PERSONAL EXPOSURE TO NITROGEN DIOXIDE AND HEALTH EFFECTS AMONG PRESCHOOL CHILDREN

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ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Medicine of the University of Helsinki for public examination in the small lecture room at the Meilahti Theoretical Institutes, Haartmaninkatu 3 on Thursday November 25th 1999, at 12 o'clock noon.

Helsinki, 1999

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To Anniina and Tuomas

ABSTRACT

A prospective panel study, in which personal exposure was monitored, was conducted among preschool children in Helsinki between October 1990 and June 1991. The aims of the study were: 1) to assess children's personal weekly exposure to nitrogen dioxide (NO_2), 2) to investigate the association of seasonal and weekly NO_2 exposure with respiratory health and 3) to compare different exposure assessment methods in regard to their association with respiratory health.

The study was carried out in eight day-care centres in Helsinki, selected on the basis of known traffic density and NO_2 concentrations obtained from the network monitoring local air quality. Four day-care centres were located in the central area and four were in suburban areas. All children aged three to six years (N=363) in these centres were invited to participate in the study. A baseline questionnaire including questions on the general health status of the children and on the sociodemographic factors and housing conditions of the families was delivered to parents. Altogether 246 (68%) questionnaires were returned, along with a written consent of participation.

The children's weekly exposure to NO_2 was assessed with passive diffusion samplers (i.e. Palmes tubes) in three different ways: personally, both inside and outside the day-care centres. A Palmes tube was fixed on the outer garments of each child for one week at a time for a total of 24 weeks. Only tubes, which had been collecting for 168 ± 24 hours were accepted for analyses. During every week of the study, one tube was placed at a height of 1.5-2 m outside the day-care centre, and another one at the same height in the playing/sleeping room of the children. In addition, NO_2 values from the fixed-site monitoring network were used in the exposure assessment.

The respiratory health of the children was followed with the help of a daily symptom diary filled in by the parents. In a subgroup of children (N=53), also peak expiratory flow (PEF) was measured at home in the mornings and evenings.

Most of the personal weekly NO_2 values of the children ranged from 10-50 $\mu g/m^3$. The median exposures in the central area (seasonal 27 $\mu g/m^3$, weekly winter 26 $\mu g/m^3$ and weekly spring 28 $\mu g/m^3$) were higher than in the suburban areas (18 $\mu g/m^3$, 18 $\mu g/m^3$ ja 17 $\mu g/m^3$, respectively). The personally measured NO_2 concentrations were generally lower than the concentrations measured simultaneously inside or outside the day-care centres or at fixed-site monitoring stations. The NO_2 concentrations outside the day-care centre, fixed-site NO_2 concentration, residential area and characteristics of the home (gas stove, smoking

inside, type of dwelling) explained 32% of the variation in personal NO_2 exposure in winter and 67% in spring.

In regard to respiratory health the children had statistically significantly more days with cough (18 vs. 15%) and a stuffy nose (26 vs. 20%) in the central area than in the suburban areas. However, the seasonal average NO_2 exposure (range 11 to 45.8 $\mu g/m^3$) did not consistently associate with the symptoms, but the personal weekly average NO_2 exposure (4-99 $\mu g/m^3$) associated with cough (RR=1.52, 95% CI 1.00-2.31). When the other exposure assessment methods were used, there was a non-significant positive trend between NO_2 concentration and cough in winter. A non-significant negative trend was found between personal NO_2 exposure and PEF.

In conclusion, the results of this study suggest that the personal NO_2 exposures of preschool children in Helsinki are associated with health outcomes, even though the NO_2 concentrations in the ambient air are mostly below the current health-based guideline values on air quality. The associations between short-term NO_2 peaks and respiratory health, and the possible long-term influence of ambient air NO_2 and pollutant mixtures on the developing lungs of children should be elucidated in future studies.

ACKNOWLEDGEMENTS

Helsinki, October 1999   Kristiina Mukala

ABBREVIATIONS

CHD	coronary heart disease
COPD	chronic obstructive pulmonary disease
$FEF_{25-75\%}$	forced expiratory flow 25-75%
FEV	forced expiratory volume
FEV_1	forced expiratory volume in one second
MMEF	maximal mid-expiratory flow
NO	nitric oxide
NO_2	nitrogen dioxide
O_3	ozone
PEF(R)	peak expiratory flow(rate)
SO_2	sulphur dioxide
TSP	total suspended particulates
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
YTV	Helsinki Metropolitan Area Council (Pääkaupunkiseudun yhteistyövaltuuskunta)

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on four original publications, referred to in the text by their Roman numerals (I-IV).

I   Alm S, Mukala K, Pasanen P, Tiittanen P, Ruuskanen J, Tuomisto J, Jantunen MJ (1998). Personal NO_2 exposures of preschool children in Helsinki. J Expos Anal Environ Epidemiol 8, 79-100.

II   Mukala K, Pekkanen J, Tiittanen P, Alm S, Salonen RO, Jantunen M, Tuomisto J (1996). Seasonal exposure to NO_2 and respiratory symptoms in preschool children. J Expos Anal Environ Epidemiol 6, 197-210.

III   Mukala K, Pekkanen J, Tiittanen P, Alm S, Salonen RO, Tuomisto J (1999). Personally measured weekly exposure to NO_2 and respiratory health among preschool children. Eur Respir J 13, 1411-1417.

IV   Mukala K, Alm S, Tiittanen P, Salonen RO, Jantunen M, Pekkanen J (1999). Nitrogen dioxide exposure assessment and cough among preschool children. Arch Environ Health, provisionally accepted

CONTENTS

1 INTRODUCTION

During the past two decades in Finland, the emissions of sulphur dioxide, nitrogen oxides and particulates from power plants and various industries have decreased considerably. This has improved the quality of ambient outdoor air. Meanwhile, the traffic has grown heavily, and since the early 1990s the emissions of carbon monoxide, nitrogen oxides, volatile organic compounds and fine particulates have increased continously. Nitrogen dioxide (NO_2) is an oxidant gas, derived from the combustion of atmospheric nitrogen and formed by oxidation of nitric oxide (NO) in the presence of ozone (O_3) and UV-light. In Finnish cities, the major outdoor sources of nitrogen oxides are gasoline and diesel-fuelled vehicles. Common indoor sources, especially in Central Europe, are unvented gas or kerosene combustion appliances, such as gas stoves and water boilers.

The health effects of inhaled NO_2 are not well understood, despite extensive research in laboratories and in various clinical and epidemiological settings (Samet and Utell 1990, Berglund et al. 1993, USEPA 1993, WHO 1997). Experimental animal studies have suggested proinflammatory toxicity in the lower respiratory tract, increased susceptibility to bacterial lung infections, and irreversible emphysema-like structural changes. The NO_2 levels used in these studies have usually been rather high (Berglund et al. 1993, USEPA 1993, WHO 1997). The health effects of the much lower NO_2 levels commonly measured in the urban air of many developed countries are difficult to characterize. This may be at least partially due to the fact that the levels of NO_2 in urban air vary with other potentially harmful pollutants (carbon monoxide, fine and ultrafine particles, hydrocarbons, O_3) and function also as an indicator of this air pollution mixture in epidemiological studies. There is evidence, however, that repeated NO_2 exposure can be associated with increased respiratory disorders and impaired lung function in children (USEPA 1993, WHO 1995).

The study presented in this thesis was planned to investigate the time-integrated NO_2 exposure and daily respiratory health among preschool children in Helsinki. The purpose was to assess exposure with personal and microenvironmental measurement techniques and to test the association between the personal NO_2 exposure and respiratory health.

2 REVIEW OF THE LITERATURE

2.1 Nitrogen dioxide (NO_2)

Nitrogen dioxide (NO_2) is a reddish brown gas with a sweet odour. It is relatively insoluble in water (0.037 ml/ml H_2O under standard conditions) and a strong oxidant. From the viewpoint of human exposure, NO_2 is the most important of a large and complex group of nitrogen oxides. In the atmosphere, nitrogen dioxide is formed from nitric oxide (NO) in photochemical reactions:

(1) $\quad 2NO + O_2 \rightarrow 2NO_2$

(2) $\quad NO + O_3 \rightarrow NO_2 + O_2$

(3) $\quad HO_2 + NO \rightarrow NO_2 + OH$

(4) $\quad RO_2 + NO \rightarrow RO + NO_2$
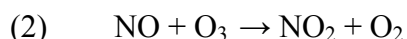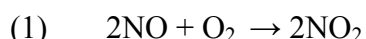
where R is a part of an organic compound; OH, HO_2 and RO_2 are reactive radicals (Berglund et al. 1993). Nitrogen dioxide is, along with NO, a chief regulator of the oxidizing capacity of the free troposphere through controlling the build up and fate of radical species, including hydroxyl radicals. It plays also a critical role in determining ozone (O_3) concentrations in the troposphere, because the photolysis of NO_2 is the key initiator of the photochemical formation of O_3 (USEPA 1993, USEPA 1995). NO_2 absorbs visible solar radiation and thus plays an important role in the formation of photochemical smog. By photolysis, NO_2 is converted to NO and atomic oxygen:

(1) $\quad NO_2 + hv \rightarrow O + NO$

(2) $\quad O + O_2 \rightarrow O_3$
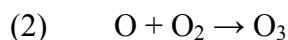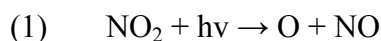
On a global scale, emissions of nitrogen oxides from natural sources far outweigh those generated by human activities. Natural sources include intrusion of stratospheric nitrogen oxides, bacterial and volcanic action and lightning. These natural emissions are responsible for the atmospheric background concentrations of nitrogen oxides, which range annually from 0.4 to 9.4 µg/m^3 (WHO 1995). The major source of anthropogenic emissions of nitrogen oxides into the atmosphere is the combustion of fossil fuels in stationary sources (heating, power generation) and vehicle engines. Common indoor sources of NO_2 are unvented combustion appliances such as gas stoves, gas-fired water heaters and kerosene space heaters (Samet 1987, Leaderer 1982). Another important indoor source of personal NO_2 exposure is cigarette smoke.

Fossil fuel power stations, motor vehicles and domestic combustion appliances emit nitrogen oxides, mostly in the form of NO, but some (usually less than 10%) as NO_2 (WHO 1997). The ambient air concentrations of NO and NO_2 tend to be highest in cities. In megacities, the contribution of mobile emissions sources of nitrogen oxides has varied between 19 to 90% in the late 1980s and early 1990s (WHO 1997). In Finland, the man-made emissions of nitrogen oxides in 1991 totalled 290 000 tons, of which road traffic emissions came to 152 000 tons (52%). In big cities, the availability of ozone slows down the formation of NO_2 from NO.

