

# Chapter 7.1

# Nitrogen dioxide

## General description

Many chemical species of nitrogen oxides ( $\text{NO}_x$ ) exist, but the air pollutant species of most interest from the point of view of human health is nitrogen dioxide ( $\text{NO}_2$ ). Nitrogen dioxide is soluble in water, reddish-brown in colour, and a strong oxidant.

Nitrogen dioxide is an important atmospheric trace gas, not only because of its health effects but also because (a) it absorbs visible solar radiation and contributes to impaired atmospheric visibility; (b) as an absorber of visible radiation it could have a potential direct role in global climate change if its concentrations were to become high enough; (c) it is, along with nitric oxide ( $\text{NO}$ ), a chief regulator of the oxidizing capacity of the free troposphere by controlling the build-up and fate of radical species, including hydroxyl radicals; and (d) it plays a critical role in determining ozone ( $\text{O}_3$ ) concentrations in the troposphere because the photolysis of nitrogen dioxide is the only key initiator of the photochemical formation of ozone, whether in polluted or unpolluted atmospheres (1, 2).

## Sources

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. Because natural emissions are distributed over the entire surface of the earth, however, the resulting background atmospheric concentrations are very small. 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 in motor vehicles (internal combustion engines).

In most ambient situations, nitric oxide is emitted and transformed into nitrogen dioxide in the atmosphere. Oxidation of nitric oxide by atmospheric oxidants such as ozone occurs rapidly, even at the low levels of reactants present in the atmosphere. Altshuller (3) calculated that 50% conversion of nitric oxide would take less than 1 minute at a nitric oxide concentration of  $120 \mu\text{g}/\text{m}^3$  (0.1 ppm) in the presence of an ozone concentration of  $200 \mu\text{g}/\text{m}^3$  (0.1 ppm). Consequently, this reaction is regarded as the most important route for nitrogen dioxide production in the atmosphere.

Other contributions of nitrogen dioxide to the atmosphere come from specific noncombustion industrial processes, such as the manufacture of nitric acid, the use of explosives and welding. Indoor sources include tobacco smoking and the use of gas-fired appliances and oil stoves. Differences in the nitrogen oxide (nitric oxide and nitrogen dioxide) emissions of various countries are due mainly to differences in the consumption of fossil fuels. Worldwide emissions of nitrogen oxides in the early 1980s were estimated at approximately  $150 \times 10^{12}$  g/year (1).

## Occurrence in air

Maximum 30-minute or 1-hour average and maximum 24-hour average outdoor nitrogen dioxide concentrations of up to  $940 \mu\text{g}/\text{m}^3$  (0.5 ppm) and  $400 \mu\text{g}/\text{m}^3$  (0.21 ppm), respectively, have been reported. Annual mean concentrations in urban areas throughout the world are generally in the range

20–90  $\mu\text{g}/\text{m}^3$  (0.01–0.05 ppm) (1, 4–6). Urban outdoor levels vary according to the time of day, the season of the year and meteorological factors. Typical daily patterns comprise a low background level on which are superimposed one or two peaks of higher levels that correspond to rush-hour traffic emissions of nitrogen oxides. Hourly average nitrogen dioxide concentrations near very busy roads often exceed 940  $\mu\text{g}/\text{m}^3$  (0.5 ppm) (7). Maximum hourly concentrations in the United Kingdom are generally of the order of 470–750  $\mu\text{g}/\text{m}^3$  (0.25–0.4 ppm) (5). From 1988 to 1990, highest 1-hour averages in the United States ranged from 75 to 1015  $\mu\text{g}/\text{m}^3$  (0.04–0.54 ppm) (1). Thus, the maximal hourly mean value may be several times the annual mean (1, 8). Long-term monitoring activities during the 1960s and 1970s indicated an increase in concentrations of nitrogen oxides in many urban areas throughout the world (9, 10). Recent reports from the United States, however, show a decrease in nationwide nitrogen dioxide concentrations, resulting from a decrease in nitrogen oxides emissions, from 1981 to 1990 (1). Whether nitrogen dioxide concentrations are increasing in urban areas in other parts of the world is unknown, although it is known that, at least in the United States, concentrations are highly correlated with population level (1) and population levels worldwide continue to grow. Nevertheless, indoor sources, such as cooking with gas or cigarette smoking, may be the main contributors to individual exposure.

Owing to the widespread use of unvented combustion appliances, nitrogen dioxide concentrations in homes may exceed considerably those found outdoors (1, 11). The average concentration over a period of several days may exceed 200  $\mu\text{g}/\text{m}^3$  (0.1 ppm) when unvented gas stoves are used for supplementary heating or clothes drying, or when kerosene heaters are used; typically, means are lower (1, 12–14). Maximum brief (minutes to 1-hour) concentrations in kitchens are in the range 230–2055  $\mu\text{g}/\text{m}^3$  (0.12–1.09 ppm) during cooking (15, 16). The highest 15-minute concentration recorded for a home with an unvented gas space heater was 2716  $\mu\text{g}/\text{m}^3$  (1.44 ppm) (17).

### Conversion factors

Nitrogen dioxide:

$$\begin{aligned} 1 \text{ ppm NO}_2 &= 1880 \mu\text{g}/\text{m}^3 \\ 1 \mu\text{g}/\text{m}^3 \text{ NO}_2 &= 5.32 \times 10^{-4} \text{ ppm} \end{aligned}$$

Nitric oxide:

$$\begin{aligned} 1 \text{ ppm NO} &= 1230 \mu\text{g}/\text{m}^3 \\ 1 \mu\text{g}/\text{m}^3 \text{ NO} &= 8.13 \times 10^{-4} \text{ ppm} \end{aligned}$$

### Routes of exposure

In the environment, nitrogen dioxide exists as a gas. Thus, the only relevant route of exposure to humans is inhalation, whether the source is outdoor air or indoor air. Occupational exposure is limited to a few industrial processes and includes a wide variety of exposures. Occupational exposures are relatively rare compared to outdoor and domestic exposures.

### Toxicokinetics

Owing, in part, to the high degree of scientific difficulties involved, very little research on the kinetics and metabolism of nitrogen dioxide has been conducted. The available information is very limited and only partially describes its deposition and fate in the respiratory tract.

On inhalation, 70–90% can be absorbed in the respiratory tract of humans, and exercise increases the total percentage absorbed (18, 19). A significant portion of the inhaled nitrogen dioxide is removed in the nasopharynx (about 40–50% in dogs and rabbits); thus, as breathing becomes more oral with exercise, an increased delivery to the lower respiratory tract can be expected (1, 4, 5, 20, 21).

Mathematical modelling studies show that the deposition of nitrogen dioxide in the tissues of the lower respiratory tract is predicted to be maximal at about the junction of the conducting airways and the gas exchange region of the lungs in humans, rats, guinea pigs and rabbits. Although the actual tissue dose at a similar starting tracheal concentration differs across species, the shape of the deposition curves is roughly equivalent. The region predicted to receive the maximal dose is that where the typical nitrogen-dioxide-induced morphometric lesion is observed in several species of animals. Using this mathematical model, it also can be predicted that, as tidal volume increases in humans (e.g. in exercise), the dose to the tissue of the gas exchange region increases substantially more than the dose to the conducting airways (22–24).

Experimental studies have shown that nitrogen dioxide or its chemical products can remain in the lung for prolonged periods; radioactivity associated with labelled nitrogen, originally within nitrogen dioxide, was detectable in extrapulmonary sites. Nitric and nitrous acids or their salts have been observed in the blood and urine after exposure to nitrogen dioxide (1, 4, 20, 21).

## Health effects

### Effects on experimental animals

Although there are exceptions, the vast majority of lung biochemical studies show effects only after acute or subchronic exposure to levels of nitrogen dioxide exceeding  $3160 \mu\text{g}/\text{m}^3$  (2 ppm) (1, 4, 5). A notable exception is the effect on lung lipid metabolism. For example, exposure of rats increases lipid peroxidation at concentrations as low as  $752 \mu\text{g}/\text{m}^3$  (0.4 ppm) (continuous, 18 months), with thiobarbituric acid reactants as an indicator, and  $75 \mu\text{g}/\text{m}^3$  (0.04 ppm) (9 months), with ethane exhalation as an indicator (25, 26). At a higher level, however, ethane had returned to normal by the 27-month point. Effects on both lipid metabolism and antioxidant metabolism showed a response pattern that depends on both concentration and duration of exposure (27). Frequently observed features at higher nitrogen dioxide levels include the induction of lung oedema, an increase in antioxidant metabolism, an increase in lung enzymes associated with cell injury, and changes in lung lipids. These alterations, although still not fully understood, may be early signs of cell lesions, which become manifest only at higher concentrations or upon longer exposure (1, 4, 5, 20, 21).

