nitrogen dioxide: evaluation of human health risks in chamber studies

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CONTENTS

SUMMARY

The World Health Organization/European region (WHO/EU) proposes a 0.11 ppm 1-hour guideline for ambient NO_2 concentrations. This guideline is based on reversible 1 hour effects on lung function of greater than 5% and increased airway responsiveness (AR) observed in mild asthmatics at 30 minute exposures to 0.2 to 0.3 ppm.

Based on the inherent variability in airways, a group mean reduction in FEV_1 of about 5% for healthy individuals and about 11% for patients with airways obstruction represents a statistically significant change from normal day-to-day variability.

Healthy subjects show no significant change in lung function from baseline at NO_2 concentrations as high as 2 ppm (percent changes in Forced Expiratory Volume in 1 sec range from -3% to +4%).

Studies of several hundred asthmatics indicate that most patients with obstructive lung disease (e.g., asthmatics) show no apparent change in lung function from baseline as a result of exposure to NO_2 concentrations ranging from 0.10 ppm to 3 ppm. The apparent exception to these results are five studies of asthmatics and COPD patients exposed to 0.3 ppm NO_2. Three of the studies were reported only in abstracts (the complete data were unavailable). These results are compared below with subjects showing no adverse effect at the highest and lowest exposure levels.

NO_2 ppm	% Change in Lung Function	Health Status	n	Type-Report
0.10	-1.8	Asthmatics	20	Contract report
0.3	-7	Asthmatics	6	Abstract
0.3	-6	Asthmatics	15	Peer-reviewed
0.3	-6	Asthmatics	12	Abstract
0.3	-2.3 to +3.5	Asthmatics	155	5 Peer-reviewed
3.0	+3.1	Asthmatics	21	Peer-reviewed
0.3	0 to -3.5	COPD	46	2 Peer-reviewed
0.3	-5.1	COPD	20	Peer-reviewed
0.3	-14	COPD	8	Abstract
2.0	-1.6	COPD	21	Peer-reviewed

The other measure of a possible adverse effect that has been used is airway responsiveness (AR), which is the percent of subjects exposed to NO_2 who show increased responsiveness to bronchoconstrictors, such as methacholine, SO_2, and cold air. About 70% of asthmatics exposed to NO_2 at rest showed increased AR, compared to about 51% exposed while exercising. About 79% of healthy subjects showed increased AR at NO_2 concentration >1.0 ppm, and less than 50% at concentration <1.0 ppm. However, the actual reduction in lung function produced by the bronchoconstrictor is generally small. The mean change in FEV_1 due to the provocative doses of irritant administered subsequent to NO_2 exposures of 0.3 ppm

to 3.0 ppm ranged from -5.5% (14 asthmatics exposed to 0.3 ppm NO_2 and breathing cold air at 30 l/minute; Bauer et al., 1986) to +1.5% (10 of the 14 asthmatics in Bauer et al. (1986) exposed to 0.3 ppm NO_2 while breathing cold air at 60 l/minute).

In summary, the chamber study data indicate little or no change in lung function as a result of short-term exposure NO_2 levels ranging from ppb to ppm concentrations for both healthy subjects and patients with asthma and obstructive airway disease. Concentrations of NO_2 likely to be found in ambient air increase airway responsiveness to irritants such as cold air, SO_2, and O_3, in both asthmatics and healthy individuals. However, the actual reduction in lung function associated with the increased responsiveness is small and not an effect that can be distinguished from background variability and the effects of exposure to air alone.

The definition of an effect level complicates interpretation of the data. The 5% reduction in FEV_1 utilized by WHO is a cutoff point for identifying normal daily changes in lung function for healthy subjects. However, patients with obstructive airways disease have more daily variability in lung function with ~95% showing an 11-17% daily change in FEV_1; therefore, it may be justified to set the criteria for an adverse effect at a higher level (i.e., 11-17% reduction in FEV_1) for patients with obstructive airways. If an 11-17% level for FEV_1 is used, then there is only one study of 8 COPD patients reporting a reduction that is possibly different than baseline (-14% to 0.3 ppm). The evidence indicates that while NO_2 exposure increases airway responsiveness (AR), this is not important clinically. NO_2, the irritant does not produce significant reductions in lung function.

1. INTRODUCTION

In 1995 the World Health Organization/European region (WHO/EU) proposed a 1-hour exposure guideline of 0.11 ppm for NO_2. This guideline is based on a 50% uncertainty factor applied to a LOAEL of 0.20 to 0.30 ppm for 1 to 2-hour experimental exposures in chamber studies.