The annual mean concentrations of NO_2 in urban areas throughout the world are generally in the range of 20-90 $\mu g/m^3$ and the hourly maximum values range from 75 to 1015 $\mu g/m^3$ (USEPA 1993, Berglund et al. 1993). Urban outdoor levels vary according to the time of day, season and meteorological factors (e.g., temperature, inversion, wind speed, sunlight). The urban background levels are superimposed by one or two higher peaks that correspond to rush-hour traffic emissions of nitrogen oxides. In Finland, the urban outdoor NO_2 levels are generally higher during winter and spring due to heating and frequent temperature inversions.

2.2 Exposure to NO_2

2.2.1 Routes of exposure and fate in humans

Nitrogen dioxide exists in the environment as a gas, and thus, the only relevant route for human exposure is inhalation. As NO_2 is rather insoluble in water, it descends to the small bronchioles and alveoli. Morphological studies in animals suggest that NO_2 is absorbed along the entire respiratory tract. At rest during normal breathing, 81-90% of the inhaled NO_2 was absorbed in healthy adults when they were exposed to NO_2 concentrations 560-13550 $\mu g/m^3$ (Berglund et al. 1993). In adult asthmatic subjects, the average absorption was 73% when they were exposed to NO_2 560 $\mu g/m^3$ (Bauer et al 1986). During exercise with maximal ventilation, the absorption was 90% for both healthy and asthmatic subjects. At rest, approximately 50% of the inhaled NO_2 was absorbed by the nasal mucosa. The nasal intake of NO_2 decreased to less than 20% with increasing oral breathing during exercise (Mohsenin 1994). Most of the absorbed NO_2 enters blood as nitrite (NO_2^-) and is excreted in urine as nitrate (NO_3^-) (Berglund et al. 1993).

2.2.2 Respiratory system

The respiratory system can be divided into three major compartments: the nasopharyngeal, tracheobronchial and pulmonary regions. The nasopharyngeal region extends from the nostrils to the larynx and consists of a well-vascularized mucosa, which warms and humidifies the inhaled air before it passes through the tracheobronchial tree. The tracheobronchial region contains the trachea, bronchi and bronchioles, which serve as conducting airways between the nasopharynx and the gas-exchange surfaces of the lungs.

The pulmonary region includes respiratory bronchioles, alveolar ducts and sacs, alveoli and associated tissues, including capillaries and lymphatic vessels. Gas exchange occurs in the pulmonary region of the lungs. The type I pneumocytes compose more than 90% of the alveolar surface area. These cells are thin and thus facilitate the exchange of gases. The cuboidal type II pneumocytes which compose only 7% of the alveolar surface area, can develop into type I pneumocytes after a pulmonary injury from inhaled toxins (Brewis and White 1995).

2.2.3 Pulmonary defense mechanisms

There are versatile defense mechanisms in the respiratory system to protect against the harmful effects of inhaled substances. The defense mechanisms include olfaction, bronchoconstriction, clearance, airway epithelium, alveolar macrophages, biochemical mechanisms, and immunological mechanisms. Some of the most important mechanisms are briefly described below.

2.2.3.1 Olfaction

The olfactory system can be viewed as an important defense mechanism from the toxicological point of view. The recognition of airborne odorants may result in avoidance or other appropriate adaptive behaviour which reduces further exposure. The threshold for the stinging, suffocating odour of NO_2 is between 200 and 400 $\mu g/m^3$ (WHO 1987).

2.2.3.2 Bronchoconstriction

Bronchoconstriction limits pulmonary exposure to inhaled toxins by reducing air flow to the lungs. Acute inhalation of high concentrations of environmental chemicals such as NO_2 and O_3 has been associated also with an increase in nonspecific bronchial responsiveness (Boushey et al. 1980). Bronchoconstriction is reflected in lung function measurements such as peak expiratory flow (PEF) and spirometry. Increased nonspecific bronchial responsiveness is reflected in increased diurnal variability of PEF, especially decreased morning PEF values, and in higher potency or greater responses to pharmacological (histamine, methacholine) or other (exercise, cold air) tests.

2.2.3.3 Clearance mechanisms

The clearance mechanisms of the respiratory system provide an important nonspecific mechanism for the removal of particulate material and dissolved chemicals from the airway surfaces. The mucociliary transport system in the tracheobronchial region moves free particles, dissolved gases and vapours, and

macrophages containing ingested particles upwards to the pharynx, from where they are swallowed or expectorated. In the pulmonary region, clearance occurs first via phagocytosis by alveolar macrophages, followed by their removal via the mucociliary escalator, blood capillaries or lymphatic vessels. The clearance depends on the physiochemical properties of the particles.

2.2.3.4 Airway epithelium

Airway epithelial cells produce many types of inflammatory mediators that can promote alterations in the microvascular permeability and recruit inflammatory cells to the site of injury. Undamaged epithelial cells produce endogenous mediators that help to maintain the normal tone of the smooth muscle of airways.

2.2.3.5 Biochemical mechanisms

Biochemical defense mechanisms in the respiratory system include protective proteins, antioxidative mechanisms, and metabolism of xenobiotics. Most studies indicate that the pulmonary and nasal tissues have a greater metabolic capacity against inhaled organic environmental chemicals than the conducting parts of the respiratory tract.

Enzyme systems are important in metabolizing compounds into more water-soluble forms, which can be eliminated more easily from the body. These systems cover a broad range of biological transformations of inhaled organic toxins. They can also bioactivate compounds with relatively low toxicity into more reactive metabolites capable of causing cellular injury, leading potentially to the induction of cancer.

Alfa$_1$-antitrypsin is a major protective protein in the lung. This molecule functions as an antiprotease. Because the exposure of alveolar surfaces to oxidants (e.g., NO_2, O_3) can alter the binding of alfa$_1$-antitrypsin and proteases (e.g., neutrophil elastase), an antioxidant system may further protect the respiratory system against chemical injury.

2.2.3.6 Immunological mechanisms

The immune system is an important defense against respiratory infections. The humoral part of this system primarily involves B-cells that function in the synthesis and secretion of antibodies (immunoglobulins G, A, M) into the blood and body fluids. The cell-mediated component primarily involves T-lymphocytes, which are responsible for delayed hypersensitivity, and defend against viral, fungal, bacterial, and neoplastic diseases. Alveolar macrophages may be responsible for the initiation of humoral and cellular immune responses as they become activated by the

ingestion of particles or soluble antigens. They can process or present antigens for specific antigen-reactive B and T cells (Berglund et al. 1993).

2.2.4 Susceptible population groups

Population groups considered susceptible to air pollution are children, elderly persons, cigarette smokers, asthmatic persons, and those with coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD). In infants, the respiratory defense mechanisms are immature, and even in older children they are still in the developing phase. Children are at increased risk to contract respiratory infections. Elderly people often have impaired respiratory defenses and reduced functional reserves. They also have an increased risk for respiratory infections, and for clinically significant effects of lung function. Cigarette smokers have impaired clearance and other defense mechanisms and possible lung injury, which may lead to increasing damage through synergism when they are exposed to air pollutants. Asthmatic subjects have a pre-existing airway inflammation and increased responsiveness, which increases the risk for exacerbation of respiratory symptoms during and after exposure to air pollution. Subjects with CHD have a reduced myocardial oxygenation capacity and their risk for myocardial ischemia is increased. In persons with COPD, several lung functions are often severely reduced, and the exposure to air pollution increases the risk for clinically significant further impairment (Utell and Samet 1995).

2.2.5 Human exposure to NO_2

People are exposed to nitrogen oxides in a number of outdoor and indoor environments. In urban areas, the major source of outdoor nitrogen oxides is traffic (WHO 1997). The NO concentrations in urban areas usually follow traffic density, while the variations in NO_2 concentrations are more determined by meteorological factors.

Indoor NO_2 levels are determined by several factors: penetration from outdoor air, presence and strength of indoor sources (e.g., gas stove, water boiler, kerosene heater), air exchange rate, and reactive decay by interior surfaces. The indoor levels of NO_2 in homes without indoor sources are generally lower than the outdoor levels, and they are higher in summer than in winter. In homes with indoor sources, the NO_2 levels are usually higher than the outdoor levels, and they are lower in summer than in winter.

The ratio between indoor and outdoor NO_2 concentrations has varied in numerous studies: 0.4-0.9 in homes without indoor sources (Melia et al. 1980, Dockery et al. 1981, Nitta et al. 1982, Spengler et al. 1983, Clausing et al. 1986, Leaderer et al. 1986, Quackenboss et al. 1986, Harlos et al. 1987, Ryan et al. 1988), 1.1-3.2 in homes with gas stove (Melia et al. 1980, Dockery et al. 1981, Spengler et al. 1983, Clausing et al. 1986, Leaderer et al. 1986, Quackenboss et al. 1986, Harlos

et al. 1987, Ryan et al. 1988), and 2.1-4.8 in homes with both gas stove and other unvented gas appliances (Hoek et al. 1984, Leaderer et al. 1986).

Direct personal exposure to outdoor NO_2 concentrations generally accounts for only a small proportion of a person's total daily exposure because of the short time typically spent outdoors. On the average, people spend about 90% of their time indoors, about 5% in transit (Chapin 1974) and 7% near smokers (Quackenboss et al. 1982). As exposure is a function of concentration and time, the integrated NO_2 exposure is the sum of the individual NO_2 exposures over all possible time intervals for all microenvironments. A microenvironment is defined as a three-dimensional space having a volume, in which the pollutant concentration is considered or assumed to be spatially uniform (USEPA 1993). The assessment of a person's total NO_2 exposure can be represented by the following basic model first described by Fugas 1975:

$$E = \Sigma_i\, E_i = \Sigma_i f_i\, c_i$$

where

E is	the total or integrated exposure to NO_2 for an individual,
E_i	the average NO_2 exposure in the ith microenvironment,
f_i	the fraction (percentage) of time spent in the ith microenvironment, and
c_i	the NO_2 concentration in the ith microenvironment (USEPA 1993).

Exposure to very high nitrogen oxide concentrations can occur in occupational environments: agriculture (225-3760 mg/m^3), military activities, chemical industry, mining fires and fire-fighting (940 mg/m^3) and welding (180 mg/m^3) (Mohsenin 1994). High levels of NO_2 have also been measured inside ice-arenas, where gas-fuelled ice resurfacers have been used. Hourly or weekly NO_2 concentrations have ranged from 100 μg/m^3 to about 8000 μg/m^3 (Berglund et al.1993, Brauer et al. 1997, Pennanen et al. 1997).

