitrógeno, sobre todo óxido nítrico (NO), y en algunos casos (normalmente menos del 10 por ciento) dióxido de nitrógeno (NO₂). En el aire se producen reacciones químicas que oxidan el NO a NO₂ y otros productos. Hay también procesos biológicos que liberan del suelo productos nitrogenados, incluso óxido nitroso (N₂O). Las emisiones de N₂O pueden producir alteraciones en la capa de ozono estratosférica.

La salud humana puede verse afectada por la presencia de concentraciones importantes de NO₂ u otros productos nitrogenados, como por ejemplo el nitrato de peroxiacetilo (NPA), el ácido nítrico (NO₃H), el ácido nitroso (NO₂H) y los compuestos orgánicos nitrogenados. Además, cuando los nitratos y el ácido nitrico se depositan en la tierra pueden tener efectos en la salud y repercusiones considerables sobre los ecosistemas.

El conjunto de NO y NO₂ suele recibir el nombre de NOₓ. Una vez liberado en el aire, el NO se oxida a NO₂ por acción de los oxidantes presentes (en particular el ozono, O₃). Esta reacción, en determinadas condiciones, es muy rápida al aire libre; en el aire de espacios cerrados suele ser un proceso mucho más lento. Los óxidos de nitrógeno son un precursor que controla la contaminación del aire por oxidantes fotoquímicos, dando lugar a la formación de ozono y de bruma; las interacciones de los óxidos de nitrógeno (excepto el N₂O) con compuestos orgánicos reactivos y la luz solar producen ozono en la troposfera y bruma en las zonas urbanas.

El NO y el NO₂ pueden sufrir asimismo reacciones que producen una serie de óxidos de nitrógeno, tanto en espacios abiertos como cerrados, entre ellos NO₃H, NO₂H, trióxido de nitrógeno (NO₃O₃), pentóxido de nitrógeno (N₂O₅), NPA y otros nitratos orgánicos. La gama compleja de óxidos de nitrógeno...
gaseosos recibe el nombre de NO\textsubscript{y} El reparto de los óxidos de nitrógeno entre estos compuestos depende fundamentalmente de las concentraciones de otros oxidantes y de los antecedentes meteorológicos del aire.

El NO\textsubscript{3}H es producto de la reacción entre el OH\textsuperscript{-} y el NO\textsubscript{2}. Es el sumidero principal del nitrógeno activo y contribuye también a la deposición ácida. Entre los posibles sumideros físicos y químicos del NO\textsubscript{3}H figuran la deposición húmeda y seca, la fotólisis la reacción con radicales OH y la reacción con amoníaco gaseoso para formar un aerosol de nitrato de amonio.

Los NPA se forman mediante la combinación de radicales peroxílo orgánicos con NO\textsubscript{2}. El NPA es el nitrato orgánico más abundante en la troposfera y puede servir como reservorio temporal de nitrógeno reactivo, que se puede transportar de una zona a otra.

El radical NO\textsubscript{3}, compuesto NO que se forma en la troposfera fundamentalmente por reacción del NO\textsubscript{2} con el O\textsubscript{3}, sufre una fotólisis rápida a la luz del día o una reacción con el NO. Durante la noche se observan concentraciones apreciables.

El N\textsubscript{2}O\textsubscript{5} es básicamente un componente nocturno del aire atmosférico, puesto que se forma a partir de la reacción del NO\textsubscript{3} con el NO\textsubscript{2}. Se forma en la atmósfera, el N\textsubscript{2}O\textsubscript{5} sufre una reacción heterogénea con el agua y forma NO\textsubscript{3}H, que a su vez se deposita.

El N\textsubscript{2}O está presente en todas partes, debido a que es un producto de procesos biológicos naturales del suelo. No se sabe, sin embargo, si interviene en alguna reacción en la troposfera. El N\textsubscript{2}O participa en reacciones de la capa superior de la atmósfera, contribuyendo a la reducción del ozono (O\textsubscript{3}) de la estratosfera, y también es un gas de efecto de invernadero relativamente potente, que contribuye al calentamiento mundial.

1.1 Transporte en la atmósfera

El transporte y la dispersión de los diversos compuestos nitrogenados en la capa inferior de la troposfera dependen de parámetros tanto meteorológicos como químicos. La advección, la difusión y las transformaciones químicas combinadas determinan los tiempos de permanencia en la atmósfera. Estos, a su vez, ayudan a establecer el alcance geográfico del transporte de un compuesto concreto. Las emisiones superficiales se dispersan en
sentido vertical y horizontal a través de la atmósfera mediante procesos mixtos turbulentos que dependen en gran medida de la estructura vertical de la temperatura y de la velocidad del viento.

Como consecuencia de los procesos meteorológicos, los NO\textsubscript{x} emitidos en las primeras horas de la mañana, en una zona urbana, se suelen dispersar en sentido vertical y desplazarse en el sentido del viento a medida que avanza el día. En los días soleados de verano, la mayoría de los NO\textsubscript{x} se habrán convertido en NO\textsubscript{2}H y NPA al atardecer, con la consiguiente formación de ozono. Una gran parte del NO\textsubscript{2}H se elimina por deposición con el transporte de las masas de aire, pero el NO\textsubscript{2}H y el NPA arrastrados a las capas altas (por encima de la capa de inversión nocturna, pero por debajo de una inversión de subsidencia superior) se pueden transportar potencialmente a grandes distancias en masas de aire ricas en oxidantes.

1.2 Medición

Son varios los métodos disponibles para medir los compuestos de nitrógeno presentes en el aire. En el presente documento se describen brevemente las metodologías utilizables o de uso general en la actualidad para la vigilancia in situ de las concentraciones en el aire en ambientes tanto externos como internos. Los compuestos examinados son el NO, el NO\textsubscript{2}, el NO\textsubscript{3}, el nitrógeno complejo reactivo total (NO\textsubscript{r}), el NPA y otros nitratos orgánicos, el NO\textsubscript{2}H, el NO\textsubscript{2}H\textsubscript{2}, el radical nitrato NO\textsubscript{3} y el N\textsubscript{2}O.