The health endpoints used by WHO are: a) small reductions in lung function (>5%* drop in FEV_1 after adjustment for air exposure), and b) changes in airway responsiveness. The 50% uncertainty factor is based on a possible (because of an "inappropriate statistical analysis") increased airway responsiveness observed in one study at 0.10 ppm (Orehek et al., 1976) and a meta-analysis suggesting increased airway responsiveness below 0.2 ppm (Folinsbee, 1992).

Asthmatics and patients with COPD are said by WHO to be "clearly more susceptible" for reductions in lung function, increased airway responsiveness, and symptoms than are healthy persons. WHO indicates the LOAEL for "possible small effects in pulmonary function of asthmatics" is about 0.21 ppm, and simultaneous and/or sequential exposures to NO_2 and an aeroallergen increase the risk of an exacerbated airway response. The LOAEL for healthy persons is greater than 1.0 ppm.

Thus, the rationale for the 0.11 ppm NO_2 proposal is to avoid changes in airway responsiveness and avoid exacerbation of airway constriction if concomitant exposure to aeroallergens and NO_2 were to occur.

* On page 8 of WHO (1995), the recommended guideline is based on <5% drop in FEV_1. This is assumed to be a mistake and should be >5% as in the referenced studies, the reduction in FEV_1 is >5%.

2. NO_2 CHAMBER STUDIES

The above conclusions are based on results from experimental exposures of volunteers in a chamber to known concentrations of NO_2. Exposure times range from 10 minutes (via mouthpiece) (Abe, 1967) to six hours (Devalia et al.) either with or without exercise. Exposure concentrations range from a low of 0.10 ppm to a high of 4.0 ppm Nearly 600 asthmatics and subjects with COPD and nearly 400 healthy subjects ranging in age from adolescence to the elderly have been studied.

The measured health effects are changes in lung function, either spirometry (FEV_1, FVC, FEF_{25-75}), airway resistance (AWR), or specific airway conductance (SGAW). The most important (and commonly reported) spirometric measure is Forced Expiratory Volume in 1 second (FEV_1). **Table 1** summarizes the typical measures of lung function used in NO_2 studies, and the direction of change in the measure if airway obstruction increases. In the chamber studies, lung function is measured prior to exposure, and at the end of exposure and sometimes during exposure. Control exposure is to air. The NO_2 effect is estimated by subtracting air exposure results from NO_2 exposure results.

A second type of measure of effect of NO_2 exposure is change in airway responsiveness (AR). The most common way to assess changes in airway responsiveness is to estimate the change in sensitivity. This can be accomplished by comparing AR tests after air and after NO_2 exposure to see whether there was an increase or decrease in airway responsiveness to the particular nonspecific challenge (e.g., methocholine) used in the study. An increase in responsiveness occurs if there is a 20% reduction in FEV_1 to the same or lower concentration of the provocative agent after NO_2 exposure compared to air exposure. So the change in direction is determined by the change in PD_{20} (or PC_{20}) where PD_{20} (or PC_{20}) is the provocative dose (or concentration) producing a 20% reduction in FEV_1.

If one assumes that by chance 50% would show an increased responsiveness and 50% a decrease, then an adverse effect would be for more than half of the tested subjects to show an increase in airway responsiveness. Directional change is the parameter used by WHO and the primary parameter evaluated in the meta-analysis by Folinsbee (1992). This is a measure of the ease of airway constriction and is here called the sensitivity.

Airway responsiveness then is divided into two components (Sterk et al., 1989). One is a measure of direction of change or "sensitivity". The comparative concentration of the challenge dose (PD_{20}) at which the defined response (20% reduction in FEV_1) is attained indicates the directional change. The response (ΔFEV_1) is the independent variable and the dose (PD_{20}) is the dependent variable (Gibbons et al., 1996). In the detection of excess bronchoconstriction, the response (percent change in FEV_1 or ΔFEV_1%) is the dependent variable, and the challenge concentration the independent variable (Gibbons et al., 1996).

Another way to assess airway responsiveness is to measure at the same challenge dose the change in response (e.g., FEV_1) after air and NO_2 exposure. This is an indirect measure of whether there is excessive bronchoconstriction and is a measure of the most "important pathophysiologic abnormality in asthma, namely, excessive bronchoconstriction which is what puts asthmatics at risk of serious illness" (Gibbons et al., 1996).

It is doubtful that AR (sensitivity) is an adequate metric for assessing excessive bronchoconstriction for several reasons:

- The relationship between sensitivity and excessive bronchoconstriction is widely variable, whereas excessive bronchoconstriction is associated with corticosteroid prescription use and appears to identify asthmatics most likely to have clinical exacerbations (Gibbons et al., 1996).