2.2.6 Exposure assessment

Exposure to NO_2 can be assessed by either direct or indirect methods. Direct methods include personal monitoring and biological markers. Indirect methods refer to the modelling of personal exposure on the basis of measurements of NO_2 concentrations in various environments.

2.2.6.1 Direct methods

2.2.6.1.1 Personal monitoring

Personal air monitoring provides a direct, individual measurement of air pollutant concentrations in the breathing zone. Personal monitors can be either passive dosimeters (e.g., Palmes tubes or Yanagisawa badges), providing integrated data on the exposure, or portable monitors with data loggers, providing nearly continuous data on the exposure. The vast majority of all personal NO_2 exposure data have been collected with passive dosimeters. The Palmes tubes (Palmes et al. 1976) and the NO_2 badges (Yanagisawa and Nishimura 1982) are passive samplers that utilize diffusion to control the delivery of gases to the collection medium. The samplers are then returned to the laboratory for chemical analysis.

Studies have been conducted to assess how well microenvironmental NO_2 measurements (outdoor, indoor average, kitchen, bedroom, living room, etc.), combined with time-activity diaries, explain the variation in daily or weekly personal NO_2 exposure as measured by passive samplers (Hoek et al. 1984, Clausing et al. 1986, Leaderer et al. 1986, Noy et al. 1986, Quackenboss et al. 1986, Harlos et al. 1987, Koo et al. 1990, Noy et al. 1990). These studies have indicated that outdoor NO_2 concentrations are usually poor predictors of personal exposure in most population groups. The average indoor residential concentrations (e.g., whole house or bedroom) tend to be the best predictors of personal NO_2 exposure, typically explaining 50 to 80% of the variation in personal exposure.

2.2.6.1.2 Biological markers

The biological markers of exposure are cellular, biochemical, or molecular measures that can be obtained from biological media such as human tissues, cells, or fluids, and they are indicative of exposure to environmental chemicals (National Research Council 1989). Biomarkers themselves do not provide information on the environment in which the exposure takes place and hence on the factors which control the exposure. Urinary hydroxyproline (breakdown product of pulmonary connective tissue) (Yanagisawa et al. 1986), nitric oxide / haeme protein-complex in bronchoalveolar lavage fluid (Maples et al. 1991) and 3-nitrotyrosine in urine (Oshima et al. 1990) have been suggested as biological markers for NO_2, but currently there are no validated biomarkers available for NO_2 exposure.

2.2.6.2 Indirect methods

Indirect estimations of personal NO_2 exposure are based on both microenvironmental monitoring and questionnaires on activities to estimate the total exposure of an individual, population group, or whole population. Time-weighted average exposure models have been developed to estimate the total personal exposure (Fugas 1975, Duan 1982, Ott 1982). The NO_2 exposure levels predicted from these

models have been shown to correlate with the exposure levels obtained from direct personal measurements (Quackenboss et al. 1986, Nitta and Maeda 1982). An indoor/outdoor residential model (Dryer et al. 1989, Billick et al. 1991) has been developed from a simplified version of the general mass-balance equation. This model has been regarded as promising, but it has not yet been adequately validated (USEPA 1993). The equation of the model is:

$$C_{in} = mC_{out} + b,$$

where

C_{in} is   the indoor NO_2 concentration ($\mu g/m^3$),

m the penetration coefficient for outdoor NO_2,

C_{out} the outdoor NO_2 concentration ($\mu g/m^3$), and

b the NO_2 concentration contribution by indoor sources ($\mu g/m^3$).

In many epidemiological studies on the health effects of NO_2 the exposure has been assessed on the basis of measurements in only one environment (outside or inside the home, ambient air monitoring station), and total exposure has not been measured. Early studies have compared the respiratory health status among children living in homes with and without gas stoves and used a simple categorical variable of the NO_2 exposure: the presence or absence of a gas cooker. The use of adequate exposure assessment is particularly important for health studies, because there are great variations in NO_2 concentrations in different outdoor and indoor microenvironments, and because the risk to human health is determined by personal NO_2 exposure.

2.3 Health effects of NO_2

The toxic effects of NO_2 have been investigated in epidemiological studies and in experimental human and animal studies. Accidental exposure to high NO_2 concentrations has caused a biphasic clinical disorder, the severity of which has depended on the intensity and duration of exposure (Jones et al. 1973). In the report of Douglas et al. (1989), the health outcomes of 17 patients examined for silo-filler's disease ranged from hypoxaemia and transient airway obstruction, even to death (NO_2 levels from 370 mg/m³ to 3760 mg/m³).

2.3.1 Epidemiological studies

The epidemiological health studies in association with NO_2 exposure cover a wide range of health outcomes, from acute irritative symptoms and lung function responses to chronic pulmonary diseases such as asthma and chronic bronchitis. Much of the present information relates to NO_2 exposure via ambient outdoor air, where NO_2 is one of several pollutants. In the past, studies on NO_2 exposure from gas

appliances have received much attention, as concomitant exposure to other pollutants is assumed to be less important indoors than outdoors. The health effects associated with outdoor NO_2 in epidemiological studies can be divided according to the investigated period of exposure into short-term and long-term effects. Short-term effects usually concern changes in mortality, symptoms and pulmonary function in association with assessed hourly peak, daily or weekly NO_2 exposure. Long-term effects are mainly reported as the prevalence of symptoms or disease in association with annual NO_2 exposure.

Symptoms and diseases of the respiratory tract, which are common in children, are often divided into upper respiratory tract symptoms/diseases and lower respiratory tract symptoms/diseases. Symptoms of the upper respiratory tract used in epidemiological studies include runny nose, cold, earache, and sore throat. Lower respiratory tract symptoms include wheezing, colds going to the chest, persistent cough and diseases such as bronchitis, asthma and pneumonia.

2.3.1.1 Effects of short-term exposure

A number of studies have focused on the associations between short-term variations in ambient air pollutant concentrations and in respiratory symptoms or lung function in children. Table 1 gives a summary of epidemiological studies on health outcomes in association with short-term NO_2 exposure among children.

Studies among adults have used somewhat different health outcomes such as mortality, hospital admissions, respiratory symptoms and lung function. A summary of epidemiological studies on health outcomes in association with short-term NO_2 exposure among adults is shown in Table 2.

TABLE 1: Health outcomes in association with short-term NO_2 exposure among children

Country	Sample	Exposure	Results	Comments	Reference
Australia	Respiratory symptoms in 388 children aged 6-11 yrs in four schools with electric heating and four with unflued gas heating in Sydney	Hourly peaks in the order ≥ 150 $\mu g/m^3$, background levels 38 $\mu g/m^3$	Mean symptom rates for sore throat, colds and absenteeism were statistically significantly higher in the high NO_2 exposure group (≥ 75 $\mu g/m^3$) than in the low exposure group (< 75 $\mu g/m^3$)	Adjusted for asthma, early severe chest illness, allergies/hay fever, geographical area, age ($<9/9+$), gender	Pilotto et al., 1997
Denmark	Home visits or telephone contacts (average 133 per day) with children 0-15 yrs All + respiratory disease contacts	Daily mean NO_2 around 40-100 $\mu g/m^3$ (Concentration values taken from a figure)	Increase of 188 $\mu g/m^3$ $NO_x \rightarrow$ increase of 6 consultations for children with respiratory illness per day	Diagnoses given only for 44% of all contacts. Diagnoses were lacking especially on days with high counts	Keiding et al., 1995
England U.K.	1025 consultations in children with wheezy episodes, and 4285 control children	1-h mean NO_2 for all seasons 60 $\mu g/m^3$	Increase in NO_2 17 $\mu g/m^3 \rightarrow$ OR 1.02 (95% CI 0.96-1.09)	Adjusted for season and temperature	Buchdahl et al., 1996
England U.K.	20 infant symptoms registered among 921 infants (3-12 mo) in Bristol	Median 2-wk NO_2 in bedrooms 13 $\mu g/m^3$ and outside homes 24 $\mu g/m^3$	No evidence of a significant association between NO_2 and respiratory symptoms	OR for diarrhoea 1.38 (95% CI 1.11-1.70)	Farrow et. al., 1997
Finland	Respiratory tract diseases and absenteeism due to febrile illness in children in day-care centres and schools in Helsinki in 1987	Weekly mean NO_2 ($\pm$SD) 47 $\pm$ 12.5 $\mu g/m^3$, range 28.5-81.1 $\mu g/m^3$	Significant association (p<0.015) with upper respiratory tract infections diagnosed at health care centres, but not with absenteeism	Sosioeconomic and demographic aspects not taken into account, limited number of air pollution monitoring sites	Pönkä 1990

Finland	PEF and respiratory symptoms (upper and lower) in children (7-12 yrs) with chronic respiratory symptoms in urban (n=85) and suburban (n=84) panels in Kuopio	Average daily mean NO_2 28 $\mu g/m^3$ in urban area and 14 $\mu g/m^3$ in suburban area	With a 2-day lag, NO_2 was negatively associated with morning PEF deviation in asthmatic children. No association between NO_2 and symptoms	Adjusted for time trend, minimum temperature, relative humidity, weekend and autocorrelation	Timonen & Pekkanen 1997
Germany	6330 cases of croup in children aged 0-6 yrs and 4755 cases of obstructive bronchitis	Study mean NO_2 14-55 $\mu g/m^3$ (of the 24-h values)	Increase in daily mean NO_2 from 10 to 70 $\mu g/m^3$ → 28% increase in croup cases. No association between NO_2 and obstructive bronchitis	Controlled for short-term weather factors and time trend. Correlation between NO_2 and TSP	Schwartz et al., 1991
Germany	Change in lung function in 467 school aged children (7-16 yrs), of which 106 had asthmatic symptoms in Freiburg	Median weekly outdoor NO_2 concentrations (Palmes tubes) during heating period (from October to April) 12-51 $\mu g/m^3$	An increase in NO_2 of 1 $\mu g/m^3$ exceeding the threshold level of 40 $\mu g/m^3$ → reduced lung function ($FEV_1\%/FVC$ decrease 4.4%) in children with asthmatic symptoms. No association between NO_2 and lung function in healthy children		Moseler et al., 1994
Germany	1854 school children aged 9-11 yrs in Leipzig	Half-hourly NO_x maxima up to 500 $\mu g/m^3$	Daily incidence of upper respiratory symptoms: OR 1.53 (95% CI 1.01-2.31) in winter and 1.82 (1.21-2.73) in summer	Values of NO_2 concentrations not given, only plotted in a figure	Von Mutius et al., 1995
Hong Kong	362 primary schoolchildren, aged 7-13 yrs	Personal daily mean NO_2: 34.5 $\mu g/m^3$ for boys, 35.7 $\mu g/m^3$ for girls	No association between personal NO_2 exposure and lower respiratory tract symptoms		Koo et al., 1990
The Netherlands	1078 schoolchildren, aged 7-11 yrs	Daily mean (±SD) NO_2 37 ± 15.8 $\mu g/m^3$, daily maximum 70 $\mu g/m^3$	An increase in NO_2 by 50 $\mu g/m^3$ → 1% decrease in FVC and FEV_1, and 2% in PEFR and MMEF. No effect on respiratory symptoms		Hoek & Brunekreef 1994