La medición de las concentraciones de óxidos de nitrógeno no es sencilla. Aunque existe un método fácil muy utilizado para la medición del NO (reacción quimioluminiscente con el ozono), es una excepción para los óxidos de nitrógeno. La quimioluminiscencia es también la técnica más utilizada para el NO\textsubscript{2}; éste se reduce en primer lugar a NO. Por desgracia, el catalizador utilizado normalmente para la reducción no es específico y tiene diversas eficacias de conversión para otros compuestos de nitrógeno oxidados. Por este motivo hay que tener mucho cuidado a la hora de interpretar los resultados del analizador común de quimioluminiscencia en cuanto al NO\textsubscript{2}, puesto que la señal puede incluir otros muchos compuestos. Se añaden nuevas dificultades por el hecho de que los óxidos de nitrógeno se pueden dividir entre las fases gaseosa y particulada tanto en la atmósfera como en el procedimiento de muestreo.
1.3 Exposición

La exposición humana y ambiental a los óxidos de nitrógeno varía mucho entre los espacios cerrados y abiertos, entre las ciudades y el campo y con la hora del día y la estación. Las concentraciones de NO y NO₂ que suelen estar presentes en los espacios abiertos de una serie de situaciones urbanas están relativamente bien definidas. Las concentraciones en los espacios cerrados dependen de los detalles específicos del tipo de los aparatos de combustión, las chimeneas y la ventilación. Cuando se utilizan aparatos de combustión para cocinar o calentar sin ventilación, las concentraciones de óxidos de nitrógeno en el interior superan en general con mucho las que hay en el exterior. En investigaciones recientes se ha comprobado que en esas circunstancias el NO₂-H puede alcanzar concentraciones considerables. En un informe se señalaba que el NO₂-H puede representar más del 10 por ciento de las concentraciones que se suelen dar como NO₂.

2. Efectos en la vegetación de los compuestos de nitrógeno de la atmósfera, en particular los óxidos de nitrógeno

La mayor parte de la biodiversidad del planeta se encuentra en ecosistemas (semi)naturales de hábitats tanto acuáticos como terrestres. El nitrógeno es el factor nutriente limitante para el crecimiento de las plantas en muchos ecosistemas (semi)naturales. La mayoría de las especies vegetales de estos hábitats están adaptadas a condiciones con escasez de nutrientes y solamente pueden competir con éxito en suelos con concentraciones bajas de nitrógeno.

Las actividades humanas, tanto industriales como agrícolas, han aumentado considerablemente la cantidad de compuestos de nitrógeno disponibles desde el punto de vista biológico, alterando así el ciclo natural del nitrógeno. Hay diversas formas de nitrógeno que contaminan el aire, sobre todo el NO, el NO₂ y el amoniaco (NH₃) como deposición sólida y los nitratos (NO₃⁻) y el amonio (NH₄⁺) como deposición líquida. El NH₃ es la suma del NH₃ y el NH₄⁺. Otra parte corresponde a la deposición oculta (niebla y nubes). Hay muchos más contaminantes del aire que contienen nitrógeno (por ejemplo N₂O₅, NPA, N₂O, aminas), pero estos no se tienen en cuenta, debido a que se considera que su contribución a la deposición total de nitrógeno es pequeña o a que sus concentraciones están probablemente muy por debajo de los umbrales con efectos.
Los contaminantes del aire con nitrógeno pueden afectar a la vegetación de manera indirecta, por medio de sus productos de reacción fotoquímica, o bien directamente, tras depositarse en la vegetación, el suelo, o la superficie del agua. La via indirecta apenas se tiene en cuenta aquí, aunque comprende procesos muy importantes y se debe tener presente al evaluar los efectos totales de los contaminantes del aire con nitrógeno: el NO₂ es un precursor del O₃ de la troposfera, que actúa como fitotoxina y como gas del efecto de invernadero.

Los efectos de la mayor deposición de nitrógeno en los sistemas biológicos pueden deberse a la absorción directa del follaje o bien a través del suelo. Si se consideran las plantas individuales, los efectos más destacados son la lesión de los tejidos, los cambios en la producción de biomasa y la mayor susceptibilidad a factores secundarios de tensión. En relación con la vegetación, el nitrógeno depositado actúa como nutriente; esto produce cambios en las relaciones competitivas entre las especies y pérdida de biodiversidad. Las cargas críticas del nitrógeno dependen de: i) el tipo de ecosistema, ii) la utilización y ordenación de la tierra en el pasado y en el presente; y iii) las condiciones abióticas (especialmente las que influyen en el potencial de nitrificación y el índice de inmovilización en el suelo).

En la superficie externa de las hojas se produce adsorción que puede ocasionar daños en las capas cérreas de la cutícula, pero todavía no se ha demostrado la importancia cuantitativa para la situación en el campo. La absorción de NO₂ y NH₃ depende del gradiente de concentración entre la atmósfera y el mesofilo. En general, aunque no siempre, está directamente determinada por la conductancia de los estomas, por lo que depende de factores que influyen en la apertura de estos. Hay cada vez más pruebas de que la absorción foliar de nitrógeno reduce la que se produce por las raíces. La absorción y el intercambio de iones a través de la superficie de las hojas es un proceso relativamente lento, de manera que unicamente tiene importancia si la superficie se mantiene húmeda durante periodos prolongados.

El NO sólo es ligeramente soluble en agua, pero la presencia de otras sustancias puede alterar la solubilidad. El NO₂ tiene una solubilidad mayor, mientras que la del NH₃ es mucho más elevada. El NO₂ (producto primario de la reacción del NO), el NH₃ y el NH₄⁺ son muy fitotóxicos y podrían ser sin duda la causa de los efectos adversos de los contaminantes del aire que contienen nitrógeno. El radical libre *N=O* puede desempeñar una función en la fitotoxicidad del NO.
Se han encontrado efectos superiores a los aditivos (sinergia) en casi todos los estudios relativos al SO₂ más NO₂. Con otras mezclas del NO₂ (NO, O₃ y CO₂), los efectos interactivos son la excepción en lugar de la regla.