- The mechanisms are different as changes in PD_{20} may not change maximal response, and vice versa. Josephs et al. (1989) conducted a prospective study of AR in asthmatics for 12-18 months and concluded that the PD_{20} shows considerable variability, and only a weak relationship with the day-to-day clinical severity of the disease.

- Measurement of AR also has the drawback that too high a provocative dose poses unacceptable risk of an excessive and dangerous airway constriction. In practice this means that data are not available on some subjects because a cutoff is imposed to avoid risk of excessive bronchoconstriction.

3. CRITERIA FOR DETERMINING SIGNIFICANT CHANGES IN LUNG FUNCTION

How much of a reduction in lung function is required to be certain the change is greater than expected and not due to just normal variability and diurnal change? Lebowitz et al. (1987) addressed this question by analyzing the amount of inherent variation in lung function measures of FEV_1 and FEF_{25-75} among healthy and asthmatic subjects. Assuming lung function changes are normally distributed, one can multiply the diurnal (within a day) coefficient of variation by 1.65 (2 standard deviations) to obtain the limit of normal daily changes.

Subjects	Coefficient of Variation (CV)	Group Variation (CV x 1.65)
Normal (2 studies)	FEV_1 :3, 5	4.95, 8.2
Normal	FEF_{25-75} :8	13.2
Obstructive patients (2 studies)	FEV_1 :7, 8.1	11.5, 13.4
Obstructive patients	FEF_{25-75} :14	23

Rodan et al. (1995) indicate similar hour-to-hour coefficients of variation for asthmatic subjects, but the day-to-day coefficient of variation was about 10 for chronic bronchitis patients with airflow obstruction. For this group of subjects with obstruction, 95% will have a day-to-day variation in FEV_1 of 16.5%. Based on this reasoning and these data, a mean decrease in FEV_1 of about 5-8% represents a change from baseline for normal subjects. Because of their larger coefficient of variation, a reduction of more than 11-17% is significant for patients with airway obstruction (e.g., asthmatics). For patients with airway obstruction, a significant change from baseline is about twice that of healthy persons using this measure. These cutoff points will be used as a guide for interpreting the chamber study results. However, it should be noted that the mean change has an unknown variability (such as mean $\pm$ standard deviation. But the standard deviation has not been taken into account in this review as in most cases it is not available and cannot be calculated. Therefore, a small difference between the cutoff point and the mean response of the group is unlikely to be statistically different.

4. CHANGES IN LUNG FUNCTION IN HEALTHY AND ASTHMATIC SUBJECTS

4.1. HEALTHY SUBJECTS (TABLE 2, APPENDIX)

FEV_1

Table 2 displays the response of healthy subjects to NO_2 exposure. Most of these studies included exercise during exposure and measurement of bronchoconstriction in the small airways. Since NO_2 is not readily soluble, it is more likely to be deposited in the deeper lung than, say the more soluble SO_2. No apparent reduction in FEV_1 or small airways obstruction is observed, and no apparent association with NO_2 concentration as high as 2.0 ppm (for FEV_1). The greatest mean reduction was 3% (Koenig et al., 1985), and the general trend was for ΔFEV% to become more positive as NO_2 concentration increased. This trend is also evident for the two studies where the same subjects were exposed to more than one concentration (Koenig, 1987; Kim, 1991). The above data suggest that the LOAEL and NOAEL is above 2.0 ppm for NO_2 exposure among healthy subjects.

Small Airways

There are relatively few data on changes in small airways (i.e., FEF_{25-75}) and a narrower range of exposure ($\leq$0.62 ppm). (see **Table 2**). The greatest reductions are about 4-5% (at 0.18, 0.50, and 0.62 ppm) and largest increases (3 to 5%) at similar concentrations (0.12, 0.18, 0.60 ppm). Thus, a clear trend is not obvious, and these mean reductions are well within the range of normal variability.

Airway Resistance

There is clearly no effect on airway resistance at concentrations as high as 4 ppm for 75 minutes with heavy exercise (Linn et al., 1985b) (see **Appendix** for data). Changes in airway resistance ranged from -24% (Bylin et al., 1985) to +9% (Koenig et al., 1987). Since airway obstruction is expressed as an increase (not decrease) in airway resistance, within the limits of these experiments NO_2 is not causing bronchoconstriction as measured by increased airway resistance.

Note in the **Appendix** that there are several studies where actual changes in lung function were not available. No significant changes were reported at NO_2 concentrations 0.30 ppm (Smeglin et al., 1985), 1 ppm (Jorres et al., 1992; Sackner et al., 1980), and 2 ppm (Mohsenin et al., 1986).