The Netherlands	Acute respiratory symptoms in 300 school children, aged 7-11 yrs	Daily maximum NO_2 62 $\mu g/m^3$	No association with incidence or prevalence of respiratory symptoms		Hoek & Brunekreef 1995
Norway	Occurence of bronchial obstruction in children below 2 yrs of age, 153 one-to-one matched pairs in Oslo	Two-week mean ($\pm$SE) NO_2 15.65 $\pm$0.60 $\mu g/m^3$ among cases and 15.37 $\pm$0.54 $\mu g/m^3$ among controls	No association with risk of developing bronchial obstruction, and no association between symptoms and proximity to car traffic		Magnus et al., 1998
Sweden	1137 emergency visits among asthmatic children (<15 yrs) from Jan 1990 to May 1993 in Halmstad	Daily mean ($\pm$SD) (6 a.m. – 6 p.m.) NO_2 17.2 $\pm$ 11.4 $\mu g/m^3$, 1-h maximum 131 $\mu g/m^3$	Daily asthma visits: high (2 to 8 cases) / low (0 to 1), mean NO_2 concentration 19.0 $\mu g/m^3$ vs 16.6 $\mu g/m^3$ (p=0.006); winter type climate/pollution	No values for particulates. Fixed-site monitoring at one station	Holmen et al., 1997
USA	1844 school children in six cities	Study median of 24-h NO_2 values 25 $\mu g/m^3$, and maximum 83 $\mu g/m^3$	An increase in NO_2 by 18.5 $\mu g/m^3$ on preceding 4-days$\rightarrow$ OR for cough incidence 1.27 (95% CI 1.04-1.56). An increase in NO_2 by 18.5 $\mu g/m^3$ on previous day $\rightarrow$ incidence of lower respiratory disorders OR 1.2 (0.98-1.47)	Adjusted for temperature, day of the week and city	Schwartz et al., 1994
USA	Lung function and respiratory symptoms in 269 schoolaged children (4th & 5th grades) in LA	Daily mean ($\pm$SD) NO_2 70 $\pm$ 28 $\mu g/m^3$	1.8 $\mu g/m^3$ increase in preceding 24-h NO_2 $\rightarrow$ 0.40mL decrease in morning FEV (95% CI –0.07-0.73).	Adjusted for year, season, day of the week, year-season interaction	Linn et al., 1996

TABLE 2: Health outcomes in association with short-term exposure to NO_2 among adults

Country	Sample	Exposure	Results	Comments	References
England U.K.	75 patients with asthma or COPD	Daily maximum NO_2 84 $\mu g/m^3$	No effect on PEFR; Effect on wheezing and bronchodilator use in a single pollutant model, disappeared with SO_2 in the same model	Strong correlation between NO_2 and SO_2, OR or CI not given	Higgins et al., 1995
England U.K.	Weekly mortality in London and England during smog episode in December 1991	Hourly average NO_2 up to 795 $\mu g/m^3$. Four daily means over 174 $\mu g/m^3$, daily mean for black smoke up to 148 $\mu g/m^3$	Relative risks (RR) for episode week versus predicted: total mortality 1.1; cardiovascular mortality 1.14; respiratory mortality 1.22; hospitalisation of people over 65 for respiratory diseases 1.19; obstructive lung diseases 1.43. No increase in hospitalisation of children (0-14 yrs)	Comparison of smog week with expected numbers for this week, calculated from the previous week of the same year and from equivalent periods in previous 4 years	Anderson et al., 1995
England U.K.	Daily mortality in London from April 1987 to March 1992	1-h maximum NO_2 696 $\mu g/m^3$, Daily maximum NO_2 342 $\mu g/m^3$, Daily mean ($\pm$SD) NO_2 70 $\pm$ 23 $\mu g/m^3$	In warm season (Apr. to Sep.) increase in daily mean NO_2 from 43 to 100 $\mu g/m^3$ $\rightarrow$ increase in cardiovascular mortality by 2.54% (95% CI 0.18-4.96%) and increase in maximum 1-hr NO_2 from 66 to 167 $\mu g/m^3$ $\rightarrow$ increase in cardiovascular and total mortality by 2.96% (0.8-5.17), and by 1.73% (-0.20-3.29), respectively and decrease in respiratory mortality by -4.54% (-8.52-0.38). No association in cool season.	Correlation with O_3 in warm season could explain the health associations. Model adjusted for secular trend, seasonal and other cyclical factors, day of the week, holidays, influenza epidemic, temperature, humidity and autocorrelation.	Anderson et al., 1996

Finland	Respiratory tract diseases and absenteeism due to febrile illness among adults in Helsinki in 1987	Weekly mean NO_2 ($\pm$SD) 47 ± 12.5 $\mu g/m^3$, range 28.5-81.1 $\mu g/m^3$	Significant association (p<0.015) with upper respiratory tract infections diagnosed at health care centres and with absenteeism	Sosioeconomic and demographic aspects not taken into account, limited number of air pollution monitoring sites	Pönkä 1990
Finland	Daily emergency room attendances (n=232) for asthma attacks among adults (aged 15-85 yrs) in Oulu during one year	Daily mean NO_2 13.4 $\mu g/m^3$ (range 0-69) and daily 1-h maximum 38.5 $\mu g/m^3$ (range 0-154) during the study year	A significant (p<0.0001) correlation between NO_2 and attendances, which was stronger in winter than in summer	Interaction with meteorological factors makes it difficult to confirm the role of any specific factor	Rossi et al., 1993
Finland	Daily hospital admissions (n=2807) of patients with chronic bronchitis and emphysema in Helsinki in 1987-1989.	Daily mean NO_2 ($\pm$SD) during the 3-year period 39 ± 16.2 $\mu g/m^3$, range 4-170 $\mu g/m^3$	With 1 to 3-day lags, a nonsignificant association between NO_2 and daily hospital admissions, and with a 6-day lag a significant association between NO_2 and daily admissions among patients >64 yrs		Pönkä & Virtanen 1994
Finland	Daily hospital admissions (n=2421) for asthma in Helsinki during 1987-1989 in age-groups 0-14, 15-64 and > 64 yrs	Daily mean NO_2 during the 3-year period 39 $\mu g/m^3$	No association was found	Health associations were sensitive to model specifications	Pönkä & Virtanen 1996
Greece	Emergency consultations and hospitalisations in greater Athens	Mean ($\pm$SD) of daily maximum 1-h NO_2 concentrations in winter 94 ± 25 $\mu g/m^3$ and in summer 111 ± 32 $\mu g/m^3$	Increase in NO_2 by 76 $\mu g/m^3$ (95th-5th percentile) in winter $\rightarrow$ increases by 11% in total, 15% in cardiac and 25% in respiratory admissions. No effect in summer	No other pollutant but NO_2 significantly associated with admissions	Pantazopoulou et al., 1995

Hong Kong	319 mothers of school children	Personal daily mean NO_2 24 – 43 $\mu g/m^3$	NO_2 associated with allergic rhinitis and chronic cough in non-smoking mothers		Koo et al., 1990
Spain	Emergency consultations among asthmatics (15-65 yrs) in Barcelona	Average 1-h maximum NO_2 in summer 104 $\mu g/m^3$ and in winter 101 $\mu g/m^3$	Increase of 25 $\mu g/m^3$ in 1-h maximum $NO_2 \rightarrow$ 4.5% increase in consultations in summer and 5.6% in winter		Castellsague et al., 1995
Sweden	2990 emergency visits of asthmatics (>15 yrs) from Jan. 1990 to May 1993 in Halmstad	Daily mean (±SD) (6 a.m. – 6 p.m.) NO_2 concentration 17.2 ± 11.4 $\mu g/m^3$	No association between NO_2 and asthma visits	No values on particulate concentrations. Fixed-site monitoring at one station	Holmen et al., 1997
USA	Daily mortality in Los Angeles County	Study mean (±SD) NO_2 130 ± 53 $\mu g/m^3$ in 1970-79	Increase of 100 $\mu g \rightarrow$ Increase of 2% in daily death counts		Kinney & Oezkaynak, 1991
USA	Hospitalisation of people over 65 yrs with cardiovascular diseases in seven large cities	Daily maximum NO_2 75-145 $\mu g/m^3$	Increase of 185 $\mu g/m^3$ in $NO_2 \rightarrow$ RR for congestive heart failure admission in Los Angeles 1.15 (95% CI 1.1-1.19), Chicago 1.17 (1.07-1.27), Philadelphia 1.03 (0.95-1.12), New York 1.07 (1.02-1.13), Detroit 1.04 (0.92-1.18), Houston 0.99 (0.88-1.10), Milwaukee 1.05 (0.89-1.23)	Association persisted in a multipollutant model only in New York. A consistent association between CO and admissions	Morris et al., 1995

2.3.1.2 Effects of long-term exposure

2.3.1.2.1 Indoor exposure

Several epidemiological studies on indoor environments have suggested that repeated NO_2 exposure increases the rate of respiratory illness in children, although often the associations have not been statistically significant (USEPA 1993). Unlike in outdoor studies, monitoring of short-term indoor exposure often reflects also the long-term exposure.

A meta-analysis of eleven indoor studies originally published between the late 1970s and early 1990s has been published by Hasselblad et al. (1992). The goal of their analysis was to estimate the odds ratio corresponding to each increase of 28.3 $\mu g/m^3$ in NO_2 exposure. Two models were employed for combining the evidence: the fixed-effects model and the random-effects model. The first model assumed that every study had estimated the same parameter, whereas the second model assumed that the parameter of interest was not fixed, but was a random variable from a distribution. The main conclusion of the meta-analysis was that each increase of 28.3 $\mu g/m^3$ in the estimated 2-week average NO_2 exposure corresponded to a 20% increased in the risk of respiratory symptoms or disease among children aged 5-12 years. The mean weekly NO_2 concentrations in bedrooms were predominantly between 15 and 122 $\mu g/m^3$ in 4 out of 11 studies reporting NO_2 levels (Hasselblad 1992). In a study in the Netherlands, there was no clear association between the measured NO_2 exposure and respiratory illness among children aged 6 years when the NO_2 levels in the kitchen were 144 $\mu g/m^3$, in the bedroom 80 $\mu g/m^3$ and in ambient air 45 $\mu g/m^3$ (Hoek et al. 1984).