The area of the lung most sensitive to morphological injury is the centriacinar region, where the conducting airways and the gas exchange area meet. In this area, type I alveolar epithelial cells and the ciliated epithelial cells seem to be particularly sensitive to nitrogen dioxide. These cells are replaced by less sensitive cells (type II cells and nonciliated bronchiolar (Clara) cells, respectively). However, these replacement cells, too, show alterations of the cytoplasm and hypertrophy after short exposure (10 days) to concentrations above  $940 \mu\text{g}/\text{m}^3$  (0.5 ppm) (1, 4, 5, 21). Both exposure regimen and time of examination can be important. In a subchronic study of lung lesions in rats, Rombout et al. (28) discovered that the concentration (C) of nitrogen dioxide had more influence than exposure duration (time, T) when  $C \times T$  was constant, and that the effect of C was

greater with intermittent exposure than with continuous exposure. Other experiments have addressed the temporal pattern of nitrogen dioxide effects and found them to be quite complex (1). For example, over a 7-day period, a wave of epithelial hyperplasia occurs, peaking by about day 2 (29). Rombout et al. (28) showed that even 2 months after a 1-month exposure ceased, some nitrogen-dioxide-induced interstitial changes were still present.

Long-term exposure leads to emphysema-like structural changes in animals, thickening of the alveolar capillary membrane, loss of ciliated epithelium, and increases in lung collagen. Such changes have been observed in mice, rats, dogs and monkeys (1, 4, 5, 20). The US Environmental Protection Agency (1) recently reviewed some 23 research reports on nitrogen dioxide exposure and emphysema to determine whether the effects reported met the US National Heart, Lung, and Blood Institute definition for human emphysema (30). Because the animal studies were of interest for the purposes of extrapolation to humans, whether or not the more rigorous definition of human emphysema (which includes destruction of alveolar walls) is met can be important. Many of the reports contained insufficient detail to permit an independent judgement as to whether “human-type” emphysema had occurred. Three studies clearly reported such emphysema: Haydon et al. (31) in which rabbits were exposed to 15 040–22 600  $\mu\text{g}/\text{m}^3$  (8–12 ppm) for 3–4 months; Freeman et al. (32) in which rats were exposed to 37 000 (reduced to 28 200 or 18 800)  $\mu\text{g}/\text{m}^3$  (20, reduced to 15 or 10 ppm) for up to 33 months; and Hyde et al. (33). In the last study, dogs exposed for 5.5 years to a nitrogen dioxide-nitric oxide mixture containing nitrogen dioxide at a concentration of 1210  $\mu\text{g}/\text{m}^3$  (0.64 ppm) and nitric oxide at 310  $\mu\text{g}/\text{m}^3$  (0.25 ppm), respectively, exhibited several decrements in pulmonary function, which continued to deteriorate compared to controls during a 2.5-year postexposure period in clean air. After this postexposure period, lung morphometry studies showed changes analogous to human centrilobular emphysema.

There is only isolated evidence of disorders of the mechanics of breathing and of ventilatory function on repeated exposure to nitrogen dioxide concentrations of 1880–9400  $\mu\text{g}/\text{m}^3$  (1–5 ppm). The only consistent observation is that nitrogen dioxide increases breathing frequency (1, 5, 20). In addition, subchronic exposures to less than 1880  $\mu\text{g}/\text{m}^3$  (1.0 ppm) can decrease lung distensibility and gas exchange (1, 4, 5).

Several types of animal study have indicated that nitrogen dioxide increases susceptibility to bacterial lung infections and perhaps viral infections (1, 4). The most extensive set of data was collected using the infectivity model, which measures the total antibacterial defences of the lungs of mice. For long-term exposures, the lowest concentration tested that had an effect was 940  $\mu\text{g}/\text{m}^3$  (0.5 ppm) for 6 months of exposure (34). After a 3-hour exposure, the lowest concentration tested with an effect was 3760  $\mu\text{g}/\text{m}^3$  (2 ppm) (35). Continuous exposure to concentrations ranging from 52 640  $\mu\text{g}/\text{m}^3$  to 940  $\mu\text{g}/\text{m}^3$  (from 28 ppm to 0.5 ppm) resulted in linear, concentration-related increases in mortality due to pulmonary infection (36). Additional studies have shown that the increase in mortality is highly dependent on the exposure regimen. Concentration is far more important than length of exposure in increasing susceptibility to infection, although duration does play a role.

These as well as other data show that peak exposures and exposure patterns are quite important in determining response (1, 37, 38). Also, some studies indicate that several months of exposure to nitrogen dioxide levels of approximately 940  $\mu\text{g}/\text{m}^3$  (0.5 ppm) can increase susceptibility to other bacteria and viruses, and that acute exposure to higher levels can decrease pulmonary bactericidal

activity and alveolar macrophage function. In summary, the body of work shows that the effects of nitrogen dioxide are due more to concentration than to duration of exposure or to total dose (expressed as  $C \times T$ ), that differences in species sensitivity exist, that the lowest effective concentration of nitrogen dioxide also depends on the microbe used in the test, and that low levels only cause effects after repeated exposures (1, 4, 5, 20). The extrapolation of these findings to humans cannot be made directly, because most of the studies used pneumonia-induced mortality as an endpoint. However, the infectivity model reflects alterations in the defence mechanisms of mice that are shared by humans. This, together with other mechanism studies, implies that the specific host defences of humans (such as alveolar macrophages) can be altered by nitrogen dioxide. However, the quantitative relationship between effective nitrogen dioxide levels in animals and in humans is unknown. Although numerous studies provide evidence of the effects on the systemic humoral and cell-mediated immune systems, these studies are difficult to interpret; reports on the pulmonary immune system have not appeared (1, 4).

To date, there are no reports that nitrogen dioxide causes malignant tumours or teratogenesis (1, 4, 39). Limited genotoxicity studies have produced mixed results with *in vitro* and high-concentration *in vivo* studies (e.g. 50 000  $\mu\text{g}/\text{m}^3$ , 27 ppm) (40). Extrapulmonary effects have also been observed but cannot be interpreted with respect to human risk (1). Numerous studies of the interaction of nitrogen dioxide with other air pollutants, predominantly ozone, show that the effects are due to ozone alone, are additive, or are synergistic, depending on the endpoint and exposure regimen (1).

In summary, acute exposures (hours) to low levels of nitrogen dioxide have rarely been observed to cause effects in animals. Subchronic and chronic exposures (weeks to months) to such levels, however, cause a variety of effects, including alterations to lung metabolism, structure and function, and increased susceptibility to pulmonary infections. Emphysema of the human type, with destruction of alveolar walls, has been reported only at very high levels relative to ambient. Animal studies indicate that the potential for human health effects is broad. At present, however, it is difficult to extrapolate quantitatively, with confidence, effective pollutant concentrations from animals to humans.

### **Controlled human clinical studies**

#### *Pulmonary function and symptoms*

Generally, concentrations in excess of 1880  $\mu\text{g}/\text{m}^3$  (1 ppm) are necessary during acute controlled exposures to induce changes in pulmonary function in healthy adults (1, 4, 5, 21). Because these concentrations almost never occur in ambient air, concern about the effects of nitrogen dioxide has been focused on people with pre-existing lung disease. There have been numerous studies of people with asthma, chronic obstructive pulmonary disease, or chronic bronchitis showing that exposure to low levels of nitrogen dioxide can cause small decrements in forced vital capacity and forced expiratory volume in 1 second ( $\text{FEV}_1$ ) or increases in airway resistance. Pulmonary function responses have been shown in three studies of asthmatics exposed to 560  $\mu\text{g}/\text{m}^3$  (0.30 ppm) while performing mild to moderate exercise. As shown in Table 1, however, these results are not always consistent with other studies of asthmatics exposed to the same or higher nitrogen dioxide concentrations.

**Table 1. Controlled studies of the effects of nitrogen dioxide in people with pre-existing disease**

Concentration		Exposure duration (and activity)	Number, sex (and age) of subject	Effect	Reference
$\mu\text{g}/\text{m}^3$	ppm				
<i>Asthmatics</i>					
188	0.1	1 hour	20 M, 34 F (18–51 years)	No effect on $\text{SG}_{\text{aw}}$ or $\text{FEV}_{1,}$ or reactivity to ragweed; variable effect (nonsignificant trend) on carbachol reactivity	(54, 55)
188	0.1	1 hour	15 M (21–46 years)	No effects on function or methacholine response	(52, 56)
207 490	0.11 0.26	1 hour	13 M, 7 F (15–44 years)	At 207 $\mu\text{g}/\text{m}^3$ , 13/20 subjects had increased carbachol reactivity; at 490 $\mu\text{g}/\text{m}^3$ , 1/4 had increased reactivity	(53)
207 (132–300)	0.11 (0.07–0.16)	1 hour	6 M, 1 F (31.1 years)	No change in $\text{SR}_{\text{aw}}$ or grass pollen reactivity in three allergic asthmatics and four allergic subjects	(57)
225	0.12	1 hour	4 M, 6 F (12–18 years)	At rest, no functional effects. With exercise, slight ( $P < 0.06$ ) decrease in $\text{FEV}_{1}$ at 338 $\mu\text{g}/\text{m}^3$	(58–60)
225	0.12	40 minutes (10 minutes of exercise at 33 litres/minute)	4 M, 6 F (11–19 years)		
338	0.18	40 minutes (10 minutes of exercise at 30 litres/minute)	7 M, 3 F (12–18 years)		
230 460 910	0.12 0.24 0.48	20 minutes	6 M, 2 F (17–45 years)	No change in $\text{SR}_{\text{aw}}$ ; increased histamine reactivity in 5/8 subjects at 910 $\mu\text{g}/\text{m}^3$	(50)