4.2. SUBJECTS WITH ASTHMA AND COPD (TABLE 3, APPENDIX)

NO_2 concentrations in over two dozen studies ranged from 0.10 to 3 ppm. FEV_1 changes ranged from -3% to +3% except for five studies at 0.3 ppm (discussed below).

Morrow and Utell (1989) reported exercising asthmatics and COPD patients exposed to 0.3 ppm for four hours showed similar reductions in FEV_1 (~4%) and FVC (~8%). Because of a lack of exercise-induced bronchoconstriction, the effect of NO_2 exposure was greater for COPD than for asthmatic patients. However, none of the airway changes were statistically significant. Adjusted FVC and FEV_1 were reduced 4.5% and 5.1%, respectively for COPD patients.

Two studies of asthmatics by Bauer et al. (1985, 1986a) showed a mean reduction in adjusted FEV_1 that was greater than 5%. The 1985 abstract reporting on six subjects also reported a significant reduction in airway conductance (unencumbered breathing). The 1986 study of 15 asthmatics reported a reduction of 6% in adjusted FEV_1, after exercising, and a 1.4% reduction while exposed at rest. FVC and airway conductance (SGAW) were unaffected by 0.3 ppm NO_2 and exercise (Bauer et al. (1986a).

Roger et al. (1985) in an abstract reported that adjusted FEV_1% was reduced 6% after 30 minutes exposure, with FEV_1 returning to baseline as exposure continued for the nearly 2-hour exposure period.

Bauer et al. (1986b) in an abstract reported that FEV_1 was reduced 14% after 1 hour and 9% after 2 hours in 8 COPD patients exposed to 0.3 ppm NO_2 with intermittent exercise. The effect on FEV_1 at the end of the 4-hour exposure was not reported. Exposure was said to have no effect on airway conductance. Obstruction was fairly severe in these older patients as the mean FEV_1/FVC ratio was 0.60, although they were able to undergo exercise that lead to an air intake which was 4 times more than at rest. The 14% reduction in FEV_1 is the only airway response reported in the literature that is clearly outside the bounds of normal variability in FEV_1 for patients with airways obstruction. These results are not consistent with Hackney et al. (1992), who showed no change in FEV_1% among 26 COPD patients with even more obstruction (FEV_1/FVC ratio = 0.56) and after 4 hours exposure to 0.3 ppm NO_2 with intermittent exercise.

Bauer et al. (1986a) suggest possible reasons why their asthmatic population show increased responsiveness to NO_2 that are somewhat different from responses reported by most other investigators. Although their study subjects were characterized as mild asthmatics, they had baseline airway obstruction as measured by an FEV_1/FVC ratio of 0.69. All were on intermittent or regular bronchodilator therapy (but not chronic corticosteroid therapy) which was discontinued 12 hours (24h for oral) prior to testing. Their subjects were not adapted to a high oxidant environment as experienced by Los Angeles residents, for example (Linn et al., 1986). This does not appear to be a valid reason as other investigators do not get a heightened response even in the same lab used by Bauer et al. (1986a) (Morrow et al., 1992; Frampton et al., 1991; Morrow and Utell, 1989) or outside California (Hazucha et al., 1983; Bylin et al., 1985; Koenig et al., 1987; Mohsehin, 1987; Roger, 1990, Kim et al., 1991).

All of the other studies of asthmatics and COPD patients show no reduction in lung function (i.e., FEV_1 or small airways) greater than normal variability and no apparent increase in airway resistance. The trends observed in these data suggest no change in FEV_1 (or the measures of small airways) as exposure to NO_2 increases up to 3 ppm (and including studies where the same subjects are exposed to two or more concentrations of NO_2). When measuring airway resistance, the trend is suggestive of a reduction in resistance with concentrations as high as 4 ppm. What is more likely is that this apparent trend is due to chance and the high variability inherent in the measurement of airway resistance, or NO_2 forms nitrite and nitrate bronchodilators (see discussion under AR).

Note that there are several studies summarized in the Appendix where actual values are not reported, but the authors state their conclusion. For asthmatics these include: no significant reduction in FEV_1 at 0.1 to 1.0 ppm NO_2 (Sackner et al., 1981) and 0.5 ppm (Mohsenin et al., 1986), and no significant effects at 1 ppm NO2 exposure for 3 hours with intermittent exercise (Jorres et al., 1992). For 20 COPD

patients exposed for 4 hours with intermittent exercise to 0.3 ppm NO_2 there was a suggestion of a 5-10% reduction in FVC, and <5% in FEV_1 (Bauer et al., 1987).