According to a meta-analysis based on studies conducted among children aged 2 years or younger, the combined odds ratio for the increase in respiratory disease per increase of 28.2 $\mu g/m^3$ in NO_2 was 1.09 (95% CI 0.95-1.26). In the studies analysed, the mean weekly NO_2 concentrations in bedrooms ranged between 9.4 and 94 $\mu g/m^3$ (WHO 1997).

2.3.1.2.2 Outdoor exposure

The associations between long-term exposure to outdoor NO_2 and respiratory health are unclear. In many outdoor studies conducted in the 1990s, statistically significant associations have been found between long-term NO_2 exposure and respiratory health both among children and adults. However, a major difficulty in the analysis of this kind of data is to distinguish the possible effects of NO_2 from those of other co-existing air pollutants. A summary of epidemiological studies on health outcomes in association with long-term exposure to outdoor NO_2 among children is shown in Table 3, and a corresponding summary of studies among adults in Table 4.

TABLE 3: Health outcomes in association with long-term exposure to outdoor NO_2 among children

Country	Sample	Exposure	Results	Comments	References
Austria	Prevalence of asthma and respiratory symptoms among 843 children (7 yrs) in eight non-urban communities	3-year mean NO_2 measured at central monitoring stations 11-32 $\mu g/m^3$	The prevalence odds ratios for "ever asthma" 1.28 (95% CI 0.20-7.98), 2.14 (0.40-11.3) and 5.81 (1.27-26.5), in low, moderate and high NO_2 communities compared with very low NO_2 communities, respectively. A similar trend for wheeze and "cough apart from colds"	Adjusted for gender, age, parental education, passive smoking, type of indoor heating and parental asthma	Studnicka et al., 1997
Sweden	199 children with hospital-treated wheezing, bronchitis or asthma, and 351 population controls (4 mo-4 yrs)	Outdoor air pollution, gas stove. Estimated outdoor NO_2 (time-weighted 99th percentile of 1 h-values): 20-205 $\mu g/m^3$ in different study subjects (mean 55 $\mu g/m^3$)	LRI[a] associated with estimated outdoor NO_2 exposure in girls (p=0.02), but not in boys. Gas stove homes: RR = 1.9 (95% CI 0.7-5.0) in girls, 1.1 (0.5-2.2) in boys.	Adjusted for asthma heredity and maternal smoking	Pershagen et al., 1995
Switzerland	Respiratory symptoms in 625 children (0-5 yrs)	Annual average NO_2 47 and 51 $\mu g/m^3$ (outdoor) and 22 and 31 $\mu g/m^3$ (indoor) in two cities	Increase in NO_2 (outdoor and indoor) by 20 $\mu g/m^3$ → OR for duration of any respiratory episode 1.11 (95% CI 1.07-1.16), and 1.16 (1.12-1.21), and for upper respiratory symptoms 1.14 (1.03-1.25) and 1.18 (1.01-1.38), respectively	TSP[b] was a predictor of both incidence and duration of respiratory symptoms. High correlation between outdoor passive NO_2 and TSP	Braun-Fahrländer et al., 1992

Switzerland	Respiratory and allergic symptoms and diseases among 4470 school children (6-15 yrs) in 10 Swiss communities	Annual mean NO_2 16-50 µg/m³	Comparison between the most and least polluted community: OR for chronic cough 1.58 (95% CI 1.14-2.18), nocturnal dry cough apart from colds 1.99 (1.51-2.61); bronchitis 1.35 (0.99-1.85), conjunctivitis 1.5 (1.15-1.96). Wheeze, asthma, hay fever and diarrhoea not significant	Adjusted for socioeconomic status, passive smoking, family history of respiratory diseases, number of siblings, gas appliances, indoor humidity	Braun-Fahrländer et al., 1997
USA	3922 children and adolescents (6-24 yrs)	Annual mean of 1-h NO_2 values of the preceding 365 days for each person: 10th and 90th percentiles 47 and 118 µg/m³ , respectively	Non-linear negative correlation (p<0.05) between FVC, FEV_1, and PEFR, and annual mean NO_2. Correlation more pronounced over NO_2 75 µg/m³	Controlled for age, height, race, sex, body mass, smoking and respiratory symptom.	Schwartz 1989

[a] LRI , lower respiratory tract illness (bronchitis, asthma, pneumonia, chest illness)
[b] TSP, total suspended particulates

TABLE 4: Health outcomes in association with long-term exposure to outdoor NO_2 among adults

Country	Sample	Exposure	Results	Comments	References
Sweden	>6000 subjects in towns	Half-year mean NO_2 in winter 10-30 μg/m^3	Throat irritation and irritating or unproductive cough during the last three months associated with NO_2 levels		Forsberg et al., 1991
USA	6340 Seventh Day Adventists in California	10 year mean: 11th percentile of study population exposure 37μg/m^3, 68th percentile 74 μg/m^3, 78th percentile 92 μg/m^3	Increase of 93 μg/m^3 in city monitor data for NO_2 → increase in incidence of COPD over 10 yrs (p=0.05, RR not given). Increase in adjusted (personal) NO_2 →RR for COPD 1.26 (95% CI 0.58-4.33)	Personal exposure estimated using life-style and housing characteristics as indoor sources. Estimation of ambient concentration from three nearest city monitors.	Abbey et al., 1993

COPD, chronic obstructive pulmonary disease

2.3.1.3 Studies on health effects in Finland

The health effects of NO_2 have been investigated in three Finnish cities. In Oulu, respiratory symptoms and PEF values were not associated with daily concentrations of SO_2 or NO_2 in a daily follow-up study among asthmatic children (Mannila 1988). In a time-series study among adult asthmatic subjects, increased daily visits to the hospital emergency clinic due to asthma attacks were associated with increased levels of air pollutants, especially NO_2 (Rossi et al. 1993).

In a time-series study in Helsinki, increased NO_2 levels were associated with absence from work among adults, but among children there was no association with absence from day-care centres or schools (Pönkä 1990). In the same study, the weekly occurrence of upper respiratory infections diagnosed at health care centres associated positively with SO_2 and NO_2 concentrations. A positive association was found between daily mean NO_2 concentration and hospital admissions of elderly subjects with chronic bronchitis and emphysema in Helsinki (Pönkä and Virtanen 1994), but not between NO_2 and daily hospital admissions of asthmatic subjects (Pönkä and Virtanen 1996).

In a panel study of children (aged 7-12 years) with chronic respiratory symptoms in Kuopio, daily NO_2 concentrations were significantly associated with declines in morning PEF among asthmatic children, but not among children with cough only (Timonen and Pekkanen 1997).

2.3.1.4 Key findings and uncertanties

According to the reviewed literature, short-term and long-term NO_2 exposure seems to be significantly associated with respiratory morbidity among children. These associations are rather consistent in studies on children aged 5 to 12 years. A seasonal difference in the association was found by von Mutius et al. (1995), and the association was stronger in summer than in winter (Table 1). In many of the reviewed studies on outdoor air pollution, there was a strong correlation between other co-existing air pollutants (especially particulates), and NO_2 (e.g., Braun-Fahrländer et al. 1992, and 1997, Linn et al. 1996), thus making it difficult to distinguish the effects of NO_2.

Among adults, the associations of short-term and long-term NO_2 exposure with respiratory health have probably not been as consistent as in children, although some studies have shown significant associations between outdoor NO_2 concentrations and respiratory mortality or morbidity.

2.3.2 Experimental human studies

In experimental human studies, volunteers have been exposed for a specified time to NO_2 in an exposure chamber, and possible effects on the respiratory system have been recorded. Both healthy persons and those with asthma or COPD have participated in these studies.

2.3.2.1 Lung function

Experimental studies have shown that NO_2 can cause bronchoconstriction in healthy, resting subjects at two-hour concentrations of 4700 µg/m^3 (WHO 1997). When the NO_2 concentrations have been below 2000 µg/m^3, there have been no significant acute effects on lung function (Berglund et al. 1993). For asthmatic subjects, the results have been contradictory: some studies have indicated statistically significant slight effects on spirometry and airway resistance at NO_2 concentrations of about 500 µg/m^3, but other studies using similar concentrations have reported no effects (Berglund et al. 1993). Patients with COPD have shown a significant further reduction in their lung function after some hours of exposure to NO_2 560 µg/m^3 (Berglund et al. 1993).

2.3.2.2 Airway responsiveness

The information on the dose and time relationships of NO_2 -induced changes in airway responsiveness are not fully understood. The meta-analysis of Folinsbee (1990) shows that there is a statistically significant effect on nonspecific bronchial responsiveness in asthmatic persons at NO_2 concentrations above 190 µg/m^3 and in normal subjects at concentrations above 2000 µg/m^3.

Tunnicliffe et al. (1994) have reported that NO_2 concentrations that can be encountered in home environments (190 µg/m^3 and 760 µg/m^3) potentiated the specific airway response of mild asthmatic subjects to inhaled house dust mite allergen. Strand et al. (1998) have shown that repeated short-lasting exposure to an ambient level of NO_2 enhances the airway response of asthmatic subjects to a non-symptomatic allergen. Among asthmatic subjects, the increased airway responsiveness appears to be influenced by the exposure protocol, most importantly the level of exercise. In a study among 15 asthmatic subjects, inhalation of NO_2 (560 µg/m^3) potentiated exercise-induced bronchospasm and airway hyperresponsiveness to subsequent cold air (Bauer et al. 1986).

In studies on cells and mediators in bronchoalveolar lavage fluid after NO_2 exposure of normal subjects, a decreased alpha-1-protease inhibitory activity was found after exposure to NO_2 6000-8000 µg/m^3 (Mohsenin and Gee 1987), but not after NO_2 3000 µg/m^3 (Johnson et al. 1990). Increased numbers of mast cells and lymphocytes were found 4, 8 and 24 hours after exposure to NO_2 8000 µg/m^3 (Sandström et al. 1990a). After seven exposures to NO_2

8000 $\mu g/m^3$ during 12 days, there were decreased numbers of mast cells, T-suppressor cells, B-lymphocytes and NK-cells (Sandström et al. 1990a and 1990b).

2.3.3 Experimental animal studies

Generally, the effects of NO_2 on experimental animals have been seen at higher levels than those causing effects on humans. There is also a clear species-specific difference in sensitivity to NO_2, which makes it difficult to extrapolate results from animals to humans. In animal studies, decreased pulmonary function has occurred in mice after chronic (>1 yr) NO_2 exposure to 375 $\mu g/m^3$ with regular peaks of 1500 $\mu g/m^3$ (WHO 1997). Morphological changes in the lungs (e.g., cell destruction, cell replacement) have been seen after subchronic exposure to 640 $\mu g/m^3$ (Berglund et al. 1993) or three-day exposure to 3760 $\mu g/m^3$ (WHO 1997).