Cuando las condiciones climáticas y el suministro de otros nutrientes permiten la producción de biomasa, tanto el NO como el NH₃ estimulan el crecimiento a concentraciones bajas y lo reducen cuando las concentraciones son más elevadas. Sin embargo, el nivel de exposición al cual se pasa del estímulo del crecimiento a su inhibición es mucho más bajo para el NO₃ que para el NH₃.

Hay pruebas de que las plantas son más sensibles con una intensidad de luz escasa (por ejemplo de noche y en invierno) y a temperaturas bajas (ligeramente por encima de 0 °C). El NO₂ y el NH₃ pueden aumentar la sensibilidad de las plantas a las heladas, la sequía, el viento y los daños de los insectos.

Existe interacción entre la química del suelo y la sensibilidad de la vegetación a la deposición de nitrógeno; este proceso está relacionado con el pH y la disponibilidad de nitrógeno.

No está clara la contribución relativa del NO y el NO₂ al efecto del NO₃ en las plantas. La inmensa mayoría de la información disponible se refiere a los efectos del NO₃, pero los datos existentes sobre el NO parecen indicar que éste y el NO₂ tienen efectos fitotóxicos comparables.

Las directrices sobre la calidad del aire se refieren a los umbrales para los efectos adversos. Existen dos tipos distintos de umbrales para los efectos: los niveles críticos y las cargas críticas. El nivel crítico se define como la concentración en la atmósfera por encima de la cual, según los conocimientos actuales, pueden producirse efectos adversos directos en los receptores, como las plantas, los ecosistemas o los materiales. La carga crítica se define como la estimación cuantitativa de una exposición (deposición) a uno o más contaminantes por debajo de la cual, según los conocimientos actuales, no hay efectos nocivos significativos en elementos sensibles específicos del medio ambiente.

De acuerdo con la práctica actual, los niveles críticos se han derivado de la evaluación de las concentraciones mínimas de exposición que causan efectos adversos en la fisiología o el crecimiento de las plantas (se excluyeron los efectos bioquímicos), utilizando un método gráfico.

535
A fin de incluir los efectos del NO, se propone un nivel crítico para el NO, en lugar de para el NO\textsubscript{2}, en este fin, se ha partido de la hipótesis de que el NO y el NO\textsubscript{2} actúan de manera aditiva. Se pueden aducir razones sólidas a favor del establecimiento de niveles críticos para la exposición a corto plazo. Sin embargo, en la actualidad no se dispone de datos adecuados para definirlos con suficiente confianza. Las pruebas actuales parecen indicar un nivel crítico aproximado de 75 µg/m\textsuperscript{3} para el NO, como media de 24 horas.

El nivel crítico para el NO\textsubscript{2} (NO y NO\textsubscript{2} añadidos en ppmm y expresados como NO\textsubscript{2} en g/m\textsuperscript{3}) se considera que es de 30 µg/m\textsuperscript{3} como media anual.

La información acerca de los organismos en el medio ambiente se limita casi exclusivamente a las plantas, con datos mínimos sobre la fauna del suelo. Por consiguiente, los valores de esta evaluación y de orientación se expresan en función de los efectos de los compuestos de nitrógeno en la vegetación. Sin embargo, cabe prever que las plantas formen el componente más sensible de los sistemas naturales y que el efecto en la biodiversidad de las comunidades vegetales sea un indicador aceptable de los efectos en todo el ecosistema.

Las cargas críticas se derivan de datos empíricos y de modelos estables del suelo. Se dan cargas críticas estimadas para la deposición total de nitrógeno en una serie de ecosistemas acuáticos y terrestres naturales. Los posibles efectos diferenciales de los compuestos de nitrógeno depositados (NO\textsubscript{x} y NH\textsubscript{3}) no se conocen suficientemente para diferenciar entre los distintos compuestos en la estimación de la carga crítica.

La gran mayoría de los ecosistemas acerca de los cuales se dispone de suficiente información para estimar las cargas críticas son de climas templados. Los escasos ecosistemas árticos y montañosos incluidos, que cabría esperar que fueran representativos de altitudes mayores, tienen la base menos fidedigna. No hay información sobre ecosistemas tropicales y es muy poca la relativa a ecosistemas de estuarios o marinos de cualquier zona climática. Es probable que los ecosistemas tropicales con escaso nitrógeno, como las selvas tropicales y los manglares pantanosos, se vean afectados negativamente por la deposición de nitrógeno. La falta de datos sobre la deposición y de umbrales de los efectos hacen que sea imposible efectuar evaluaciones del riesgo para esas regiones climáticas.
Los ecosistemas más sensibles (turberas ombrotróficas, lagos poco profundos de agua blanda y brezales árticos y alpinos) para los que pueden estimarse umbrales de los efectos muestran cargas críticas de 5-10 kg de N/ha/año, tomando como base la menor diversidad biológica de las comunidades vegetales. Un valor más medio para la gama limitada de ecosistemas estudiados es de 15-20 kg de N/ha/año, que es aplicable a los árboles de los bosques.

La química atmosférica de los óxidos de nitrógeno comprende la capacidad de generación de ozono en la troposfera, la reducción del ozono en la estratosfera y la contribución al calentamiento mundial como gases del efecto de invernadero. Los óxidos de nitrógeno y el amoníaco contribuyen a la acidificación del suelo (junto con los óxidos de azufre) y, por consiguiente, al aumento de la biodisponibilidad de aluminio.

Los efectos fitotóxicos de los óxidos de nitrógeno en las plantas tienen escaso interés directo para las cultivadas cuando las concentraciones superan marginalmente el nivel crítico. Sin embargo, la función del NO₂ en la generación de ozono y otras sustancias fitotóxicas, por ejemplo nitratos orgánicos, da lugar a la pérdida de cultivos. El nitrógeno depositado en las plantas en fase de crecimiento representa un aumento muy pequeño del nitrógeno total disponible en comparación con el que se añade como fertilizante.