Concentration		Exposure duration (and activity)	Number, sex (and age) of subject	Effect	Reference
$\mu\text{g}/\text{m}^3$	ppm				
260 140 1015	0.14 0.27 0.54	30 minutes	8 M, 12 F (17–56 years)	Decline in $\text{SR}_{\text{aw}}$ over time unrelated to $\text{NO}_2$ ; tendency towards increased histamine reactivity in 14/20 subjects at 140 $\mu\text{g}/\text{m}^3$ only	(61)
376	0.2	2 hours (1 hour of exercise at 20 litres/minute)	12 M, 19 F (18–55 years)	No effects on spirometry or $\text{SR}_{\text{aw}}$ ; variable increase in methacholine reactivity	(62)
470	0.25	30 minutes	10 M, 4 F (20–55 years)	No effect on $\text{SR}_{\text{aw}}$ ; increased reactivity to sulfur dioxide	(63)
470	0.25	30 minutes (10 minutes of exercise at 30 litres/minute)	9 M, 2 F (18–55 years)	Mouthpiece exposure; no effects on methacholine reactivity	(64)
560 1130	0.3 0.6	2 hours (1 hour of exercise at 40–41 litres/minute)	27 M, 32 F (18–50 years)	No significant effects on $\text{SR}_{\text{aw}}$ ; possible increased cold air response at 560 $\mu\text{g}/\text{m}^3$ only	(65)
560	0.3	2.5 hours (1.5 hours of exercise at 30 litres/minute)	24 M, 10 F (10–16 years)	At 60 minutes, decrease in $\text{FEV}_1$ , FVC, and PEF; increase in $\text{SR}_{\text{aw}}$ ; no change in cold air response; after 2.5 hours of exposure, no effects	(66)
560	0.3	0.5 hour (10 minutes of exercise at 30 litres/minute)	15 (20–45 years)	No effect at rest; increased cold air response.	(19)
560 1880 5640	0.3 1.0 3.0	1 hour (30 minutes of exercise at 41 litres/minute)	15 M, 6 F (20–34 years)	No effects on function or reactivity to cold air	(41)
560	0.3	225 minutes (30 minutes of exercise at 30–40 litres/minute)	10 M, 10 F (19–54 years)	No group change in function, symptoms or carbachol reactivity, but previously studied subjects (19) had possible $\text{NO}_2$ responses	(44)
560	0.3	110 minutes (1 hour of exercise at 42 litres/minute)	13 M (19–35 years)	After first 10 minutes of exercise, decreased $\text{FEV}_1$ ; smaller change later	(43)

Concentration		Exposure duration (and activity)	Number, sex (and age) of subject	Effect	Reference
$\mu\text{g}/\text{m}^3$	ppm				
282 560 1130	0.15 0.30 0.60	75 minutes (30 minutes of exercise at 42 litres/minute)	21 M (19–30 years)	No effect on FEV <sub>1</sub> or SR <sub>aw</sub> ; 2 hours after exposure, no change in methacholine reactivity	
560	0.3	30 minutes (20 minutes of exercise at »30 litres/minute)	5 M, 4 F (23–34 years)	No effect on function, SR <sub>aw</sub> , reactivity to SO <sub>2</sub> or symptoms	(67)
940	0.5 + 0.3 SO <sub>2</sub>	2 hours (1 hour of exercise at »20 litres/minute)	7 M, 12 F (33 years)	No effect	(68)
940	0.5	1 hour	10 (22–44 years)	No symptoms; no functional changes; decreased methacholine reactivity.	(69)
940	0.5	21 (15 minutes of exercise)	9 M, 4 F (»71 years)	No effect	(70,71)
7520	4.0	75 minutes (15 minutes of exercise at 25 or 49 litres/minute)	12 M, 11 F (18–34 years)	No effect on SR <sub>aw</sub> , symptoms, heart rate or skin conductance; small decrease in systolic blood pressure	(42)
<i>Chronic obstructive pulmonary disease</i>					
560	0.3	225 minutes (21 minutes of exercise at 25 litres/minute)	13 M, 7 F (47–70 years)	Decreased FVC (after exposure) and FEV <sub>1</sub> (after »4 hours of exposure).	(44)
940	0.5	2 hours (15 minutes of exercise at 25 litres/minute)	7 (24–53 years) chronic bronchitis	No effects on bronchitis alone; possible decrease in quasistatic compliance	(70)
940 1880 3760	0.5 1.0 2.0	1 hour (30 minutes of exercise at 16 litres/min)	13 M, 9 F (48–69 years) 6 chronic bronchitis, 21 emphysema, 4 asthma	No change in spirometry; SR <sub>aw</sub> tended to increase after first exercise period; no change in symptoms; no change in SaO <sub>2</sub>	(49)
940–9400	0.5-5	15 minutes	88 chronic bronchitis	Decreased earlobe PO <sub>2</sub> $\geq 7520 \mu\text{g}/\text{m}^3$ ; increased R <sub>aw</sub> $\geq 3000 \mu\text{g}/\text{m}^3$	(46,47)

Concentration		Exposure duration (and activity)	Number, sex (and age) of subject	Effect	Reference
$\mu\text{g}/\text{m}^3$	ppm				
1880–9400	1–5	30 breaths (15 minutes)	84 M (30–72 years)	Increased $R_{aw}$ above 2820 $\mu\text{g}/\text{m}^3$	(48)
9400	5	1 hour	Chronic obstructive pulmonary disease	Decreased $\text{PO}_2$ (earlobe)	
1880–15 040	1–8	5–60 minutes	130 (25–74 years) 116 chronic nonspecific lung disease 14 chronic bronchitis	At 7520–9400 $\mu\text{g}/\text{m}^3$ for 15 minutes, decreased $\text{PaO}_2$ ; at $\geq 3000 \mu\text{g}/\text{m}^3$ , increased $R_{aw}$ .	(45)

Note:  $\text{FEV}_1$  = forced expiratory volume in 1 second;  $\text{FVC}$  = forced vital capacity;  $\text{PaO}_2$  = arterial partial pressure of oxygen;  $\text{PEFR}$  = peak expiratory flow;

$\text{PO}_2$  = partial pressure of oxygen;  $R_{aw}$  = airway resistance;  $\text{SaO}_2$  = arterial saturation of oxygen;  $\text{SG}_{aw}$  = specific airway conductance;  $\text{SO}_2$  = sulfur dioxide;

$\text{SR}_{aw}$  = specific airway resistance.

For example, Linn et al. (41) and Linn & Hackney (42) found no pulmonary function responses at concentrations of 1880–7520  $\mu\text{g}/\text{m}^3$ . Even within the same laboratory, results have not been replicated with different groups of asthmatics (43, 44). In concentration–response studies, Von Nieding and colleagues (45–48) found that brief exposures to levels of 3000  $\mu\text{g}/\text{m}^3$  (1.6 ppm) increased airway resistance in people with chronic obstructive pulmonary disease. At similar concentrations in mildly exercising subjects exposed for 1 hour, however, no responses were seen (49). Longer exposures (4 hours) have caused functional effects in chronic obstructive pulmonary disease patients at lower levels (560  $\mu\text{g}/\text{m}^3$ ; 0.3 ppm) (44). The reasons for these mixed results have not been clarified by further research, although it has been suggested that nitrogen-dioxide-induced increases in airway resistance at ambient concentrations may not show the expected monotonic concentration–response relationship (50). Other possible explanations include differences in methods, subject selection (e.g. diagnosis of asthma) and the statistical power of studies with small numbers of subjects.

The lowest level of nitrogen dioxide exposure reported in more than one laboratory to show a direct effect on pulmonary function in asthmatics was a 30-minute exposure, with intermittent exercise, to 560  $\mu\text{g}/\text{m}^3$  (0.3 ppm) (43, 51). Although similar but statistically nonsignificant trends have been observed in other controlled human studies performed at lower concentrations (50, 52, 53), the small size of the decrements and questions regarding the statistical significance of some of these results together suggest that caution should be exercised in accepting these findings as demonstrating acute effects.

There were no significant symptomatic complaints by either asthmatics or healthy subjects exposed to concentrations of  $\leq 1880 \mu\text{g}/\text{m}^3$  (1 ppm). Symptomatic complaints have been made by healthy subjects exposed to 7520  $\mu\text{g}/\text{m}^3$  (4 ppm) but not by asthmatics exposed to the same concentration. At lower levels (less than 940  $\mu\text{g}/\text{m}^3$ ; 0.5 ppm), the changes in pulmonary function of asthmatics are small, although sometimes statistically significant. It should be noted that most of the asthmatic volunteers had only a “mild” level of disease according to the US National Heart, Lung, and Blood Institute classification (1). The impact of nitrogen dioxide on people with more severe asthma has not been studied adequately.