Nitrogen dioxide has increased the susceptibility of animals to bacterial and viral pulmonary infections. The murine infectivity model, used in several studies to investigate the effects on defence mechanisms, has demonstrated an increased susceptibility to bacterial infections after one-year exposure to NO_2 375 $\mu g/m^3$ with regular peaks of 1500 $\mu g/m^3$ (USEPA 1993, WHO 1997). A decreased function of alveolar macrophages (reduced phagocytic activity and mobility) has been seen after 13 days of exposure to NO_2 500 $\mu g/m^3$ (WHO 1997). Nitrogen dioxide has induced also several biochemical alterations (e.g., lipid peroxidation, antioxidant defense mechanisms, protein function) but the relevance of these findings to the observed toxicity of NO_2 in vivo is not known (Berglund et al. 1993).

Long-term exposure to high NO_2 concentrations ($\geq$9400$\mu g/m^3$) has caused emphysema-like structural changes in animals: thickening of alveolar capillary membrane, loss of ciliated epithelium, and increase in lung collagen (Berglund et al. 1993, USEPA 1993, WHO 1997). The type of emphysema has been similar to that in human lungs.

The body of experimental animal work comparing different exposure regimens has clearly shown the respiratory effects to depend on the NO_2 concentration, exposure duration and exposure profile, rather than simply on the cumulative NO_2 exposure (concentration x duration) (USEPA 1993, WHO 1997).

3 AIMS OF THE STUDY

The general aim of this thesis was to investigate the personal exposure of preschool children to NO_2 in Helsinki, and the possible health effects caused by this exposure.

The specific aims were:
1.   to assess the weekly NO_2 exposure of preschool children and the determinants of this exposure (I),

2.   to investigate the association between seasonal (II) and weekly (III) average NO_2 exposures, and the respiratory health of preschool children ,

3.   to compare different exposure assessment methods with regard to their association with respiratory health (IV).

4 MATERIALS AND METHODS

4.1 Study area

Helsinki, the capital of Finland, is located at 60° North latitude, 0-35 meters above sea level. The total area of the city is 185 km^2. The centre of the city is situated on a peninsula with an area of 23 km^2. The main sources of air pollution in Helsinki are motor traffic, energy production and industry. Because more than 90% of the buildings are connected to the municipal district heating system, local heating emissions are almost nonexistent. Traffic remains the most important air pollution source at ground level, causing almost all carbon monoxide (CO) emissions, over 90% of nitrogen oxide emissions, and most of the emissions of hydrocarbons and suspended particulates. The air quality has been monitored in Helsinki since 1983. In 1991, there were nine permanent, multicomponent air quality monitoring stations installed at heights ranging from 2 to 10 m above street level. A regular diurnal concentration pattern was seen at these stations, with peak values of CO, NO, NO_2 and hydrocarbons coinciding with rush hours. In 1991, the highest 24-h average NO_2 concentration was 115 µg/m^3 and the corresponding 24-h average sulphur dioxide (SO_2) concentration was 131 µg/m^3. The concentration of total suspended particulates (TSP) was measured every third day, and the annual average concentration at different monitoring sites varied between 37 and 102 µg/m^3. The annual average concentration of inhalable particulates (PM_{10}) varied between 18 and 30 µg/m^3 (Aarnio et al. 1992)

4.2 Selection of study population

Eight municipal day-care centres were selected from Helsinki; four in suburban areas and the other four in the central area. All 363 children from these day-care centres were asked to participate in the study. A total of 246 (68%) children, aged three to six years, returned the baseline questionnaire and the letter of consent signed by the parents. The questionnaire inquired the sociodemographic factors and home characteristics of the family and the health status of the child. Most of the children lived within 2 km of their day-care centre, and spent there 6-9 hours on weekdays. The characteristics of the children are shown in Table 1/I.

4.3 Study design

The prospective panel study was conducted between October 1990 and June 1991. It was divided into three eight-week periods: autumn, winter and spring. In each period the children's exposure to NO_2 was measured as a weekly average with Palmes tubes, and their

symptoms were followed with a daily symptom diary filled in by the parents. Lung function was followed among a subgroup of children conducting PEF measurements at home (II).

4.4 NO$_2$ exposure assessment

The integrated weekly average NO$_2$ exposure of the children was assessed by using passive diffusion samplers (Palmes tubes) in three different ways: personally, outside the day-care centre and inside the day-care centre. The accepted collection time was 168 ± 24 h. The personal NO$_2$ measurements were conducted by fixing the tubes on the outer garments of the children near the breathing zone. Outside the day-care centres, the tubes were placed in the children's playground at the height of 1.5-2 m. Inside the day-care centres, the tubes were placed in the playing/sleeping room at the same height as outside. All the tubes were changed by the researchers once a week: on Mondays in the central area and on Tuesdays in the suburban areas. The absorbed NO$_2$ was determined as nitrite by ion chromatography (I). Ambient air NO$_2$ concentrations were monitored with chemiluminescence analyzers (Environnement AC 30 M) at three fixed sites of the Helsinki Metropolitan Area Council (YTV) network (I).

4.5 Recording of symptoms

The parents were asked to record their child's symptoms every day in a diary. The symptoms included runny nose, stuffy nose and nasal discharge, dry cough, phlegm from the chest, breathing difficulties (dyspnoea, wheezing) at rest and during exercise, ear-ache and discharge, itching and redness of the eyes, watering of the eyes and eye discharge, fever, abdominal pain, diarrhoea, and emesis. The severity of the symptoms was graded into three categories: 1 = weak, hardly noticeable symptoms; 2 = moderate symptoms not restricting the child's daily activities; and 3 = severe symptoms restricting the child's daily activities. If the child had none of these symptoms on a particular day, this was marked in a separate category of "no symptoms". Visits to a physician and administration of drugs were also recorded in the diary. To encourage the keeping of the diaries, they were distributed and collected once a week at the day-care centres at the same time as the samplers.

4.6 PEF measurements

Measurements of PEF were performed among 53 children using the mini-Wright meters (Airmed, Clement Clarke International Ltd., Essex, England). The recordings were taken twice a day at home: morning PEF after getting up and evening PEF at bedtime (III). On both occasions, the parents were asked to record the highest value from three successful PEF measurements.

4.7 Data analysis

The NO_2 and health data from six weeks in winter and seven weeks in spring were included in the final analyses for publications I-IV due to quality assurance measures in the databases (I-IV).

The selection of the study population in the original publications II-IV on health effects was basically the same, but the inclusion criteria in the last two publications contained more variables than in publication II. Consequently, there were fewer subjects in the statistical analyses of publications III and IV. Symptoms were recorded on a daily basis and NO_2 exposure was assessed on a weekly basis. In publication II on seasonal NO_2 exposure, the inclusion criteria were at least 60% of the daily diary data per season and 2/6 (winter) or 3/7 (spring) Palmes tubes per season. In publication III on weekly NO_2 exposure, the inclusion criteria were as follows: 1) at least 60% of daily diary data per season, 2) personal NO_2 measurement and diary data from the same study week, 3) complete diary data for every day of the week, 4) complete questionnaire data, and 5) the last week of both seasons was excluded, because there were only two children fulfilling the criteria 1-4. In publication IV comparing of different NO_2 exposure methods, the inclusion criteria were the same as in publication III, but complete data on NO_2 measurements both the inside and outside the day-care centres were also required.

The SAS$^©$ statistical package was used in the statistical analysis (SAS Institute Inc., Cary, NC, USA) (SAS Institute Inc. 1989). Differences in NO_2 exposure between different exposure groups (defined by smoking, stove type and living area) were tested with t-test (I). The between and within-children variance components of NO_2 exposure were estimated with PROC NESTED (SAS, 1989) (I). The association between the NO_2 exposure and personal covariates was examined with multiple linear regression (I).

The Mann-Whitney U-test was used to test the differences in seasonal median NO_2 exposure between central and suburban areas (II). The corresponding differences in symptom outcomes were tested with the Chi-square test (II). The associations between each risk factor and the incidence and prevalence of cough and nasal symptoms were examined by using the general estimating equations approach (GEE) (Zeger and Liang 1986) (II, III, IV). The analyses on the association between NO_2 exposure and symptoms in publications II-IV were adjusted for stove type, smoking, parental education and area (II) or day-care centre (III-IV), and in publications III and IV also for allergy.

Multiple linear regression was used to examine the association between the personal NO_2 exposure and the weekly average PEF deviation (the MIXED procedure)(SAS Institute Inc., 1996) (III). The analyses on the association between NO_2 exposure and PEF were adjusted for stove type, smoking, parental education, area, allergy and first-order autocorrelation (III).

5 RESULTS

5.1 Exposure

5.1.1 Levels of personal NO$_2$ exposure (*I-III*)

Palmes tubes were feasible for measurements of personal NO$_2$ exposure among 3 to 6 year-old preschool children in low ambient air NO$_2$ levels (I). The NO$_2$ exposure in the central area was statistically significantly higher than in the suburban area, most likely reflecting a difference in the density of motor traffic (I, II, III). The seasonal median NO$_2$ exposure was 27.4 μg/m^3 in the central area and 18.2 μg/m^3 in the suburban areas (range 11-45.8 μg/m^3) (II). Similar average NO$_2$ exposures were observed also at the weekly level: 26 μg/m^3 and 18 μg/m^3 during the winter season and 28 μg/m^3 and 17 μg/m^3, during the spring season, respectively (III).

There was no difference in the geometric mean NO$_2$ exposures between boys and girls (I). Smoking at home increased significantly the children's NO$_2$ exposure (24.6 μg/m^3 vs 20.4 μg/m^3, p<0.0001) (I). In the central area, the NO$_2$ exposure of children living in homes with gas stoves was significantly higher than the exposures of children living in homes with electric stoves (29.5 μg/m^3 vs 24.9 μg/m^3, p<0.0001) (I).

5.1.2 Comparison of weekly NO$_2$ concentrations measured with different methods (*I, IV*)

The comparison of the fixed-site, outside the day-care centre, inside the day-care centre, and personal NO$_2$ concentrations between winter and spring, showed that in the central area the fixed-site monitoring levels were lower in the spring, but that all other NO$_2$ measurements displayed lower levels in winter (Table 6/I, Table 1/IV). During both seasons, the median NO$_2$ concentration measured with personal tubes was lower than the median NO$_2$ concentration measured with other methods both in the central and suburban areas (Table 6/I, Table 1/IV).