3. Efectos de la exposición al dióxido de nitrógeno en la salud

Se han realizado numerosos estudios con objeto de evaluar los efectos del NO₂ para la salud. De los compuestos del NOₓ, el más estudiado ha sido el NO₂. El examen de esta sección se concentra en el NO₂, el NO, el NO₂H y el NO₃H, mientras que los nitratos se mencionan brevemente.

3.1 Estudios sobre los efectos de los compuestos de nitrógeno en animales de experimentación

La extrapolación a las personas de los datos obtenidos en animales tiene componentes tanto cualitativos como cuantitativos. Como se señala a continuación de manera resumida, el NO₂ produce una serie de efectos en varias especies animales, en particular sobre las defensas del huésped frente a las enfermedades infecciosas pulmonares, en el metabolismo/bioquímica de los pulmones, la función de éstos y su estructura. Debido a las
analogías fisiológicas, metabólicas y estructurales básicas de todos los mamíferos (animales de laboratorio y personas), el conjunto de las observaciones realizadas en varias especies animales lleva a la conclusión razonable de que el NO₂ podría ocasionar tipos parecidos de efectos en las personas. Sin embargo, debido a las diferencias entre las especies de mamíferos, no se sabe todavía con exactitud qué exposiciones darían lugar en la práctica a esos efectos. Este es el aspecto de la extrapolación cuantitativa. Las limitadas investigaciones sobre la creación de modelos relativos al aspecto dosimétrico (es decir, la dosis que realmente produce toxicidad en el tejido/célula destinatario) de la extrapolación cuantitativa parecen indicar que la distribución de la deposición de NO₂ en el aparato respiratorio de los animales y las personas es analoga, aunque no se dispone todavía de valores adecuados que puedan utilizarse para la extrapolación de los animales a las personas. Por desgracia, es muy poca la información disponible sobre el otro aspecto básico de la extrapolación, la sensibilidad específica (es decir, la respuesta de los tejidos de distintas especies a una dosis determinada). Así, gracias a los estudios sobre animales actualmente disponibles sabemos qué efectos puede tener el NO₂ para la salud humana. No estamos en condiciones de definir con gran precisión los efectos que produce realmente una dosis determinada de NO₂ inhalada.

Teniendo en cuenta lo expuesto, a continuación se resume la base de datos sobre la toxicología del NO₂ en los animales, de acuerdo con las principales clases de efectos y los temas de especial interés. Aunque es evidente que los efectos de la exposición al NO₂ van más allá de los límites de los pulmones, no está clara la interpretación de estos efectos sistémicos en relación con el posible riesgo para la salud humana. Por consiguiente, no se sigue hablando de ellos aquí, sino que se examinan en capítulos posteriores. Aunque las interacciones del NO₂ y otros contaminantes que lo acompañan, como el O₃ y el ácido sulfúrico (SO₂H₂), pueden ser bastante importantes, especialmente si se produce simergia, la base de datos no permite todavía llegar a conclusiones a partir de las cuales se puedan evaluar las interacciones potenciales en la realidad.

3.1.1 Mecanismos de acción bioquímicos y celulares de los óxidos de nitrógeno

El NO₂ actúa como oxidante fuerte. Los lipidos insaturados se oxidan fácilmente, con peróxidos como producto predominante. Tanto el ácido ascórbico (vitamina C) como el α-tocoferol
Resumen

(vitamina E) inhiben la peroxidación de los lipidos insaturados. Cuando el ácido ascórbico queda encerrado herméticamente dentro de liposomas de doble capa, el NO₂ oxida con rapidez el ácido ascórbico englobado. Los efectos protectores del α-tocoferol y el ácido ascórbico en los animales y las personas se deben a la inhibición de la oxidación por el NO₂. Éste también oxida las proteínas de las membranas. La oxidación de los lípidos o las proteínas de las membranas provoca la pérdida del control de la permeabilidad celular. Los pulmones de las personas y de los animales experimentales expuestos al NO₂ tienen cantidades mayores de proteínas en el lumen. La aparición de células inflamatorias y los cambios en los pulmones se deben a esa acción.

Las propiedades oxidantes del NO₂ también inducen la vía de destoxicificación de los peróxidos de la glutatión peroxidasa, la glutatión reductasa y la glucosa-6-fosfato deshidrogenasa. Tras la exposición al NO₂, se registra una relación exposición-respuesta en el aumento de la vía de destoxicificación de los peróxidos en los animales.

El mecanismo de acción del NO es menos claro. Se oxida fácilmente a NO₂ y luego se produce una peroxidación. Debido a que en las exposiciones a NO hay también presente algo de NO₂, es difícil distinguir los efectos de ambos. El NO actúa como segundo mensajero intracelular que modula una gran variedad de enzimas esenciales e inhibe su propia producción (por ejemplo, mediante retroinhibición). El NO activa la guanilato ciclasa, que a su vez eleva los niveles de GMPc intracelular. Un posible mecanismo de acción de los nitratos se puede producir por medio de la liberación de histamina de los gránulos de los mastocitos. Los contaminantes atmosféricos nitrogenados ácidos, en particular el NO₃H, pueden actuar alterando el pH intracelular.

El NPA se descompone en el agua, formando peróxido de hidrógeno. Apenas se conoce el mecanismo de acción, pero es probable que haya presión oxidativa para el NPA y las sustancias analógas.

Los nitratos inorgánicos pueden actuar mediante alteraciones del pH intracelular. El ión nitrato se transporta a las células alveolares de tipo 2 y las acidifica. También moviliza la histamina de los mastocitos. El NO₃H podría actuar también alterando el pH intracelular, pero este mecanismo no está claro.
No se conocen los mecanismos de acción de los demás óxidos de nitrógeno.