Findings on the effects of nitric oxide on human pulmonary function at concentrations higher than those described for nitrogen dioxide have been reported (72). One study indicates a possible increase in one pulmonary function variable in some healthy persons tested after 2 hours of exposure to nitric oxide concentrations of about 1200  $\mu\text{g}/\text{m}^3$  (1 ppm) with light exercise, but the effects probably occurred by chance (73). When Kagawa (74) evaluated the effects of a 2-hour exposure to a mixture of nitric oxide (740  $\mu\text{g}/\text{m}^3$ , 0.6 ppm) and nitrogen dioxide (560  $\mu\text{g}/\text{m}^3$ , 0.3 ppm) with mild exercise, there were no changes in function or in nonspecific bronchial responsiveness. Furthermore, Frostell et al. (75) found no bronchoconstriction with inhalation of nitric oxide at 18  $\text{mg}/\text{m}^3$  (10 ppm); higher concentrations (6–100  $\text{mg}/\text{m}^3$ ) may cause bronchodilation (4). Because exposure to nitric oxide without simultaneous contamination with nitrogen dioxide is difficult to accomplish, these findings will need further verification.

The effects of nitric acid vapour on adolescent asthmatics have been studied (76, 77). Generally, FEV<sub>1</sub> was decreased slightly, and total respiratory resistance was increased in some but not all of the studies.

#### *Airway responsiveness*

A significant amount of research has been directed at evaluating the effect of nitrogen dioxide on airway responsiveness to pharmacological, physical (e.g. cold air) or natural (i.e. allergens) bronchoconstrictors. Generally, concentrations higher than 1880 µg/m<sup>3</sup> (1.0 ppm) are required to increase responsiveness to bronchoconstrictors in healthy adults (1, 4). Of greater interest are the responses of people with pre-existing lung disease, such as asthmatics who have a markedly elevated baseline responsiveness to bronchoconstrictors. As shown in Table 1, results with nitrogen dioxide are mixed.

Bauer et al. (51) found that cold-induced increased airway constriction in asthmatics was potentiated by nitrogen dioxide at a concentration of 560 µg/m<sup>3</sup> (0.3 ppm). However, in a further illustration of the response paradox, Avol et al. (65) found possible increases in reactivity to cold air at 560 µg/m<sup>3</sup> (0.3 ppm) but not at 1130 µg/m<sup>3</sup> (0.6 ppm), and no such effect was found in a follow-up study (66). No exacerbation of bronchial reactivity to natural allergens was found in asthmatics on exposure to nitrogen dioxide, but this may be because of the low levels used (190 µg/m<sup>3</sup>; 0.1 ppm) (54, 55, 57). A meta-analysis (78) of the bronchoconstrictor studies indicated that of the 105 subjects exposed to < 376 µg/m<sup>3</sup> (0.2 ppm), 67 had increased reactivity and 38 had decreased reactivity. For all the nitrogen dioxide studies, the percentage increase in airway responsiveness was 59%, primarily due to the results in subjects exposed at rest.

#### *Lung lavage and host defences*

More recently, investigators have sought to evaluate the effects of nitrogen dioxide on measures other than on pulmonary function (1). The key studies are summarized here. Analysis of lung lavage from healthy humans indicated that high levels (5640–7520 µg/m<sup>3</sup>; 3–4 ppm) reduce the activity of alpha-1-protease inhibitor, a protein that acts to protect the lung from the proteolytic enzyme elastase by inhibiting connective tissue damage. However, 2820 µg/m<sup>3</sup> (1.5 ppm) had no such effect (1).

Goings et al. (79) evaluated the effects of nitrogen dioxide on antiviral defences. Healthy subjects were exposed to 1880–5600 µg/m<sup>3</sup> (1–3 ppm) for 2 hours/day for 3 days and to an attenuated influenza virus. There was a nonsignificant trend for increased infectivity, but the results are inconclusive because the study had insufficient power to detect small differences in infectivity owing to the small number of subjects. Although several studies of alveolar macrophages and other cells and mediators in fluid lavaged from the lungs of exposed subjects found no significant or remarkable changes, there were a number of interesting findings (1, 5, 80). In a study of healthy subjects exposed to 3760 µg/m<sup>3</sup> (2.0 ppm) for 4 hours (with intermittent exercise), bronchoalveolar lavage revealed an increase in the number of polymorphonuclear leukocytes, but no elevation in lactate dehydrogenase activity or protein that would be indicative of cell damage (81). However, alveolar macrophage phagocytic activity appeared to be decreased, and less superoxide anion was released from these cells. In another study using a longer exposure (6 hours) to the same nitrogen dioxide

level with lavage 18 hours after exposure, there was an increase in polymorphonuclear leukocytes but no change in macrophage production of superoxide anion (82). Boushey et al. (83) found no such responses in lavage fluid after repeated 2-hour exposures (four exposures in 6 days) to  $1130 \mu\text{g}/\text{m}^3$  (0.6 ppm). In another study (84), healthy subjects were exposed to  $7520 \mu\text{g}/\text{m}^3$  (4.0 ppm) with intermittent exercise for 20 minutes/day, every other day for 12 days. One day after the series of exposures, mast cells and certain classes of lymphocytes were decreased in number and, although the number of alveolar macrophages was decreased, their phagocytic activity (i.e. number of yeast particles phagocytized) was increased.

Generally, these studies on humans indicate the potential for nitrogen dioxide to affect antimicrobial host defences and perhaps cause mild inflammation. However, the exposure regimens and endpoints examined are too limited to draw quantitative exposure–response conclusions about these types of effects in humans.

#### *Interaction of nitrogen oxides with other air pollutants*

There are at least 17 controlled studies of the interaction of nitrogen dioxide with other common air pollutants (1, 4, 5, 20). The results indicate that mixtures of nitrogen dioxide and ozone result in responses that are similar to those to ozone alone. However, Hazucha et al. (85) observed increased airway responsiveness to methacholine in subjects who were first exposed to nitrogen dioxide ( $1130 \mu\text{g}/\text{m}^3$ ; 0.6 ppm) and then ozone. In general, from the results of other studies of air pollution mixtures containing nitrogen dioxide, no additional effects of nitrogen dioxide could be discerned. However, the number of endpoints examined has been limited, and, from present knowledge of the toxicity of common air pollutants, it may be assumed that interactions occur. With so little information, quantitative evaluation relative to guidelines is not possible.

#### **Epidemiological studies**

There have been a number of epidemiological studies of associations between nitrogen dioxide exposure and morbidity indicators, undertaken principally in Germany, the Netherlands, Switzerland, the United Kingdom and the United States (1, 4, 5). Several of these studies compared populations of children in communities that had contrasting ambient air levels of nitrogen dioxide, sulfur oxides and particulate matter. Generally, epidemiological studies attempting to correlate outdoor pollutant levels of nitrogen dioxide and health effects are not as quantitative as the indoor studies, but their results tend to be qualitatively similar. One of the difficulties in deriving quantitative estimates of health risks associated specifically with outdoor nitrogen dioxide exposures is in separating its relative contributions from those of other major pollutants (ozone, sulfur dioxide, particulate matter, etc.) often also present in urban ambient air mixtures; hence nitrogen dioxide might best be considered as just one indicator of polluted ambient air, especially where it is present in traffic-dominated urban air pollution mixes. Great care is therefore needed in interpreting available outdoor epidemiology studies, so as not to ascribe an undue degree of confidence to reported health associations with nitrogen dioxide concentrations as being specifically due to that compound as compared to the overall ambient air mix.

Indoor studies have compared groups exposed to nitrogen dioxide emitted from the combustion of gas inside buildings to groups in homes without such sources and hence with lower levels of nitrogen

dioxide. The limited studies of adults have tended to show no relationship between the use of gas for cooking and respiratory symptoms or lung functions. Although an epidemiological study of elderly women cooking with gas stoves found an increase in asthma symptoms and shortness of breath, there were no significant changes in FEV<sub>1</sub> and FVC measurements (86). Thus, the following discussion focuses on children. For a more comprehensive discussion of both indoor and outdoor epidemiological studies, see recent reviews (1, 4, 5).

Numerous epidemiological studies have shown or suggested associations between living in homes with gas stoves and increased respiratory illness in children. Respiratory illness is a general term that refers to symptoms measured in most of the epidemiological studies, such as colds going to the chest, chest congestion, phlegm with colds, wheeze, asthma and cough. The evidence from individual studies is mixed, making it difficult to draw overall conclusions and making a recent meta-analysis of interest. Hasselblad et al. (87) performed a meta-analysis of studies in homes with gas stoves that met several criteria. The endpoint was the presence of lower respiratory symptoms and disease in children aged 5–12 years. Given the nature of the studies evaluated, the goal was to estimate the odds ratio of an increase in nitrogen dioxide concentration of 28.3 µg/m<sup>3</sup> (0.015 ppm). Table 2 provides a summary of the studies used.