5.1.3 Correlation between personal and stationary NO$_2$ data (*I, IV*)

In the analysis of individual levels, the NO$_2$ concentrations measured outside the day-care centres usually correlated most strongly with the personal NO$_2$ concentrations (Table 2/IV). At the population level analysis, however, the concentrations measured outside and inside the day-care centres correlated about equally strongly with the personal concentrations (Fig. 5/I; Fig. 2/IV). The latter correlations were usually stronger than the correlations between the fixed-site NO$_2$ concentrations from the YTV monitoring network and the personal NO$_2$ concentrations (Table 7/I; Table 2/IV; Fig. 2/IV). All correlations between the

personal and stationary NO_2 concentrations were much stronger in spring than in winter (Table 7/I, Table 2/IV, Fig. 2/IV).

5.1.4 Within and between-children variation of NO_2 exposure (*I*)

The total variation in personal NO_2 exposure was due more to the differences in the weekly exposure of each child (59%) than to the differences between children (41%) (Table 5/I). In the central area, the differences between the children were greater and contributed more to the total variation in exposure than in the suburban areas (28% vs 14 %) (Table 5/I). The between-children variation was greater in spring than in winter, whereas the within-child variation was greater in winter (Table 5/I).

5.1.5 Seasonal exposure models (*I*)

Seasonal exposure models were established from the questionnaire data and the NO_2 concentrations measured inside and outside the day-care centres and at the fixed-site monitoring stations. The spring model explained much better the variance in the average personal NO_2 exposure than the winter model ($R^2 = 0.67$ vs 0.32) (Table 9/I).

5.2 Health outcomes
5.2.1 Symptom prevalence (*II*)

The proportion of days with eye symptoms, ear symptoms, or breathing difficulties was small in both areas during both seasons (Table 2/II). The children in the central area had statistically significantly ($p < 0.001$) more days with cough (18% vs. 15%) and a stuffy nose (26% vs. 20%) than the children in the suburban areas (Table 2/II).

5.2.2 Seasonal association between personal NO_2 exposure and cough and nasal symptoms (*II*)

No consistent association was found between the personal NO_2 exposure and the prevalence or incidence of cough or nasal symptoms. At the individual level, there was a non-significant positive correlation between personal NO_2 exposure and cough in spring (Table 4/II).

5.2.3 Association between personal weekly NO_2 exposure and nasal symptoms (*III*)

Because there was a seasonal difference in the association between the personal NO_2 exposure and nasal symptoms at the weekly level, the winter and spring data were analyzed separately. In both seasons, there was no increase in the risk of lag0 (exposure and

symptoms from the same week) nasal symptoms with increasing NO_2 exposure (Table 4/III). The risk of lag2 (the symptom period starting two days after the beginning of the exposure period) nasal symptoms tended to increase in the higher NO_2 categories during the winter season, but the association was not statistically significant. During spring, there was a non-significant negative association between the personal NO_2 exposure and the risk of nasal symptoms (both lag0 and lag2 data) (Table 4/III).

5.2.4 Association between personal weekly NO$_2$ exposure and cough (*III*)

At the weekly level, an increased risk of cough (lag0 data) was associated with increasing personal NO_2 exposure (RR=1.52, 95% CI 1.00-2.31) (Table 4/III). With lag2 data, the risk was slightly lower and statistically non-significant (Table 4/III). The adjusted risk ratio (lag0 data) in the highest NO_2 category was slightly reduced when SO_2 or O_3 was added to the model (RR=1.50, 95% CI 1.00-2.26 and RR=1.47, 95% CI 0.96-2.25, respectively), and slightly increased when TSP was added to the model (RR=1.55, 95% CI 1.01-2.40) (III).

5.2.5 Different exposure assessment methods and their association with cough (*IV*)

When the associations between different exposure assessment methods and cough were examined, there was a statistically significant association between the personal weekly NO_2 exposure and cough in winter (Table 3/IV). There were positive non-significant trends between cough and NO_2 concentrations measured outside the day-care centres, inside the day-care centres, or at the fixed-site monitoring stations in winter (Table 3/IV). In spring, the risk of cough increased non-significantly in the highest NO_2 category of all other exposure assessment groups, except the fixed-site monitoring group (Table 3/IV).

5.2.6 Lung function (*III*)

In spring, there was a non-significant negative trend between the personal weekly NO_2 exposure and weekly average PEF deviations in the morning and evening. In winter, there was no clear association (Table5/III).

6 DISCUSSION

6.1. Exposure to NO$_2$

6.1.1 Feasibility

Palmes tubes have been widely used in measurements of NO$_2$ concentrations (Spengler et al. 1983, Clausing et al. 1986, Quackenboss et al. 1986, Harlos et al. 1987, Brunekreef et al. 1990; Noy et al. 1990). In this study, it was feasible to measure the time-weighted average NO$_2$ exposure of preschool children with passive diffusion samplers, but it was difficult to control the proper use of the tubes during the weekly measurement periods. Consequently, a large number of tubes had to be rejected for a number of reasons, which, however, could be expected in this kind of long-term study (I). A disadvantage of passive samplers is that they give a time-weighted average exposure but no information on short-term peak exposures. It has been suggested that short-lasting peaks might actually be more harmful to respiratory health than somewhat elevated long-term average levels. (WHO 1987, USEPA 1993, Berglund et al.1993, WHO 1995).

6.1.2 NO$_2$ concentrations

The measured ambient air NO$_2$ concentrations in Helsinki are similar to corresponding concentrations in major central European cities (Kukkonen et al. 1999). In this study, all measurement methods gave higher weekly NO$_2$ concentrations in the central area than in the suburban areas. This reflects most likely the difference in the density of motor traffic, because other sources of nitrogen oxides are scarce. However, all of the few children living in homes with gas stoves were from the central area. In the suburban areas, the NO$_2$ concentrations were higher in winter than in spring, as reported in previous studies (Berglund et al. 1993). In the central area, however, the NO$_2$ concentrations were higher in spring when measured with methods other than fixed-site monitoring. Nitrogen oxide emissions are mainly in the form of nitric oxide, which is rapidly oxidized in the atmosphere into NO$_2$ in the presence of O$_3$ and UV-light. Consequently, the formation of NO$_2$ levels off when there is no O$_3$ available, and near a high density of mobile emission sources the NO$_2$ concentration is lower than the emissions would predict. This is true especially in the central area of Helsinki during the winter season when there is less UV-light and O$_3$ available. The fixed-site monitors in the present study were situated at a height of 2 to 10 m above street level, where somewhat more O$_3$ might have been available, thus explaining the differences in NO$_2$ concentrations between the different methods in winter. According to the fixed-site monitoring data collected during our study, the ratio of NO/NO$_2$ was constantly greater than one in the central area, and usually less than one in the suburban area.

6.1.3 Determinants of exposure

A gas stove and smoking at home increased personal NO_2 exposure, which is in agreement with previous studies (Dockery et al. 1981, Noy et al. 1986, Quackenboss et al. 1986, Clausing et al. 1986, Noy et al. 1990, Spengler et al. 1994, Good et al. 1982, Leaderer et al. 1986, Neas et al. 1991). In the central area, the median NO_2 exposure of children living in homes with gas stoves was among the lowest of previously published results (29 $\mu g/m^3$ vs. 25-80 $\mu g/m^3$), which might have been due to a more effective local venting of indoor air pollution caused by gas-stove cooking. In contrast, the median NO_2 exposure of children living in homes with electric stoves was among the highest of previous results (25 $\mu g/m^3$ vs. 9-26 $\mu g/m^3$) (I). There was an overlap in the exposure of children from gas stove and electric stove homes, which would have led to a misclassification of exposure, if the personal exposure category had been decided only on the basis of stove type (I). The contribution of smoking to the personal NO_2 exposure was statistically significant contrary to some previous studies. These above-mentioned findings might have been due to differences in building construction and ventilation and to scarcity of other indoor sources of nitrogen oxides in Finnish homes (I).

6.1.4 Correlation between data from personal and stationary samples

Successful personal NO_2 monitoring provides the most accurate estimate of total exposure, but it can be used only in selected epidemiological studies (WHO 1997). In order to compare different exposure assessment methods in a health effect study, we measured the children's NO_2 exposure in three different ways with passive samplers: personally, inside the day-care centre and outside the day-care centre. In addition, we used NO_2 data received from the municipal air quality monitoring network. In this study, the personal NO_2 exposures of the children were systematically below the ambient air NO_2 concentrations, which is consistent with the results from other studies in the absence of indoor sources (Dockery et al. 1981, Clausing et al. 1986, Quackenboss et al. 1986, Berglund et al. 1993). In addition, the mean personal exposures were lower than the NO_2 concentrations inside and outside the day-care centres (Table 6/I; Table1/IV).

According to the present study, the NO_2 levels inside and outside the day-care centres explained the weekly population exposure far better than the corresponding data from the fixed-site monitoring stations (Fig.5/I; Fig. 2/IV). However, there were a seasonal differences, so that the correlations between the personal NO_2 concentrations and NO_2 concentrations measured outside and inside the day-care centres and at the fixed-site monitoring stations were higher in spring than in winter.

The NO_2 concentrations measured outside the day-care centres explained best the personal NO_2 exposure, both in winter (r=0.48, p<0.0001) and in spring (r=0.74, p<0.0001) (Table 7/I; Table 2/IV). Compared with previous studies, the correlation was at the same level in winter, but was better in spring (Spengler et al. 1994, Raaschou-Nielsen et al. 1997).

In general, outdoor NO_2 measurements (home, school-yard, ambient air monitoring site) (Nitta et al. 1982, Quackenboss et al. 1982, Leaderer et al. 1986) are regarded as poorer predictors of long-term average personal exposure than measurements inside homes (Quackenboss et al. 1982, Noy et al. 1986, Noy et al. 1990). However, the usefulness of indoor measurements at work, at school or in day-care centres is still uncertain, and Linaker et al. (1996) have recommended personal exposure monitoring in studies investigating potential health effects of NO_2 in children.

6.1.5 Within and between-children variation of exposure

The NO_2 exposures of children living in gas stove homes showed greater week-to-week variation than the exposures of children living in homes with electric stoves which corresponds with previous studies (Quackenboss et al. 1982, Spengler et al. 1983, Clausing et al. 1986, Quackenboss et al. 1986, Spengler et al. 1994).

The between-children variation (41% of the total variation) was slightly less than the within-children variation (59%). When the central area and suburban area groups were analyzed separately, the between-children variations fell to 28% and 14%, respectively. The greater between-children variation in the central area was probably due to the greater heterogeneity of indoor sources.