La exposición aguda al NO$_2$ a una concentración de 750 µg/m$^3$ (0,4 ppm) puede dar lugar a una peroxidación de los lípidos. El NO$_2$ puede oxidar los ácidos grasos poliinsaturados de las membranas celulares, así como grupos funcionales de proteínas (proteínas solubles de la célula, como las enzimas, o bien proteínas estructurales, como los componentes de las membranas celulares). Tales reacciones de oxidación (con la intervención de radicales libres) son un mecanismo mediante el cual el NO$_2$ produce una toxicidad directa en células pulmonares. Este mecanismo de acción se ha comprobado en estudios con animales, en los que se pone de manifiesto la importancia de las defensas antioxidantes de los pulmones, tanto endógenas (por ejemplo el mantenimiento de los niveles de glutatión de los pulmones) como exógenas (por ejemplo las vitaminas C y E de la alimentación) en la protección frente a los efectos del NO$_2$. En numerosos estudios se ha observado que diversas enzimas de los pulmones, entre ellas la glutatión peroxidasa, la superoxído dismutasa y la catalasa, pueden actuar también defendiendo los pulmones del ataque de los oxidantes.

3.1.2 Efectos en la defensa de los huéspedes

Aunque la función primaria de las vías respiratorias es asegurar un intercambio eficaz de gases, este sistema orgánico proporciona también al cuerpo la primera línea de defensa frente a los agentes presentes en la atmósfera, viables y no viables, que se inhalan. En una amplia base de datos se pone claramente de manifiesto que la exposición al NO$_2$ puede provocar la disfunción de estas defensas del huésped, aumentando la susceptibilidad a las enfermedades infecciosas de las vías respiratorias. Los parámetros de defensa del huésped afectados por el NO$_2$ incluyen la actividad funcional y bioquímica de las células de los pulmones, los macrófagos alveolares, la competencia inmunológica, la susceptibilidad a infecciones de las vías respiratorias inducidas experimentalmente y la tasa de eliminación mucociliar.

Los macrófagos alveolares se ven afectados por el NO$_2$. Estas células se encargan de mantener la esterilidad de la región pulmonar, eliminando las partículas de ella y participando en las funciones inmunológicas. Entre los cambios funcionales que se han descrito cabe mencionar los siguientes: supresión de la capacidad fagocítica y del estimulo de la limpieza de los pulmones a 560 µg/m$^3$ (0,3 ppm) dos horas/día durante 13 días; disminución de la
actividad bacteriana a 4320 μg/m³ (2,3 ppm) durante 17 horas; y
una disminución de la respuesta al factor de inhibición de la
migración a 3760 μg/m³ (2,0 ppm) ocho horas/día y cinco
días/semana durante seis meses. El aspecto morfológico de estas
células de defensa cambia tras la exposición crónica al NO₂.

La importancia de las defensas del huésped se pone de
manifiesto cuando los animales tienen que hacer frente a
infecciones pulmonares inducidas en el laboratorio. Los animales
expuestos a NO₂ sufren de infecciones bacterianas o viricas
de manera dependiente de la concentración. También aumenta la
mortalidad con la elevación de la concentración de NO₂ o la
duración de la exposición. Tras una exposición aguda, se observan
efectos a concentraciones de apenas 3760 μg/m³ (2 ppm). La
exposición a concentraciones de sólo 940 μg/m³ (0,5 ppm) produce
efectos en el modelo de infectividad después de seis meses.

La exposición al NO₂ modifica tanto el sistema de defensa
humoral como el celular. En los casos en que se ha investigado el
sistema inmunológico, se han observado efectos tras una exposición
breve a concentraciones 9400 μg/m³ (5 ppm). Los efectos son
complejos, puesto que la dirección del cambio (es decir, el
aumento o disminución) depende de la concentración de NO₂ y de
la duración de la exposición.

3.1.3 Efectos de la exposición crónica en la evolución de las neumopatías
crónicas

Las personas están crónicamente expuestas al NO₂. Por
consiguiente, dicha exposición se ha estudiado en animales con
bastante detenimiento, normalmente utilizando métodos
morfológicos y/o morfométricos. Esta investigación ha demostrado
en general que en los pulmones se producen diversas alteraciones
estructurales, acompañadas de otras funcionales. Algunos de estos
cambios pueden ser reversibles cuando cesa la exposición.

La función pulmonar de animales experimentales se puede
alterar tras la exposición crónica al NO₂. Después de una
exposición a 7520 μg/m³ (4,0 ppm) de NO₂ durante cuatro meses
se registró un desequilibrio del intercambio de gases, y esto se puso
de manifiesto en una menor tensión arterial de O₂, una
disminución del rendimiento físico y un aumento del metabolismo
anerobio.
Aunque el NO₂ produce cambios morfológicos en las vías respiratorias, la base de datos es a veces confusa, debido a la variabilidad cuantitativa y cualitativa de la capacidad de respuesta en distintas especies, e incluso en la misma. La rata, que es el animal experimental más utilizado en evaluaciones morfológicas de la exposición, parece ser relativamente resistente al NO₂. La exposición de corta duración a concentraciones de 9400 μg/m³ (5,0 ppm) o menores tiene en general escasos efectos en la rata, mientras que exposiciones similares en el cobaya pueden producir algunos daños en el epitelio centriacinar.

La exposición de más larga duración provoca lesiones en algunas especies con concentraciones de solo 560–940 μg/m³ (0,3–0,5 ppm). Estas se caracterizan por una modificación del epitelio parecida a la descrita más arriba, pero con la intervención de vías respiratorias más proximales y el engrosamiento del intersticio. Sin embargo, muchos de estos cambios desaparecen incluso con una exposición continuada, necesitándose una exposición de larga duración a niveles por encima de un valor aproximado de 3760 μg/m³ (2,0 ppm) para que aparezcan cambios más extensos y permanentes en los pulmones. Algunos efectos son relativamente persistentes (por ejemplo la bronquiolitis), mientras que otros tienden a ser reversibles y limitados, incluso con una exposición continuada. En cualquier caso, parece que tanto en la exposición de corta duración como en la larga la respuesta depende más de la concentración que del tiempo de exposición.

Hay pruebas bastante convincentes de que la exposición de larga duración de varias especies de animales de laboratorio a concentraciones elevadas de NO₂ da lugar a lesiones morfológicas en los pulmones. En un número limitado de estudios bastante fidedignos se ha descrito la destrucción de las paredes alveolares de los pulmones de animales como otro criterio esencial para el enfisema humano. A partir de estos estudios publicados no se puede determinar la concentración más baja de NO₂ para la duración más breve de exposición que provoca lesiones pulmonares enfisematosas.