Exclusive of Bauer et al. (1986b), and using an 11% reduction in FEV_1 as a point to separate changes outside the range of normal variation, the above data are suggestive that short-term exposure to NO_2 concentrations below 1 ppm or higher and after adjustment for air exposure does not cause significant airway obstruction in most patients with asthma or COPD. Most of the data suggest there is little difference in acute response to NO_2 between healthy and asthmatic subjects when measured as changes in FEV_1, small airways or airway resistance.

5. AIRWAY RESPONSIVENESS (AR)

AR is characterized by increased sensitivity and increased maximal response of the airways. Increased sensitivity is measured as a reduction in the provocative dose needed to produce an effect such as a 20% reduction in FEV_1 (PD_{20}). Increased sensitivity is probably associated with epithelial damage, inflammation or abnormal autonomic control, and in asthmatics is largely controlled with bronchodilators (Bel et al., 1991).

An increased maximal response can result in excessive degrees of airway narrowing in asthmatics and is controlled mainly by corticosteroids. Maximal response may occur because of enhanced shortening of airway smooth muscles and/or thickening of the airway wall because of inflammation. It may be measured by comparing mean airway obstruction at the same PD. It is the excessive airway narrowing, not the increased AR, that is potentially dangerous for asthmatic patients (Bel et al., 1991).

Sensitivity

The effect of NO_2 on AR has been summarized in a "meta-analysis" by Folinsbee (1992). He assessed the effect of NO_2 on AR by comparing the direction of change (increase or decrease) in response to the post-exposure challenge. Results were also grouped by asthmatic and healthy subjects, and whether exercise was part of the exposure. The basic design of these studies is similar to that of the usual chamber study. Subjects are exposed to air or NO_2 and pre- and post-exposure lung function is measured. After post-exposure lung function measures are completed, the challenge irritant is administered in increasing doses till the response (e.g., PD_{20}) is attained.

The results (Folinsbee, 1992) are summarized in **Table 4**. Folinsbee (1992) concludes that the data support the hypothesis that NO_2 increases AR in both healthy and asthmatic subjects. He also indicates factors that contribute to the uncertainty of this conclusion, although it is not clear these factors always detract from the hypothesis.

- There was a considerable range in severity of asthma in the various studies.
- Eight different provocative agents were used (histamine, methacholine, cold air, carbachol, acetylcholine, ragweed pollen, grass pollen, SO_2). Hackney et al. (1992) used ozone as a provocative agent.
- Procedures were quite diverse. Measurements after exposure ranged from 0 to 60 minutes and exposure duration ranged from 20 to 225 minutes.

A puzzling aspect of these results is that exposure during rest appears to increase AR more than during exercise. Dose of NO_2 is greater during exercise because of greater total volume of air breathed so more air goes deeper in the lung. Also, the exercise studies were on average 2.3 times longer. Miller et al. (1982) estimate that NO_2 is not deposited in the lung until about the first branching of the conducting airways which is near the terminal bronchioles. As total volume increased from a 500 ml (resting) to 2500 ml (exercise) the percent uptake in the respiratory airway tissue increases from about 70% to nearly 100%, with corresponding decline in uptake by mucus in the tracheobronchial region.

Folinsbee (1992) hypothesizes that NO_2 may not only induce increased AR, but also relax airway smooth muscles, thereby, reducing airway resistance (and increasing FEV_1). This could perhaps be accomplished by the transformation of NO_2 into nitrates and nitrites when dissolved in lung fluids. Nitrites have a direct relaxing effect on smooth muscles, including airway smooth muscles. The formation of nitrites has been observed in vitro (Postlethwait and Mustafa, 1981), and perhaps could explain the reduction in blood pressure observed by Linn et al. (1985) and the apparent tendency for FEV_1 to increase (and airway resistance decrease) as NO_2 concentrations increased in the chamber studies. Saul and Archer (1983) found a linear relationship between ambient NO_2 and urinary nitrite. Their data suggest NO_2 interaction in the lung is largely with oxidizable tissue components, such as proteins, lipids, or amines to form nitrite. In the rat, 70% of the blood nitrite is recovered in the urine as nitrate. Postlethwait and Bidani (1989) present a representation of the chemical fate of inhaled NO_2 (based on the perfused rat lung model). About 70% of the NO_2 is absorbed and quickly forms HNO_2 (via interaction with organics in the respiratory epithelium) and transformation into the nitrite ion via HNO_2 dissociation. About 50% of the nitrite gets into the blood stream and the remaining fraction into the lung tissue. It is not clear whether the NO_2 concentrations in chamber studies and the time of exposure are sufficient to produce adequate quantities of bronchodilators to cause the observed effects.