According to the present study, the NO_2 exposure levels on a particular week for two children living in the same area are closer to each other than the exposure of a single child on two different weeks. This temporal variation in NO_2 exposure, and in respiratory health, suggests that exposure should be considered as a spatial phenomenon, in which repeated NO_2 measurements in relevant microenvironments should be preferred rather than single personal NO_2 measurements when investigating the association between exposure and health effects. This conclusion is also supported by the present finding that the best model for personal exposure estimation was based on the stationary measurements in the children's neighbourhood (inside and outside the day-care centres and fixed-site monitors) and on the information of home characteristics. The important contribution of indoor sources to the personal NO_2 exposure has been shown in many studies (WHO 1997), but gas appliances and smoking inside homes seem to be relatively infrequent in Finland.

6.1.6 Exposure models

As mentioned in the previous section, the best model for personal exposure estimation among preschool children was based on the stationary measurements in the children's neighbourhood, and on the information on home characteristics (I). This finding agrees with other studies, in which the best models have also included both the home characteristics and the NO_2 concentrations measured inside and/or outside homes. The previous models have explained 60-87% of the exposure variation (Dockery et al. 1981,

Clausing et al. 1986, Quackenboss et al. 1986, Noy et al. 1986, Harlos et al. 1987, Noy et al. 1990, Xue et al. 1993).

In the present study, there was a seasonal difference in the explanatory power of the best models: 71% of the personal exposure variation in spring, and 33% in winter. This seasonal difference is important and should be taken into account in epidemiological studies using stationary NO_2 monitoring. Nitrogen dioxide measurements conducted only in winter may lead to overestimation of long-term average exposure and thus underestimation of health effects per unit change in NO_2 exposure. When models of this kind are used for personal NO_2 exposure estimation, a separate model should be made for each season on the basis of personal measurements carried out in a subgroup of the study population.

6.2 Respiratory health effects of NO_2

6.2.1 Cough

An increased risk of cough was associated with personal NO_2 exposure among preschool children in Helsinki, where the ambient air NO_2 concentrations are usually below the present guideline values. The recently updated Finnish 1-h and 24-h guideline values for NO_2 are 150 $\mu g/m^3$ and 70 $\mu g/m^3$ (Council of State Decree 1996). The corresponding WHO annual guideline value is 40 $\mu g/m^3$ and the 1-h value is 200 $\mu g/m^3$. During the study year, 1991, the annual average NO_2 concentration in Helsinki was between 31 and 47 $\mu g/m^3$ at different municipal monitoring stations. According to the WHO report (1997) a no-effect level for subchronic or chronic NO_2 exposure cannot be determined according to the present scientific data.

The recent results from NO_2 health studies among children are not consistent, but in a majority of studies there have been significant associations between the estimated exposure and health outcomes (Schwartz et al. 1991, Schwartz et al. 1994, von Mutius et al. 1995, Buchdahl et al. 1996, Braun-Fahrländer et al. 1997, Holmen et al. 1997). The ambient air NO_2 was not associated with the incidence or prevalence of respiratory symptoms among children aged 7 to 11 years in the Netherlands, when the daily maximum NO_2 concentration was 62 $\mu g/m^3$ (Hoek et al. 1995). In the present study, there was a positive association between the personal NO_2 exposure and cough, both in the seasonal and weekly analyses, but the association was significant only at the weekly level. The addition of other air pollutants to the model either reduced (SO_2, O_3) or increased (TSP) the risk ratio. The concentrations of ambient air pollutants tend to be highly intercorrelated, and therefore it is difficult to pin-point the contribution of one air pollutant to a health outcome. Braun-Fahrländer et al. (1997) have formulated this problem as follows: "As long as only one-pollutant models can be considered, every pollutant variable stands for the complex mixture of pollutants in the outdoor air rather than for one specific component."

Cough is a non-specific and common symptom, and in fact it is one of the defense mechanisms of the airways. It is, however, biologically plausible that there was a significant association between personal NO_2 exposure and cough, but not between exposure and nasal

symptoms. Nitrogen dioxide is a strong oxidant, and relatively insoluble in water. Consequently, the primary deposition site of NO_2 in the respiratory tract is the centri-acinar region (Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society 1996) thus making NO_2 a deep lung irritant with a potential of inducing cough through chemoreceptors.

6.2.2 Nasal symptoms

The non-significantly increased risk of nasal symptoms after a 2-day lag in winter might have been partly due to both the NO_2 exposure and the northern climatic conditions, because exposure to cold dry air in physically active subjects causes respiratory irritation. There is no clear explanation for the decreased risk of nasal symptoms with increasing NO_2 exposure in spring. Nasal symptoms are nonspecific, and they may be attributed to a variety of causes, e.g., pollen. However, adjusting for the pollen counts did not change the risk estimates.

6.2.3 Lung function

In the present study, there was a non-significant trend of lower PEF values with increasing personal weekly NO_2 exposures. This finding is consistent with previous studies, in which there have been no clear associations between low NO_2 concentrations and PEF values. (Vedal et al. 1987, Dijkstra et al. 1990). In the Netherlands, two weeks after an air pollution episode, a significant decrease was noted in PEF among children aged 6-12 years (Brunekreef et al. 1989), but after a second, milder episode, there were no significant changes in PEF among children aged 7-12 years (Hoek and Brunekreef 1993). The 1-h maximum NO_2 concentrations were 250 $\mu g/m^3$ and 127 $\mu g/m^3$, respectively.

The use of weekly average morning and evening PEF deviations in the present study might have been a too crude and insensitive way to estimate changes in lung function; and this may have weakened the association. The maturation of children´s lungs continues up to school age, and the resistance of the peripheral airways constitutes a large part of the total airway resistance in children under six years of age. It is possible that the PEF measurements reflected changes mainly in the calibre of the larger central airways and the coordination and strength of respiratory muscles, while the main target of NO_2 is the small bronchioles. In a Dutch study, a statistically significant association was found between the NO_2 concentrations measured in school and forced expiratory flows of 25-75% ($FEF_{25-75\%}$) in children living within a 300-m from a motorway, but the association between NO_2 concentration and PEF was not significant (Brunekreef et al. 1997). In order to clarify the association between NO_2 exposure and lung function among children, more epidemiological studies are needed, especially on short peak exposures.

6.2.4 Association with different exposure assessment data

Errors in measuring exposure potentially pose one of the most important methodological problems in epidemiological studies on NO_2 (USEPA 1993, WHO 1997). In several studies, NO_2 exposure has been assessed by categorising the existence of a gas stove (Hasselblad 1992). Moreover, in many studies utilizing direct NO_2 measurements, short measurement periods of e.g. 1 to 2 weeks have been taken to represent children's exposures over much longer periods (WHO 1997). This is also a potential source of misclassification, as the within-children variation usually composes a larger part of the total exposure variation than the between-children variation.

Misclassification of exposure can bias the estimation of effect measures, and affect the power of statistical tests (Bross 1954, Copeland et al. 1977, Shy et al. 1978, Greenland 1980, Lebret 1990, Navidi and Lurmann 1995). If the exposure measurement error is equally likely in both directions, and independent of the outcome (nondifferential), this usually reduces the possibility to find an association between exposure and health outcome (Shy et al. 1978, Greenland 1980, Lebret 1990). In the present study, we used personal passive diffusion samplers for all children over the entire study period, and the exposure measurement error was equally likely in both directions and independent of health outcome, i.e., cough.

In the seasonal analyses, there was a non-significant positive association between NO_2 exposure and cough. In these analyses, the children's exposure was calculated as the seasonal average of their personal weekly NO_2 concentrations during six to seven weeks. This procedure reduced the temporal variation of exposure and decreased the accuracy of exposure assessment, thus increasing the risk of misclassification. All in all, it weakened our possibilities to find an association between NO_2 exposure and cough.

In the weekly analyses, the association between fixed-site NO_2 concentrations and cough was seen only as a positive trend in winter. The NO_2 concentrations inside and outside day-care centres were regarded as microenvironmental data, giving a more accurate estimate of the exposure. In fact, the associations between these exposure estimates and cough were stronger than the association of cough with the fixed-site monitoring estimates. However, even the former associations were non-significant. Only when NO_2 exposure was assessed by using personal measurement data, was the association with cough statistically significant in winter with relatively broad confidence intervals (IV). The results thus demonstrated that the more accurate the exposure assessment is in the analyses, the stronger is the association between NO_2 exposure and cough.

At present, there is little information on the relative importance of peak vs. average NO_2 exposures in association with respiratory effects in humans (WHO 1997). If the health effects were largely associated with short-term NO_2 peaks, then the use of integrated weekly estimates of exposure would increase the measurement error. Although personal monitoring methods give a good estimate of the NO_2 exposure, they are still only a surrogate of the effective NO_2 dose in individual subjects.

7 CONCLUSIONS AND PUBLIC HEALTH IMPLICATIONS

During the study period (January 1991 to June 1991), the ambient air NO_2 concentrations in Helsinki were mostly below the recently updated Finnish air quality guideline values (1-h value 150 μg/m^3, 24-h value 70 μg/m^3) (Council of State Decree 1996). The personal weekly exposures of preschool children were below the corresponding ambient air values, and the exposures were significantly higher in the central area than in the suburban areas of Helsinki. The main determinants of personal NO_2 exposure were: NO_2 concentrations outside and inside the day-care centres, fixed-site NO_2 concentrations, residential area and home characteristics (gas stove, smoking inside, type of dwelling). The exposure of children to NO_2 derived from gas stoves is minimal in Finland, because mainly electric stoves are used for cooking. Also other indoor sources of NO_2 (gas heaters, water boilers) are scarce, and the prevalence of smoking inside the home is much lower than in many other countries. As the major point sources of NO_2 (power plants) are relatively well controlled, the most important way to reduce children's exposure to NO_2 both outdoors and indoors in Finnish cities is to reduce exhaust emissions from motor traffic.

In the seasonal analyses, there was no consistent association between the personal NO_2 exposure and the occurrence of cough or nasal symptoms, whereas in the weekly analyses there was a significant positive association between exposure and the risk of cough. This finding suggests that personal NO_2 exposure among susceptible population groups, such as small children, can lead to health outcomes even at low ambient air NO_2 concentrations which do not exceed current health-based air quality guidelines.

Personal and microenvironmental NO_2 monitoring methods should be used more often in epidemiological health studies, because they give a better estimate of the human exposure than does fixed-site NO_2 monitoring. The associations between short-term NO_2 peaks and respiratory health, and the possible long-term influences of ambient air NO_2 and pollutant mixtures on the developing lungs of children, should be elucidated in future studies.

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9 APPENDICES (! not available with this electronic version)

Appendix 1   Basic questionnaire

Appendix 2   Daily symptom diary

Appendix 3   PEF diary