Although two analytic models (fixed and hierarchical) were used, they gave about the same results (Fig. 1). The combined odds ratio was about 1.2, with confidence intervals ranging from about 1.1 to 1.3. Thus, the analysis estimated an increased risk of approximately 20% for respiratory symptoms and disease for each increase of 28.3 µg/m<sup>3</sup> (0.015 ppm) (2-week average), where average weekly bedroom concentrations were between 15 and 122 µg/m<sup>3</sup> (0.008 and 0.065 ppm) (1, 87). Several uncertainties exist in this analysis, and exposure measurement errors are present in the studies. For example, monitoring data for a few rooms are not the same as actual human exposure, and knowing a time-weighted average is not the same as knowing the baseline and peaks of exposure.

To further illustrate the indoor epidemiological findings, the study of Neas et al. (95) is described in further detail here. It was selected because it is relatively recent and because the individual symptom results are consistent with the magnitude of effects found in the British studies and other analyses of the data from six United States cities. The authors evaluated 1286 white children (7–11 years) from the larger six-city study. For the children selected there was complete covariate information and at least one indoor measurement of nitrogen dioxide (Palmes passive diffusion tubes for 2 weeks during the heating season and 2 weeks during the cooling season at three sites: kitchen, activity room and the child's bedroom). Parents completed a questionnaire on symptoms during the previous year. An increase in symptoms was estimated for an additional exposure of 28.3 µg/m<sup>3</sup> (0.015 ppm). A multiple logistic model was used. The odds ratios were as follows: shortness of breath, 1.23 (95% confidence interval (CI), 0.93–1.61); persistent wheeze, 1.16 (CI, 0.89–1.52); chronic cough, 1.18 (CI, 0.87–1.60); chronic phlegm, 1.25 (CI, 0.94–1.66); and bronchitis, 1.05 (CI, 0.75–1.47). When the authors performed a multiple logistic regression of the combined lower respiratory symptom measure (i.e. the presence of any of the above-mentioned symptoms), they obtained an odds ratio of 1.40 (CI, 1.14–1.72).

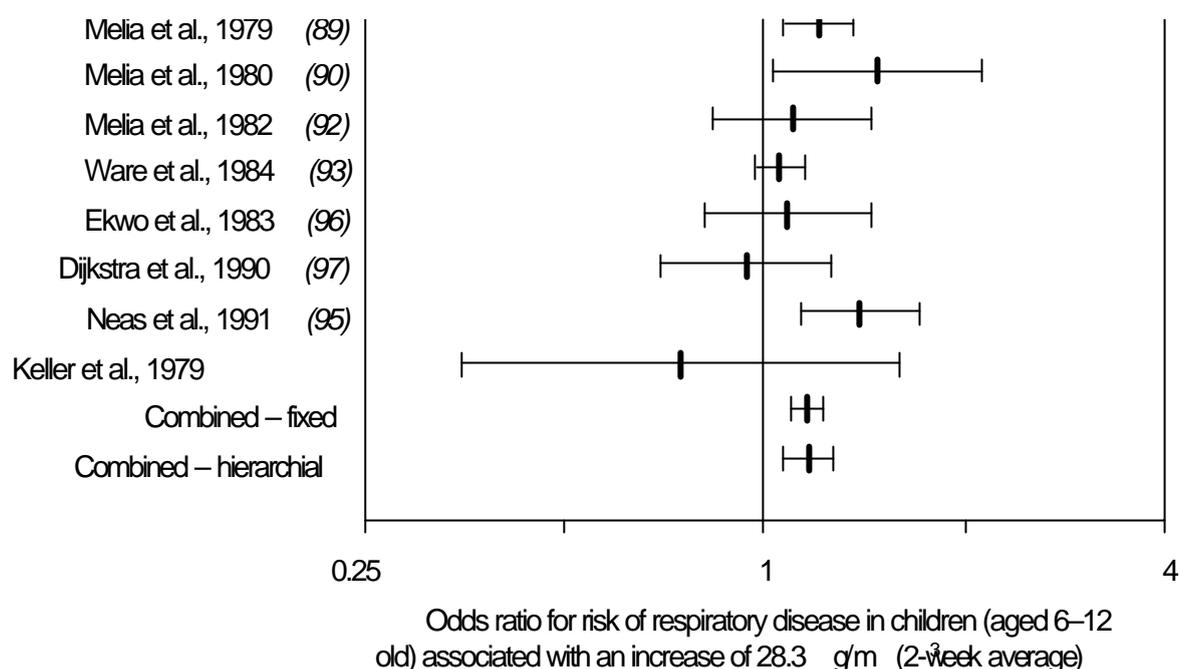
**Table 2. Summary of epidemiology studies of nitrogen dioxide exposure effects on respiratory disease in children aged 5–12 years used in a meta-analysis<sup>a</sup>**

Reference	Health outcome used	Method <sup>b</sup>	NO <sub>2</sub> exposure measure used in analysis	Covariates <sup>c</sup>			Age and sample size	Location (and year)
				SES	Parental Smoking	Sex		
(88)	Colds going to chest showed a prevalence of 26.8–19.8%.	Symptoms during past 12 months recalled by child's parent in completing respiratory symptoms questionnaire.	Gas stove compared to electric stove.	A	NM	A	6–11 years 5658	28 areas of England and Scotland (1973)
(89)	Responses to respiratory questions grouped into (a) none or (b) one or more symptoms or disease types. Colds going to chest (26.4–19.6%) showed the highest prevalence, followed by wheeze (10.1–6.2%), cough and episodes of asthma or bronchitis in previous year.	As above.	Gas stove compared to electric stove.	A	M	A	5–10 years 4827	27 areas of England and Scotland (1977)
(14, 90, 91)	Group response to respiratory questions as above.	As above.	NO <sub>2</sub> measured with Palmes tubes. Gas stove homes only.	M	M	A	6–7 years 103	Middlesborough, England (1978)
(92)	As above.	As above.	NO <sub>2</sub> measured with Palmes tubes. Gas stove homes only.	M	M	A	5–6 years 188	Middlesborough, England (1980)
(93)	Lower respiratory illness index (index of respiratory health) indicating during past year the	Questionnaire (94) completed by parent for symptoms during	Gas stove compared to electric stove	M	M	M	6–10 years 8240	Six United States cities (1974–1979)

	indicating during past year the presence of (a) bronchitis, (b) respiratory illness that kept the child home 3 days or more, or (c) persistent cough for 3 months of the year.	symptoms during previous 12 months.	electric stove					(1974–1979)
(95)	Combined indicator of one or more lower respiratory symptoms as defined. The highest prevalences were for chronic phlegm and wheeze. The other symptoms in the index are shortness of breath, chronic cough and bronchitis. Chest illness reflects a restriction of the child's activities for 3 or more days.	Symptom questionnaire completed by parent for the year during which measurements of NO <sub>2</sub> were taken.	NO <sub>2</sub> measured with Palmes tubes. Gas and electric stoves.	A	A	A	7–11 years 1286	Six US cities (1983-1986)
(96)	Chest congestion and phlegm with colds.	Questionnaire (ATS) completed by parent.	Gas stove compared to electric stove.	NM	A	M	6–12 years 1138	Iowa City, United States
(97, 98)	Respiratory illness combination variable of presence of one or more of cough, wheeze, or asthma.	Questionnaire (WHO) completed by parent.	NO <sub>2</sub> measured with Palmes tubes. Gas and electric appliances.	M	M	M	6–12 years 775	Netherlands (1986)
(99, 100)	Respiratory illness.	Telephone interview by nurse epidemiologist.	Gas stove compared to electric stove.	M	NM	M	<12 years 176	Columbus, Ohio, United States (1978)

<sup>a</sup>Source: US Environmental Protection Agency (1).

<sup>b</sup>Note: A = covariate included in study and meta-analysis; ATS = American Thoracic Society; M = measured in study but data not available for meta-analysis; NM = not measured in study; SES = socioeconomic status.

**Fig. 1. Meta-analysis of indoor epidemiological studies of nitrogen dioxide exposure effects on respiratory disease in children aged 5 to 12 years (1)****Table 3. Odds ratio and 95% confidence interval for the effect of nitrogen dioxide exposure on the prevalence of lower respiratory symptoms in children (95)**

Nitrogen dioxide concentration ( $\mu\text{g}/\text{m}^3$ )		Number of children	Odds ratio	95% Confidence interval
Range	Mean			
0–9	7	263	1.00	
9–19	14	360	1.06	0.71–1.58
19–37	27	317	1.36	0.89–2.08
38–147	58	346	1.65	1.03–2.63

Table 3 shows the adjusted odds ratios for combined lower respiratory symptoms for ordered nitrogen dioxide exposure categories. These findings are consistent with a linear concentration–response relationship.