Folinsbee (1992) also wonders whether increased AR is an adverse effect. While NO_2 could potentially cause an acute inflammatory response, temporary exacerbations of asthma symptoms, and increased medication usage, the latter two possibilities were not reported in the chamber studies. Sandstrom et al. (1990) observed signs of inflammation (increased lymphocytes and mast cells) following 20 minutes of exercising and exposure to 4 ppm NO_2 to 32 healthy subjects. All numbers returned to baseline at 72 hours. Effect of control exposures was not evaluated, however.

Maximal Response

The possibility of NO_2 causing a potentially dangerous maximal response on the airways can be better assessed by the magnitude of the reduction in lung function produced by the PD following NO_2 exposure. In this instance the PD_{20} determined prior to exposure to air and NO_2 in the chamber, were administered after exposure to air and NO_2. Then the response to the previously determined PD_{20} is measured. These results are summarized in **Table 5**.

All the study groups but two showed changes in adjusted FEV_1 that ranged from -3.9% to +1.5%. The group of asthmatics reported on by Morrow and Utell (1989) showed a 10% reduction in FEV_1 attributed to increased response to carbachol following exposure to 0.3 ppm. This group comprises nine asthmatics. One patient in the group was reported to have a 58% increase in FEV_1 as a result of carbachol challenge immediately after exposure to air. The actual FEV_1 was 4.18 l (carbachol) vs. 3.01 l (baseline), an increase of 38.9% (not 58.3% as reported). However, 4.18 l FEV_1 is 96% of the baseline FVC for that exposure period. Such a high FEV_1/FVC ratio is highly unlikely, and furthermore, no FVC was reported for the carbachol challenge. Because of these discrepancies, this person is deleted from the calculations. Now the mean air/NO_2 difference in ΔFEV_1% after carbachol challenge is -3.3% to -7.1%, or an adjusted ΔFEV_1% of -3.9%, which is the value reported in **Table 5**.

Of the group of 15 asthmatics studied by Bauer et al. (1986a), 14 underwent cold air challenge at resting ventilation and 30 l/minutes hyperventilation, for a mean reduction of -5.5%. Four of the subjects experienced a reduction in FEV_1 of >10%

and an SGAW of >40% at 30 l/minutes ventilation and so were not tested at 60 l/minutes ventilation. This group of 10 that remained showed a mean improvement in FEV_1 of 1.5% at 60 l/minutes ventilation while inhaling cold air.

Summary

The subjects studied by Bauer et al. (1986) appear to be among the most sensitive subjects reported on, both for reduction in FEV_1 and for AR to cold air; (75% with increased reactivity versus less than 50% by other investigators) (Avol et al., 1988, 1989). More study could be helpful in assessing the reasons for the increased response to NO_2 in this population of asthmatics, which seems contrary to the findings observed in the other asthmatic populations studied. The largest reduction after NO_2 exposure was only 5.5% greater than after air exposure, and well within the normal range of daily variation.

6. UNCERTAINTIES OF NO_2 CHAMBER STUDY DATA

There are several unresolved questions that make clearcut conclusions problematic.

1. What is a meaningful increase in airway obstruction that should be protected against? Increases in airway obstruction that are outside the normal range of variability is an effect level we have used to evaluate the chamber study results. If this definition is accepted, then a larger response (greater reduction in FEV_1) is accepted for obstructive subjects with already compromised lung function, and therefore, least able to cope with the reduced FEV_1. If this definition is not accepted, than a 5-10% reduction in FEV_1 for obstructive patients may be normal variability and not caused by NO_2. Also, the normal variability in the subjects from which the group mean is derived has not been reported, and therefore cannot be used in assessing statistical differences between response and effect level. Therefore, the estimate of effect is conservative.

   Maximum response is suggested as a more appropriate measure than sensitivity as an effect measure for AR. This maximum response measure of AR was not considered by WHO Also, the use of reductions in FEV_1 rather than FVC used by Gibbons et al. (1994) needs verification in the context of chamber studies. In the absence of FVC values, we used FEV_1 and assumed that similar results are likely because of their generally high correlations as well as the supposition that airway obstruction is the relevant response which is adequately measured by FEV_1.

2. The possible small improvements in lung function observed with increasing NO_2 exposure (both at rest and exercise) is not characteristic of the usual exposure-response relationship. If this increase in lung function is not a chance finding, research is needed to test the hypothesis that it is caused by nitrite bronchodilators formed from NO_2. If it is not a chance finding, then exposure longer than an hour is unlikely to cause increased airway obstruction and may even result in a further improvement in lung function at NO_2 concentrations found in ambient air.