3.1.4 Posibles efectos carcinógenos o cocarcinógenos

Se ha demostrado que el NO₂ tiene una acción mutagénica sobre la bacteria Salmonella, pero dicha acción no se puso de manifiesto en un estudio realizado con un cultivo de células de mamífero. En otros estudios realizados con cultivos de células se han descubierto intercambios entre cromatidios hermanos y roturas...
Resumen

de cadenas sencillas de ADN. No se han observado efectos genotóxicos in vivo en relación con linfocitos, espermatocitos o células de la médula ósea, aunque en dos estudios de inhalación con concentraciones elevadas (50 760 y 54 400 µg/m³, 27 y 30 ppm) durante 3 y 6 horas, respectivamente, se han demostrado dichos efectos en las células pulmonares.

En la bibliografía no se han encontrado informes publicados de estudios sobre el NO₂ utilizando bioensayos crónicos clásicos de carcinogénesis con animales enteros. Las investigaciones con ratones que espontáneamente tenían un índice elevado de tumores eran equivocas. En un estudio con una concentración de NO₂ de 18 800 µg/m³ (10 ppm) se detectó un ligero aumento de la frecuencia de adenomas pulmonares en una raza de ratones sensible (A/J). Si bien se han realizado varias investigaciones de cocarcinogénesis, no se ha podido sacar ninguna conclusión debido a problemas de metodología e interpretación. Los informes sobre si el NO₂ facilita la formación de metástasis de tumores en los pulmones son también insuficientes para sacar conclusiones. Otras investigaciones se han concentrado en la posibilidad de que el NO₂ forme nitratos y nitritos que, al reaccionar con las aminas del organismo, podían producir nitrosaminas. En un pequeño número de estudios parece que se forman nitrosaminas en organismos tratados con dosis elevadas de aminas y expuestos a NO₂, pero en otros estudios se ha señalado que no es probable la formación de nitrosaminas.

3.1.5 Susceptibilidad en función de la edad

Las investigaciones sobre la dependencia de la edad no son suficientes y los resultados hasta ahora son equivocos.

3.1.6 Influencia de las modalidades de exposición

En varios estudios toxicológicos realizados con animales se ha puesto de manifiesto la relación entre concentración (C) y duración (T) de la exposición, indicando que esta es compleja. En la mayor parte de estas investigaciones se ha utilizado el modelo de la infectividad.

En los primeros estudios de C x T se demostró que la concentración tenía más efectos en la mortalidad que la duración de la exposición. Una evaluación de la toxicidad de la exposición al NO₂ no se puede definir por la relación C x T.
3.2 Estudios de exposición humana controlada a óxidos de nitrógeno

Se han evaluado las respuestas humanas a una serie de compuestos de nitrógeno oxidado. Con diferencia, la base de datos más amplia y la más adecuada para la evaluación del riesgo es la disponible para exposiciones controladas al NO\(_2\). La base de datos sobre la respuesta humana al NO, NO\(_2\)H gaseoso, NO\(_2\)H gaseoso y aerosoles de nitratos inorgánicos no es tan amplia. Se han examinado varios subgrupos sensibles o potencialmente sensibles, incluidos adolescentes y adultos asmáticos, ancianos, y pacientes con neumopatía obstructiva crónica e hipertensión pulmonar. El ejercicio durante la exposición aumenta la absorción total y altera la distribución del material inhalado dentro de los pulmones. La proporción relativa del NO\(_2\) depositado en las vías respiratorias inferiores aumenta también con el ejercicio. Esto puede acentuar los efectos de los compuestos citados anteriormente en personas que están en movimiento durante la exposición.

Como suele ocurrir en la respuesta biológica humana a partículas y gases inhalados, la correspondiente al NO\(_2\) es variable. Las personas sanas tienden a ser menos receptivas a los efectos del NO\(_2\) que las que padecen enfermedades pulmonares. Los asmáticos son claramente el grupo más sensible al NO\(_2\) entre los estudiados hasta ahora. Las personas con neumopatía obstructiva crónica pueden ser más sensibles que las sanas, pero tienen una capacidad de respuesta limitada al NO\(_2\), por lo que son difíciles de evaluar las diferencias cuantitativas entre este tipo de pacientes y otras personas. No se dispone por el momento de información suficiente para determinar si la edad y el sexo desempeñan una función en la respuesta al NO\(_2\).

Las personas sanas pueden detectar el olor del NO\(_2\) en algunos casos a concentraciones inferiores a 188 \(\mu g/m^3\) (0,1 ppm). En general, la exposición a este compuesto no aumentó los síntomas respiratorios en ningún de los grupos sometidos a prueba.

El NO\(_2\) provoca una disminución de la función pulmonar, en particular una mayor resistencia al paso del aire en personas sanas en reposo sometidas a concentraciones de sólo 4700 \(\mu g/m^3\) (2,5 ppm) durante dos horas. Los datos disponibles son insuficientes para determinar la naturaleza de la relación concentración-respuesta.

La exposición de personas no fumadoras sanas en movimiento a concentraciones de NO\(_2\) de sólo 2800 \(\mu g/m^3\) (1,5 ppm) durante
una hora o más produce una mayor sensibilización de las vías respiratorias a los agentes broncoconstrictores.

La exposición de asmáticos al NO₂ causa, en algunos pacientes, una mayor sensibilización de las vías respiratorias a diversos mediadores reactivos, incluidos productos químicos colinérgicos e histaminérgicos, el SO₂ y el aire frío. En presencia de estas respuestas parece que influye el procedimiento de exposición, en particular el hecho de que ésta tenga lugar con ejercicio o sin él. Las respuestas pueden comenzar a concentraciones de apenas 380 μg/m³ (0,2 ppm). De un metanálisis parece desprenderse que los efectos pueden presentarse a concentraciones incluso más bajas. Sin embargo, se ha observado una relación concentración-respuesta inequívoca entre 350 y 1150 μg/m³ (0,2 a 0,6 ppm).