Seven studies focused on younger children (less than 2 years old) living in homes with gas stoves, compared to children in homes without gas stoves (93, 96, 101–105). The most extensive of these studies, involving over 1000 infants aged up to 18 months, was conducted in Albuquerque, New Mexico, by Samet et al. (105). The 10th and 90th percentiles of the weekly nitrogen dioxide concentrations measured weekly in the children’s bedrooms were 9.4 and 94  $\mu\text{g}/\text{m}^3$  (0.005 and 0.05 ppm), respectively. These bedroom exposures correlated well with short-term personal

exposure of infants in pilot studies. Few of the children spent time in the kitchen during cooking, so peak exposures were likely to be rare. The experimental design and exposure assessment were rigorous, but no significant effect was found. Other studies were performed in England, Scotland and other United States cities. Meta-analysis of these seven studies shows a combined odds ratio of 1.09 (for an increase in respiratory disease per increase of  $28.3 \mu\text{g}/\text{m}^3$ ) with a 95% confidence interval of 0.95–1.26, indicating that there was no statistically significant increase in respiratory disease (1).

In relation to outdoor nitrogen dioxide as a potential indicator for traffic-dominated urban air pollution, several epidemiological studies undertaken in Europe and Japan provide suggestive evidence for respiratory effects being related to living near busy roads, presumably in part due to associated higher exposures to traffic-generated nitrogen dioxide. For example, Wjst et al. (106) reported increased respiratory symptoms (e.g. recurrent wheeze) in children aged 9–11 years as a function of traffic density on main roads through their school districts in Munich, Germany; since there was no use of home addresses and no information was provided on the actual distances of the schools from the indicator roads, however, the results are open to question owing to possible exposure misclassification. Similarly, only limited confidence can be accorded to the findings from a case–control study in the United Kingdom conducted by Edwards et al. (107), which compared children (aged over 5 years) admitted to hospital with asthma compared to other “hospital” and “community” control subjects. After stratification for distance from the road (based on relating the postal code of the home address, good to within 100 m of its true location, to indicator roads), children admitted with asthma were more often found to reside near roads with high traffic density than control children.

Somewhat better exposure classification was probably achieved by Oosterlee et al. (108) in a Netherlands study that compared adults and children living on busy roads to those on quiet roads, selected on the basis of nitrogen dioxide concentrations estimated from a model used to predict traffic-related air pollution in city streets (109). Consistent with the above reports, wheeze was more often reported for children (almost entirely restricted to girls) living along busy roads; but no relationship was found between traffic density and respiratory symptoms in adults. A similar approach was used by Pershagen et al. (110) in a case–control study in Sweden that compared children (aged 4–48 months) admitted to hospital with wheezing to aged-matched population controls from the hospital catchment area. Air pollution dispersion models were used to estimate outdoor nitrogen dioxide concentrations at home and day-care centre addresses; hospital admission case subjects (again mainly the girls) were found to have been exposed to higher estimated outdoor concentrations than control children. A positive association was also found for girls and the presence of gas stoves in their homes.

In Japanese studies mainly focused on adults, Nakatsuka et al. (111) reported more respiratory symptoms (e.g. cough and phlegm) among adults (especially women) who self-reported living alongside heavy traffic density roads. Also, Nitta et al. (112) found that Japanese women living within a few metres of roads carrying heavy traffic reported significantly more cough, phlegm, wheeze and chest colds with phlegm than did those living farther away. Suspended particulate matter and nitrogen dioxide concentrations measured at curbside and 20, 50 and 150 m from the roads indicated higher curbside measurements but not much of a nitrogen dioxide gradient beyond,

providing empirical evidence to suggest an etiological role for nitrogen dioxide in the reported respiratory symptoms.

In a cross-sectional study performed in 1987 on 4855 children aged 6 years in Stuttgart, Germany, asthma prevalence was investigated in relation to outdoor pollution, based on annual measurements where the children lived (13). A significantly elevated relative risk of 2.28 was found if the upper tercile (nitrogen dioxide concentration, 60–70  $\mu\text{g}/\text{m}^3$ ) was compared with the lower tercile (40–50  $\mu\text{g}/\text{m}^3$ ). A similarly elevated relative risk was seen for the corresponding comparison with nitric oxide, carbon monoxide or traffic counts on the road, suggesting that nitrogen dioxide was an indicator for the traffic effect in this study. Another study in Duisburg, Germany (114) investigated the influence of living by the side of roads with busy traffic on the airways of 10-year-old children. Nitrogen dioxide (14-day average) was measured indoors (child's bedroom) and outdoors (outside bedroom). Airway responsiveness to cold-air challenge and spirometry were measured. Children exposed to an outdoor concentration of 49  $\mu\text{g}/\text{m}^3$  (90th centile) compared to 25  $\mu\text{g}/\text{m}^3$  (10th centile) showed slightly reduced spirometric parameters. The results became clearer and statistically significant if the outdoor concentrations of a traffic index composed of nitrogen dioxide, toluene and benzene was used in the analysis.

Other community epidemiology studies that included actual measurements of ambient nitrogen dioxide have yielded mixed results, but overall findings were generally consistent with the picture emerging from the above studies. For example, a relationship between daily nitrogen dioxide levels and daily cases of croup (children's visits for croup symptoms at paediatricians' offices and in hospitals) was observed in a study in five German cities (115). After controlling for short-term weather factors, an increase from 10  $\mu\text{g}/\text{m}^3$  to 70  $\mu\text{g}/\text{m}^3$  (daily average) was associated with a 28% increase in croup cases. A similar increase in total suspended particulates was associated with a similar increase in croup. There was colinearity of total suspended particulates and nitrogen dioxide in the model, but the data suggested that the former may be the more likely pollutant of interest. No pollutant studied was associated with daily cases of obstructive bronchitis.

Two non-European studies reported significant positive associations between monitored outdoor nitrogen dioxide concentrations (24-hour) and total mortality in Los Angeles (116) and mortality due to respiratory disease in children in Sao Paolo (117). In each case, however, it is very difficult to separate the relative contributions of nitrogen dioxide from those of co-occurring particulate matter; the latter was more likely to have been the causative agent, given typically much stronger and consistent associations of particulate matter with increased mortality risks in these and other urban areas.

Recently reported time-series analyses of air pollution effects from a European multi-city epidemiology project (118) also found the following very mixed results.

- There was no association with any cause of death for nitrogen dioxide or ozone in Lyon, France, where daily nitrogen dioxide concentrations averaged 70  $\mu\text{g}/\text{m}^3$  (maximum 324  $\mu\text{g}/\text{m}^3$ ) and both sulfur dioxide and particulate matter were significantly related to mortality from cardiovascular and respiratory causes (119).
- No relationship between daily nitrogen dioxide levels and daily mortality was found in Cologne, Germany, where the all-year median for daily nitrogen dioxide was 45  $\mu\text{g}/\text{m}^3$  (maximum

176  $\mu\text{g}/\text{m}^3$ ) and both sulfur dioxide and particulate matter were significantly related to increased mortality risk (120).

- In Paris, there were no significant effects of photo-oxidant pollutants (nitrogen dioxide, ozone) on daily mortality risk, but there was a significantly increased hospital asthma admission relative risk (RR) of 1.175 per 100  $\mu\text{g}/\text{m}^3$  increase in nitrogen dioxide concentration above the reference value. The mean 24-hour nitrogen dioxide concentration averaged 45  $\mu\text{g}/\text{m}^3$  (99th centile 108  $\mu\text{g}/\text{m}^3$ ) and the mean 1-hour maximum was 74  $\mu\text{g}/\text{m}^3$  (99th centile 203  $\mu\text{g}/\text{m}^3$ ). Particulate matter and sulfur dioxide significantly increased daily mortality from respiratory causes and hospital admissions for all respiratory disease (121).
- Negative (sometimes statistically significant) effects of nitrogen dioxide in decreasing respiratory hospital admissions (RR = 0.86–0.89) were found in Amsterdam for adults (aged 15–64 years) and in chronic obstructive pulmonary disease admissions for all ages (RR = 0.795–0.948), but there were some positive effects on respiratory admissions for the elderly (65+ years) and on asthma admissions (RR = 0.902–1.062). The mean 24-hour nitrogen dioxide concentration averaged 50  $\mu\text{g}/\text{m}^3$  (95th centile 84  $\mu\text{g}/\text{m}^3$ ) and the mean 1-hour maximum was 75  $\mu\text{g}/\text{m}^3$  (95th centile 127  $\mu\text{g}/\text{m}^3$ ). In addition, ozone showed nonsignificant positive effects on summer respiratory admissions for the elderly (65+ years) but neither particulate matter nor sulfur dioxide showed any clear effects on hospital admissions (122).
- Rotterdam showed contrasting results, i.e. predominantly positive (albeit few significant at  $P < 0.05$ ) effects of nitrogen dioxide in terms of relative risk increases for all respiratory admissions for all age groups and increases (mostly significant) for chronic obstructive pulmonary disease admissions for all ages. The mean 24-hour nitrogen dioxide concentration averaged 54  $\mu\text{g}/\text{m}^3$  (95th centile 86  $\mu\text{g}/\text{m}^3$ ) and the mean 1-hour maximum averaged 82  $\mu\text{g}/\text{m}^3$  (95th centile 138  $\mu\text{g}/\text{m}^3$ ). Ozone and particulate matter (as black smoke) generally increased the risk of hospital admissions, and sulfur dioxide showed mixed results (122).
- Ambiguities regarding the effects of ambient nitrogen on hospital admissions for asthma were found in Helsinki (daily mean in the range 34–44  $\mu\text{g}/\text{m}^3$ ). Pönkä & Virtanen (123) reported asthma admissions for children (aged 0–14 years) to be related to 8-hour ozone levels and for adults (15–64 years) to 24-hour sulfur dioxide levels, but provided no results for nitrogen dioxide. This was in contrast to an earlier report (124) of significant associations between hospital admissions for asthma and nitrogen dioxide, sulfur dioxide, ozone and total suspended particulates based on different statistical modelling.
- In London, the effects of nitrogen dioxide and sulfur dioxide on daily mortality were significant but smaller and less consistent than the effects of ozone and particulate matter (as black smoke) on respiratory, cardiovascular and all-cause mortality, the effects being greater in the warm season (April–September) and independent of other pollutants (125).
- Also in London, a significant ( $P < 0.05$ ) association of respiratory hospital admissions for the elderly (65+ years) with 24-hour nitrogen dioxide concentration lagged by 2 days (greater in the warm than in the cool season), possibly attributable to the high correlation of nitrogen dioxide with ozone (which showed much more consistent, larger effects on respiratory admissions for all