3. The largest reduction in FEV_1 occurred at 0.3 ppm NO_2 in a group of eight COPD patients (Morrow and Utell, 1989). The reductions in FEV_1 among 21 asthmatic patients are between -6% and -7% (Bauer et al., 1985, 1986a; Roger et al., 1985). All of these reports are abstracts except the study of 15 asthmatics (Bauer et al., 1986a). There is no clear explanation as to why the response of these subjects (particularly the COPD patients) are different than other obstructive subjects exposed to concentrations both lower and higher than 0.3 ppm. Further examination of the characteristics of these populations compared to the nonrespondent populations might be helpful. At present there are hardly enough available details to even speculate beyond what Bauer et al. (1986a) have already done.

   If a -11% reduction in FEV_1 is a cutpoint for normal range of variability in FEV_1, there is only one study reporting a mean adverse reduction in FEV_1, namely, the eight COPD patients reported by Bauer et al. (1986b).

7. SUMMARY AND CONCLUSIONS

- Measures of response in NO_2 chamber studies include increased airway obstruction measured as changes in lung function (e.g., changes in FEV_1 and changes in small airways), increases in airway responsiveness, and exacerbations of response to nonspecific bronchoconstrictors.

- For the purposes of this review, the reasoning of Lebowitz et al. (1987) was used to define a group effect outside the range of normal variability as follows:

  ⇒ FEV_1%: greater than a 5% reduction for normal subjects and about 11% reduction for asthmatic/COPD patients. WHO appears to have used a 5% or greater reduction in FEV_1 among both normal, asthmatic and COPD patients as an indication of an effect.

  ⇒ FEF_{25-75}%: for normal subjects a greater than 13% reduction; for COPD patients a greater than 20% reduction. (WHO did not consider this endpoint.)

  ⇒ Airway responsiveness: more than 50% showing increased responsiveness is greater than expected (WHO appears to also have used the criteria for defining an adverse effect). However, AR may not be highly correlated with exacerbations or clinical severity and may not be useful as a measure of risk for an asthmatic. Therefore, it is not clear that AR >50% is a meaningful measure of an effect with a significant impact on health.

  ⇒ Maximal response to nonspecific bronchoconstrictors may be a better measure of risk of exacerbation. An increased mean response greater than a 5% (healthy person) to 11% (COPD subjects) reduction in FEV_1 after adjustment for control exposure is considered outside the range of normal day-to-day variation. (WHO did not consider this measure.)

- Healthy subjects exposed to concentrations below 2 ppm NO_2 do not experience adverse reductions in FEV_1 (changes in FEV_1 range from -3% to +4%).

- Over 90% of asthmatic subjects exposed to concentrations below 3 ppm NO2 do not experience reductions in FEV_1 that are outside normal variability (changes in FEV_1 range from -4% to +4%), and a small proportion of asthmatics show reductions in FEV_1 between 5 and 7% at concentrations of 0.3 ppm NO_2. Less than 10% of COPD patients show reductions in FEV_1 (-14%) greater than the lower limit but still within the range of normal variation.

- Exercise may reduce the response to NO_2 compared to exposure at rest.

- There is no apparent exposure-response trend of NO_2 concentration and reduced FEV_1. There is a suggestion of an inverse exposure-response trend for the airways to become less obstructed as NO_2 concentration increases. The possible formation of bronchodilators such as nitrites needs further study to explore the plausibility of such a possibility.

- AR is increased in exercising asthmatics at concentrations $\leq$0.30 ppm, and for asthmatics at rest and NO_2 concentrations up to about 0.50 ppm, the highest concentration tested. AR is not increased for healthy subjects exposed to NO_2 concentrations less than 1.0 ppm.

- Nonspecific bronchoconstrictors have not generally produced reductions in FEV_1 that are more than 5% above the effect of control exposures for either asthmatics or normal subjects exposed to NO_2 concentration as high as 3 ppm and 1.5 ppm, respectively.

The conclusions of Kleinman (1983) over a decade ago appear to be supported by more recent data:

"The practical question of health risks to asthmatics from exposure to low-level ambient NO_2 is still open, but these results do not strongly support the need for special protective efforts (of course, caution should be exercised in generalizing results from relatively small groups of experimental subjects to the larger and diverse populations of asthmatics... [The] effect of NO_2 on methacholine response [or other nonspecific PD]...seems to be small and/or inconsistent enough that it's health importance could be questioned."

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GLOSSARY

AR Airway Responsiveness. Airway responsiveness measures airflow obstruction in response to nonspecific irritants such as methacholine, histamine, cold air, etc.