Los efectos de esta tendencia general no están claros, pero una mayor sensibilización de las vías respiratorias podría producir en potencia una respuesta más intensa a los alérgenos del aire o un recrudecimiento del asma, lo cual posiblemente llevaría a un aumento de la medicación o incluso de las hospitalizaciones.

Puede producirse un incremento moderado de la resistencia de las vías respiratorias en pacientes con neumopatía obstructiva crónica sometidos a exposiciones breves (15-60 minutos) a concentraciones de NO₂ de sólo 2800 μg/m³ (1,5 ppm), y también puede observarse una disminución en las mediciones espirométricas de la función pulmonar (cambio del 3 al 8% en el VEF₁ (volumen espiratorio forzado en un segundo)) con exposiciones más prolongadas (3 horas) a concentraciones de sólo 600 μg/m³ (0,3 ppm).

La exposición a concentraciones de NO₂ superiores a 2800 μg/m³ (1,5 ppm) puede alterar el número de células inflamatorias y sus tipos en las vías distales o los alveolos. También pueden modificar el funcionamiento de las células dentro de los pulmones y la producción de mediadores que pueden ser importantes para las defensas pulmonares del huésped. El conjunto de cambios en dichas defensas, las alteraciones de las células pulmonares y de sus actividades y los cambios en los mediadores bioquímicos están en consonancia con los hallazgos epidemiológicos de una mayor susceptibilidad del huésped relacionada con la exposición al NO₂.

En estudios sobre mezclas de NO₂ con otros contaminantes no se ha observado que éste aumente la respuesta frente a los demás contaminantes presentes por encima del nivel que se detectaría si...
estos se encontraran solos. Una excepcion importante es la
observacion de que una exposicion previa al NO₂ de personas sanas
en movimiento potencibia los cambios inducidos por el ozono en
la sensibilizacion de las vías respiratorias cuando posteriormente
se las someta a una exposicion al ozono. Esta observacion parece
poner de manifiesto la posibilidad de respuestas retardadas o
persistentes al NO₂.

Dentro de la gama de concentraciones de NO₂ que puede
interesar con vistas a la evaluacion del riesgo (es decir,
100-600 µg/m³), los datos disponibles no permiten determinar las
caracteristicas de la relacion concentracion–respuesta para cambios
drasticos de la funcion pulmonar, la capacidad de respuesta de las
vías respiratorias a agentes broncoconstrictores o los sintomas.

A partir de un efecto a 400 µg/m³ y de la posibilidad de
efectos a niveles mas bajos, tomando como base un metanalisис, se
recomienda a titulo indicativo para un periodo breve un promedio
diario de una hora a una concentracion maxima de NO₂ de
200 µg/m³ (0,11 ppm).

Se sabe que el NO es un segundo mensajero endogeno
importante en varios sistemas del organismo. La inhalacion de
concentraciones de NO superiores a 6000 µg/m³ (5 ppm) puede
producir vasodilatacion en la circulacion pulmonar sin afectar a la
sistematic. No se ha determinado la concentracion eficaz minima.
La informacion sobre la funcion pulmonar y las defensas de los
pulmones del huesped despues de la exposicion al NO es
demasiado limitada para que por el momento se puedan sacar
conclusiones. No se ha informado de reacciones secundarias tras la
utilizacion, en aplicaciones clinicas, de concentraciones
relativamente altas (> 40 000 µg/m³) durante periodos breves
(<1 hora).

Concentraciones de acido nitrico del orden de 250-500 µg/m³
(97-194 ppm) pueden producir cierta respuesta en la funcion
pulmonar de adolescentes asmaticos, pero no en adultos sanos.

De la limitada informacion disponible sobre el NO₂H cabe
deducir que puede causar inflamacion ocular a 760 µg/m³ (0,40
ppm). En la actualidad no existen datos publicados sobre respuesta
pulmonar del ser humano al NO₂H.

Los limitados datos sobre nitratos inorganicos de que se
dispone indican que los aerosoles de nitratos a una concentracion
de 7000 µg/m³ o inferior no tienen efectos en la funcion pulmonar.

546
3.3 Estudios epidemiológicos sobre el dióxido de nitrógeno

Los estudios epidemiológicos sobre los efectos de los óxidos de nitrógeno en la salud se han concentrado principalmente en el NO₂. Se han realizado numerosos estudios epidemiológicos en espacios cerrados y abiertos para determinar los efectos del NO₂ en la salud. En general se consideran dos mediciones del estado de salud en la exposición al NO₂: las mediciones de la función pulmonar y los síntomas y enfermedades de las vías respiratorias.

Las pruebas de estudios individuales de los efectos del NO₂ sobre los síntomas y las enfermedades de las vías respiratorias inferiores de niños en edad escolar son algo confusas. Se examinó la concordancia de los estudios y las pruebas se resumieron en un análisis cuantitativo combinado (metanalisis) de ellos. En la mayoría de los estudios realizados en espacios cerrados se observó una mayor morbilidad de las vías respiratorias inferiores en niños asociada a exposiciones prolongadas al NO₂. En la mayoría de los estudios en los que se notificaron los niveles de NO₂ las concentraciones medias semanales en los dormitorios fueron predominantemente de 15 a 122 µg/m³ (0,008 y 0,065 ppm). La combinación de los estudios en espacios cerrados como si los resultados finales fueran semejantes da una razón de posibilidades estimada de 1,2 (límites de confianza del 95 por ciento de 1,1 y 1,3) para el efecto de un aumento de la concentración de NO₂ de 28,3 µg/m³ (0,015 ppm) en la morbilidad de las vías respiratorias inferiores. Esto indica que, teniendo en cuenta la hipótesis hechas para el análisis combinado, a cada aumento de 28,3 µg/m³ (0,015 ppm) en la exposición media estimada al NO₂ durante dos semanas corresponde un aumento de alrededor del 20 por ciento en las posibilidades de síntomas y enfermedades en las vías respiratorias inferiores. Así pues, las pruebas combinadas confirman los efectos de la exposición estimada para el NO₂ en los síntomas y las enfermedades de las vías respiratorias inferiores de niños con edades comprendidas entre cinco y 12 años.