age groups). Daily average nitrogen dioxide concentrations were 23–53 ppb in the warm season and 25–50 ppb in the cool season (126).

Other European studies have reported associations between daily (24-hour) nitrogen dioxide concentrations and visits to the emergency department by asthmatics in Barcelona (127), emergency hospitalizations for asthma and home visits for asthma in Paris (128), and emergency hospitalizations and consultations in hospitals for overall respiratory causes in Athens (129). Typically, the relative risks for one or another of these endpoints have been reported to fall in the range 1.4–3.3% increases in hospitalizations or visits per 10  $\mu\text{g}/\text{m}^3$  24-hour increment in nitrogen dioxide concentration above the reference values cited in these studies.

Several epidemiological investigations have evaluated long-term ambient (outdoor) nitrogen dioxide exposure effects in children. For example, two studies in the United States reported a higher relative risk for wheezing dyspnoea (RR = 2.25;  $P < 0.05$ ) among children aged 9–11 years, comparing areas of high and low annual average nitrogen dioxide exposure in Ohio (130), and higher rates (RR = 1.4;  $P < 0.001$ ) of acute respiratory disease in high compared with low exposure areas in Tennessee (131). While these studies indicate qualitative effects of nitrogen dioxide on respiratory symptoms in children, quantitative estimates of associated nitrogen dioxide levels cannot be relied on owing to the use of measurement methods that are influenced by temperature and humidity. Kagamimori et al. (132) reported associations between annual mean nitrogen dioxide levels and wheezing in Japanese children with positive skin reactions to house-dust extract ( $P < 0.01$ ) but not in children with negative skin reactions in areas with concentrations ranging from 20 to 60  $\mu\text{g}/\text{m}^3$ . Braun-Fahrländer et al. (133) found the duration of any respiratory episode among Swiss children (aged 0–5 years) to be increased by 13% per 20  $\mu\text{g}/\text{m}^3$  increase in outdoor nitrogen dioxide levels (annual averages were 47 and 52  $\mu\text{g}/\text{m}^3$  in the two cities studied). Another recent study (134) of schoolchildren in 10 Swiss communities (annual averages ranged from 12 to 50  $\mu\text{g}/\text{m}^3$ ) found increases of 58% for chronic cough and 35% for chronic bronchitis in the previous year comparing communities with the highest pollution levels to those with the lowest levels. A recent study in Stockholm (135) found a statistically significant increased risk of wheezy bronchitis in girls to be associated with an estimated winter half-year mean outdoor nitrogen dioxide concentration of 27  $\mu\text{g}/\text{m}^3$  (whole-year annual average probably higher when warm season nitrogen dioxide is included). Lastly, on the basis of US NHANES data, Schwartz (136) reported nonlinear associations of decreases in lung function among 3900 children with increasing nitrogen dioxide concentrations, the clearest effects being seen at annual average levels exceeding 75  $\mu\text{g}/\text{m}^3$ .

Various other studies have evaluated long-term exposure effects on adults (137–141). Most report evidence of increased respiratory symptoms (mainly bronchitic) being associated with exposures to higher levels. Nevertheless, particular study designs, exposure estimate methods and/or other considerations related to statistical analysis preclude any confident identification of specific long-term (annual average) concentrations likely to be associated with the reported effects.

The most consistent general impression given by the epidemiological studies is that nitrogen dioxide may increase respiratory illness in older children (5–15 years). These findings are of concern because repeated respiratory illness in children (independent of nitrogen dioxide) is associated with increased lung damage in later life (142). Thus any increase in such illness associated with nitrogen dioxide could have later as well as immediate consequences.

## Sensory effects

Nitrogen dioxide has a stinging, suffocating odour. The odour threshold has been placed by various authors at between  $100 \mu\text{g}/\text{m}^3$  (0.05 ppm) and  $410 \mu\text{g}/\text{m}^3$  (0.22 ppm) (4). Owing to adaptation, however, no odour was perceived during a gradual (15-minute) increase in concentration from 0 to  $51\,000 \mu\text{g}/\text{m}^3$  (0–27 ppm).

## Evaluation of human health risks

### Exposure evaluation

Levels of nitrogen dioxide vary widely because a continuous baseline level is frequently present, with peaks of higher levels superimposed. Natural background annual mean concentrations are in the range  $0.4\text{--}9.4 \mu\text{g}/\text{m}^3$ . Outdoor urban levels have an annual mean range of  $20\text{--}90 \mu\text{g}/\text{m}^3$  and hourly maxima in the range  $75\text{--}1015 \mu\text{g}/\text{m}^3$ . Levels indoors where there are unvented gas-combustion appliances may average more than  $200 \mu\text{g}/\text{m}^3$  over a period of several days. A maximum 1-hour peak may reach  $2000 \mu\text{g}/\text{m}^3$ . For briefer periods, even higher concentrations have been measured.

### Critical concentration–response data

Monotonic concentration–response data are available from only a few animal studies. Thus, this section will focus on lowest-observed-effect levels and their interpretation.

#### *Short-term exposure effects*

Available data from animal toxicology experiments rarely indicate the effects of acute exposure to nitrogen dioxide concentrations of less than  $1880 \mu\text{g}/\text{m}^3$  (1 ppm). Normal healthy people exposed at rest or with light exercise for less than 2 hours to concentrations of more than  $4700 \mu\text{g}/\text{m}^3$  (2.5 ppm) experience pronounced decrements in pulmonary function; generally, such people are not affected at less than  $1880 \mu\text{g}/\text{m}^3$  (1 ppm). One study showed that the lung function of people with chronic obstructive pulmonary disease is slightly affected by a 3.75-hour exposure to  $560 \mu\text{g}/\text{m}^3$  (0.3 ppm). A wide range of findings in asthmatics has been reported; one study observed no effects from a 75-minute exposure to  $7520 \mu\text{g}/\text{m}^3$  (4 ppm), whereas others showed decreases in FEV<sub>1</sub> after 10 minutes of exercise during exposure to  $560 \mu\text{g}/\text{m}^3$  (0.3 ppm).

Asthmatics are likely to be the most sensitive subjects, although uncertainties exist in the health database. The lowest concentration causing effects on pulmonary function was reported from two laboratories that exposed mild asthmatics for 30–110 minutes to  $560 \mu\text{g}/\text{m}^3$  (0.3 ppm) during intermittent exercise. However, neither of these laboratories was able to replicate these responses with a larger group of asthmatics. One of these studies indicated that nitrogen dioxide can increase airway reactivity to cold air in asthmatics. At lower concentrations, the pulmonary function of asthmatics was not changed significantly.

Nitrogen dioxide increases bronchial reactivity as measured by pharmacological bronchoconstrictor agents in normal and asthmatic subjects, even at levels that do not affect pulmonary function directly in the absence of a bronchoconstrictor. Asthmatics appear to be more susceptible. For example, some but not all studies show increased responsiveness to bronchoconstrictors at nitrogen dioxide levels as low as  $376\text{--}560 \mu\text{g}/\text{m}^3$  (0.2–0.3 ppm); in other studies, higher levels had no such effect. Because the actual mechanisms are not fully defined and nitrogen dioxide studies with allergen challenges showed no effects at the lowest concentration tested ( $190 \mu\text{g}/\text{m}^3$ ; 0.1 ppm), full

evaluation of the health consequences of the increased responsiveness to bronchoconstrictors is not yet possible.

#### *Long-term exposure effects*

Studies with animals have clearly shown that several weeks to months of exposure to nitrogen dioxide concentrations of less than  $1880 \mu\text{g}/\text{m}^3$  (1 ppm) cause a plethora of effects, primarily in the lung but also in other organs such as the spleen, liver and blood. Both reversible and irreversible lung effects have been observed. Structural changes range from a change in cell types in the tracheo-bronchial and pulmonary regions (lowest reported level  $640 \mu\text{g}/\text{m}^3$ ) to emphysema-like effects (at concentrations much higher than ambient). Biochemical changes often reflect cellular alterations (lowest reported levels for several studies  $380\text{--}750 \mu\text{g}/\text{m}^3$  (0.2–0.4 ppm) but isolated cases of lower effective concentrations). Nitrogen dioxide levels as low as  $940 \mu\text{g}/\text{m}^3$  (0.5 ppm) also increase susceptibility to bacterial and viral infection of the lung.

There are no epidemiological studies that can be confidently used quantitatively to estimate long-term nitrogen dioxide exposure durations or concentrations likely to be associated with the induction of unacceptable health risks in children or adults. Because homes with gas cooking appliances have peak nitrogen dioxide levels that are in the same range as levels causing effects in some animal and human clinical studies, epidemiological studies evaluating the effects of nitrogen dioxide exposures in such homes have been of much interest. In general, epidemiological studies on adults and on infants under 2 years showed no significant effect of the use of gas cooking appliances on respiratory illness; nor do the few available studies of infants and adults show any associations between pulmonary function changes and gas stove use. Nevertheless, children aged 5–12 years are estimated to have a 20% increased risk for respiratory symptoms and disease for each increase in nitrogen dioxide concentration of  $28.3 \mu\text{g}/\text{m}^3$  (2-week average) where the weekly average concentrations are in the range  $15\text{--}128 \mu\text{g}/\text{m}^3$  or possibly higher. The observed effects, however, cannot clearly be attributed to one or another of either the repeated short-term high level peak exposures or long-term exposures in the range of the stated weekly averages (or possibly both).

As hinted at by the indoor studies, the results of outdoor studies tend to point consistently towards increased respiratory symptoms, their duration, and/or lung function decrements being qualitatively associated in children with long-term ambient nitrogen dioxide exposures. Outdoor epidemiology studies, as with indoor studies, however, provide little evidence for the association of long-term ambient exposures with health effects in adults. None of the available studies yields confident estimates of long-term exposure–effect levels, but available results most clearly suggest respiratory effects in children at annual average nitrogen dioxide concentrations of  $50\text{--}75 \mu\text{g}/\text{m}^3$  or higher.

#### **Health risk evaluation**

Small, statistically significant, reversible effects on lung function and airway responsiveness have been observed in mild asthmatics during a 30-minute exposure to nitrogen dioxide concentrations of  $380\text{--}560 \mu\text{g}/\text{m}^3$  (0.2–0.3 ppm). The sequelae of repetitive exposures of such individuals or the impact of single exposures on more severe asthmatics are not known. In most animal experiments, however, 1–6 months of exposure to  $560\text{--}940 \mu\text{g}/\text{m}^3$  are required to produce changes in lung structure, lung metabolism and lung defences against bacterial infection. Thus, it is prudent to avoid exposures in humans, because repetitive exposures in animals lead to adverse effects. Animal toxicology studies of lung host defence and morphology suggest that peak concentrations contribute

more to the toxicity of nitrogen dioxide than does duration, although duration is still important. Nitrogen dioxide puts children at increased risk for respiratory illness. This is of concern because repeated lung infections in children can cause lung damage later in life.

Nitrogen dioxide presents a dilemma with respect to guidelines. It is clear that the public should be protected from excessive exposure, but the recommendation of a guideline is complicated owing to the difficulties posed by the uncertainties in exposure–response relationships for both acute (< 3-hour) and long-term exposure and the uncertainties in establishing an appropriate margin of protection. Studies of asthmatics exposed to 380–560  $\mu\text{g}/\text{m}^3$  indicate a change of about 5% in pulmonary function and an increase in airway responsiveness to bronchoconstrictors. Asthmatics are more susceptible to the acute effects of nitrogen dioxide. They have a higher baseline airway responsiveness. Thus, a nitrogen-dioxide-induced increase in airway responsiveness is expected to have clinical implications for exaggerated responses to a variety of provocative agents, such as cold air, allergies or exercise. Concern about asthmatics is also enhanced, considering the increase in the number of asthmatics in many countries (many countries have 4–6% asthmatics). A number of epidemiological studies of relatively large populations exposed indoors to peak levels of nitrogen dioxide from gas-combustion appliances have not provided consistent evidence of adverse pulmonary function effects. In one study, elderly women who used gas stoves had a high prevalence of asthma. Nevertheless, the human clinical studies of function and airway reactivity do not show monotonic concentration responses, and the studies are not internally consistent. Animal studies do not provide substantial evidence of biochemical, morphological or physiological effects in the lung following a single acute exposure to concentrations in the range of the lowest-observed-effect level in humans. On the other hand, the mild asthmatics chosen for the controlled exposure studies do not represent all asthmatics, and there are likely to be some individuals with greater sensitivity to nitrogen dioxide. Furthermore, subchronic and chronic animal studies do show significant morphological, biochemical and immunological changes.

The epidemiological studies discussed show increased risk of respiratory illness in children at an increase in nitrogen dioxide level of about 30  $\mu\text{g}/\text{m}^3$ ; most studies measured 2-week averages on personal samplers. It is not known, however, whether the effect was related to this 2-week average, the actual pattern (baseline and peaks) over the 2 weeks, the peaks over the 2 weeks, or some other index for a longer time-frame prior to the study measurement. It is also not possible to clearly discern the relative contributions of indoor and outdoor levels of nitrogen dioxide.

### Guidelines

Despite the large number of acute controlled exposure studies on humans, several of which used multiple concentrations, there is no evidence for a clearly defined concentration–response relationship for nitrogen dioxide exposure. For acute exposures, only very high concentrations (1990  $\mu\text{g}/\text{m}^3$ ; > 1000 ppb) affect healthy people. Asthmatics and patients with chronic obstructive pulmonary disease are clearly more susceptible to acute changes in lung function, airway responsiveness and respiratory symptoms. Given the small changes in lung function (< 5% drop in FEV<sub>1</sub> between air and nitrogen dioxide exposure) and changes in airway responsiveness reported in several studies, 375–565  $\mu\text{g}/\text{m}^3$  (0.20 to 0.30 ppm) is a clear lowest-observed-effect level. A 50% margin of safety is proposed because of the reported statistically significant increase in response to a bronchoconstrictor (increased airway responsiveness) with exposure to 190  $\mu\text{g}/\text{m}^3$  and a meta-analysis suggesting changes in airway responsiveness below 365  $\mu\text{g}/\text{m}^3$ . (The significance of the

response at  $190 \mu\text{g}/\text{m}^3$  (100 ppb) has been questioned on the basis of an inappropriate statistical analysis.)

On the basis of these human clinical data, a 1-hour guideline of  $200 \mu\text{g}/\text{m}^3$  is proposed. At double this recommended guideline ( $400 \mu\text{g}/\text{m}^3$ ), there is evidence to suggest possible small effects in the pulmonary function of asthmatics. Should the asthmatic be exposed either simultaneously or sequentially to nitrogen dioxide and an aeroallergen, the risk of an exaggerated response to the allergen is increased. At 50% of the suggested guideline ( $100 \mu\text{g}/\text{m}^3$ , 50 ppb), there have been no studies of acute response in 1 hour.

Although there is no particular study or set of studies that clearly support selection of a specific numerical value for an annual average guideline, the database nevertheless indicates a need to protect the public from chronic nitrogen dioxide exposures. For example, indoor air studies with a strong nitrogen dioxide source, such as gas stoves, suggest that an increment of about  $30 \mu\text{g}/\text{m}^3$  (2-week average) is associated with a 20% increase in lower respiratory illness in children aged 5–12 years. However, the affected children had a pattern of indoor exposure that included peak exposures higher than those typically encountered outdoors. Thus the results cannot be readily extrapolated quantitatively to the outdoor situation. Outdoor epidemiological studies have found qualitative evidence of ambient exposures being associated with increased respiratory symptoms and lung function decreases in children (most clearly suggestive at annual average concentrations of 50–75  $\mu\text{g}/\text{m}^3$  or higher and consistent with findings from indoor studies), although they do not provide clear exposure–response information for nitrogen dioxide. In these epidemiological studies, nitrogen dioxide has appeared to be a good indicator of the pollutant mixture. Furthermore, animal toxicological studies show that prolonged exposures can cause decreases in lung host defences and changes in lung structure. On these grounds, it is proposed that a long-term guideline for nitrogen dioxide be established. Selecting a well-supported value based on the studies reviewed has not been possible, but it has been noted that a prior review conducted for the Environmental Health Criteria document on nitrogen oxides recommended an annual value of  $40 \mu\text{g}/\text{m}^3$  (143). In the absence of support for an alternative value, this figure is recognized as an air quality guideline.

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