En estudios individuales con niños de dos años o menos en espacios cerrados no se encontró una relación uniforme entre las estimaciones de la exposición al NO₂ y la prevalencia de síntomas y enfermedades de las vías respiratorias. Tomando como base un metanalisis de estos estudios realizados con niños en espacios cerrados, en función de las hipótesis hechas para el metanalisis, la razón combinada de posibilidades para el aumento de las enfermedades respiratorias con incrementos de 28,2 µg/m³ (0,015 ppm) de NO₂ fue de 1,9, con un intervalo de confianza del 95 por
ciento de 0,95 a 1,26, siendo las concentraciones semanales medias de NO₂ en los dormitorios predominantemente de 9,4 a 94 µg/m³ (0,005 y 0,050 ppm). El aumento de riesgo fue muy pequeño y no en todos los estudios se notificaron resultados uniformes. No se puede concluir que las pruebas indiquen un efecto en los niños pequeños comparable al observado en otros de más edad. No están claras las razones de estas diferencias relacionadas con la edad.

En los estudios de medición del NO₂ se obtuvo una razón de posibilidades superior a las estimaciones sustitutivas, lo que concuerda con un efecto de error de medición. El efecto de haber ajustado covariantes como la situación socioeconómica, la condición de fumador y el sexo fue que en los estudios en que se ajustó una variante particular se encontraron razones de posibilidades más altas que en los otros.

Si bien en muchos de los estudios epidemiológicos con niveles conocidos de NO₂ solo se realizaron mediciones durante una o dos semanas, estos niveles se utilizaron para caracterizar las exposiciones de los niños durante un periodo mucho más largo. El cuestionario normalizado sobre síntomas respiratorios utilizado en la mayor parte de estos estudios resume la información sobre el estado de salud durante todo un año. La diferencia de 28,2 µg/m³ (0,015 ppm) en los niveles de NO₂ utilizados en los metaanálisis corresponde a la diferencia en la exposición media anual en el hogar entre las cocinas de cocinar de gas y eléctricas. En algunos estudios las concentraciones de NO₂ se midieron sólo durante el invierno y puede haber una sobreestimación de la exposición media anual. Esto podría inducir a subestimar el efecto sobre la salud de la diferencia de 28,2 µg/m³ (0,015 ppm) en la exposición anual al NO₂. En un estudio basado en dicha exposición en el hogar, medida tanto en invierno como en verano, se puso de manifiesto un efecto sobre la salud mayor que en muchos de los otros estudios. Se desconoce el periodo de exposición verdaderamente importante desde el punto de vista biológico, pero estas exposiciones se prolongaban durante un periodo largo, que podía durar incluso toda la vida del niño.

De las investigaciones actuales no es posible deducir una relación clara entre el NO₂ de los espacios abiertos y la salud de las vías respiratorias. Hay algunas pruebas que demuestran que la duración de estas enfermedades puede aumentar en ambientes con concentraciones de NO₂ más altas. Una dificultad importante para el análisis de los estudios realizados en estos espacios radica en la distinción entre los posibles efectos debidos al NO₂ y los de otros contaminantes que lo acompañan.
Hay que considerar varios aspectos dudosos a la hora de interpretar los estudios y metanálisis expuestos más arriba. El error en la medición de la exposición es posiblemente uno de los problemas metodológicos más importantes en los estudios epidemiológicos del NO₂. Si bien hay pruebas de que los síntomas están relacionados con indicadores de la exposición al NO₂, la calidad de estas estimaciones de la exposición puede ser insuficiente para determinar una relación cuantitativa entre la exposición y los síntomas. En la mayor parte de los estudios en que se midió la exposición al NO₂ se hizo sólo por periodos de una a dos semanas y se dieron los valores como promedios. En pocos de los estudios se intentó relacionar los efectos observados con las modalidades de exposición (por ejemplo, niveles máximos transitorios de NO₂). Además, es posible que la concentración de NO₂ medida no sea una dosis biológicamente importante; la estimación de la exposición real exige el conocimiento de los tipos de contaminantes, sus niveles y las pautas correspondientes de la actividad humana. Sin embargo, los datos disponibles sobre actividad y aerométricos en los que se examinen dichos factores son muy limitados. La extrapolación a posibles pautas de exposición ambiental es difícil. Además, aunque el nivel de semejanza y de elementos comunes entre las medidas de los resultados en los estudios del NO₂ proporcione cierta confianza en su uso en el análisis cuantitativo, los síntomas y las enfermedades combinados son en cierto sentido diferentes y podrían reflejar de hecho procesos básicos distintos. Así pues, hay que ser prudentes a la hora de interpretar los resultados del metanálisis.

En otros estudios epidemiológicos se ha tratado de relacionar alguna medida de la exposición al NO₂ en espacios cerrados y/o abiertos con cambios en la función pulmonar. Estos cambios fueron marginalmente significativos. En la mayoría de los estudios no se encontró efecto alguno, lo que corrobora los datos obtenidos en los estudios de exposición humana controlada. Sin embargo, no hay pruebas epidemiológicas suficientes que permitan sacar conclusiones sobre los efectos a largo o corto plazo del NO₂ en la función pulmonar.

A partir de un nivel básico de 15 µg/m³ (0,008 ppm) y del hecho de que con un nivel adicional de 28,2 µg/m³ (0,015 ppm) o más se producen efectos negativos considerables en la salud, se propone un valor orientativo anual de 40 µg/m³ (0,023 ppm). Este valor evitará las exposiciones más graves. Hay que subrayar el hecho de que no se haya determinado todavía un nivel sin efectos para concentraciones de exposición al NO₂ subcrónicas o crónicas.
3.4 Valores orientativos basados en la salud para el dióxido de nitrógeno

A partir de los estudios de exposición humana controlada, se recomienda como valor orientativo en periodos breves una concentración máxima diaria media de NO$_2$ de 200 μg/m$^3$ (0,11 ppm) durante una hora. El valor orientativo para periodos prolongados, basado en estudios epidemiológicos del aumento del riesgo de enfermedades respiratorias en niños, es de un promedio anual de 40 μg/m$^3$ (0,023 ppm).
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