COPD Chronic Obstructive Pulmonary Disease, e.g., asthma

FEF_{25-75} Forced Expiratory Flow at 25-75% of FVC or maximal midexpiratory flow rate which is the average flow between 25 and 75% of FVC

FEV_1 Forced Expiratory Volume at 1 second

FEV_1/FVC Ratio of FEV_1 and FVC. Healthy persons can exhale 70-80% of their FVC in one second

$\Delta FEV_1\%$ Percent Change in FEV_1 =

$$\left(\frac{\text{post-exposure } FEV_1 - \text{pre-exposure } FEV_1}{\text{pre-exposure } FEV_1} \right) \times 100$$

FVC Forced Vital Capacity

LOAEL Lowest Observable Adverse Effect Level

NOAEL No Observable Adverse Effect Level

PC_{20} or PD_{20} (FEV_1) Provocative Concentration or Provocative Dose of a nonspecific irritant (such as methacholine) that causes a 20% reduction in FEV_1 when measuring airway responsiveness

PFT Pulmonary Function Test, e.g., FEV_1, FEF_{25-75}, AWR

SGAW Specific Airway Conductance (is the inverse of AWR). Specific airway conductance (SGAW) relates airway conductance to lung volume

SRAW or AWR Airway Resistance = the pressure difference between mouth (atmospheric pressure) and the aveoli. It is elevated in asthma, emphysema, and chronic bronchitis

TABLE 1

SUMMARY OF MAJOR RESPONSE MEASURES USED TO ASSESS EFFECT OF NO_2 IN CHAMBER STUDIES

Response	Directional Change Indicating Obstruction	Group* Effect Level Different than Normal Variability	Comment	Relative Importance
Spirometry				
FEV_1 Forced Expiratory Volume in 1.0 second	Reduction	Normal: >5% Obstructive: >11% After adjustment for control air exposure	Integrated measure of obstruction in both large and small airways; least variable and most reproducible measure of airway obstruction, and test most commonly utilized; often the most sensitive test	+++ Most important; >5% change used by W.H.O.
FVC Forced Vital Capacity	Reduction	Similar to FEV_1	Measure of lung volume; similar to FEV_1 in variability and reproducibility, but less sensitive to airway obstruction. One study suggests most important test of maximal response to irritant in measure of airway responsiveness	+ Less important because of few data and low sensitivity
Small Airways (FEF_{25-75}, FEF_{50}) Forced Expiratory Flow at mid-range of FVC	Reduction	Normal: >13% Obstruction: >23% After adjustment for control air exposure	Measure of flow rate at the middle portion of the forced vital capacity, and therefore, flow in small or peripheral airways. Theoretically is thought to be the most sensitive measure of response, but there are few data, low reproducibility	+ Most useful as supplemental information to ΔFEV_1
Airway Resistance (inverse = airway conductance)	Increase (decrease)	? (?)	Measure of central airways obstruction; more variable, less reproducible, and sensitive than FEV_1; not effort dependent, but measured less often because requires body box that measures small changes in pressure	+ Most useful as supplemental information to ΔFEV_1

TABLE 1 (cont'd)

SUMMARY OF MAJOR RESPONSE MEASURES USED TO ASSESS EFFECT OF NO_2 IN CHAMBER STUDIES

Response	Directional Change Indicating Obstruction	Group* Effect Level Different than Normal Variability	Comment	Relative Importance
Airway Responsiveness				
Sensitivity	Increased responsive-ness measured as a decrease in challenge dose	>50% in group showing increased responsiveness	Measured as the reduction in provocative dose of an irritant needed to produce a 20% reduction in FEV_1 (PD_{20}). Low correlation with exacerbations or severity of obstruction	+ Used by W.H.O. Not sure whether direction alone is an adverse effect. The importance of quantitative reduction in challenge dose has not been estimated
Maximal Airway Response	Increase	Same as FEV_1	Measured as the reduction in FEV_1 as result of provocative challenge dose. Not reported as often as sensitivity, but correlates better with severity of obstruction and exacerbations of asthmatic response	+++ Considered more relevant than sensitivity as excessive bronchoconstriction is what puts person with airway obstruction at risk of serious illness

*Adjusted for control = % NO_2 response - % air response

$$= \%\ \text{response} \left(\frac{\text{post exposure response - pre-exposure response}}{\text{pre-exposure response}} \right) \times 100$$

TABLE 2

CHANGES IN LUNG FUNCTION (ΔFEV_1%, Δ SMALL AIRWAY %) AMONG HEALTHY SUBJECTS EXPOSED TO NO_2

NO_2 Concentration (ppm)	Air/NO_2 (adjusted ΔPFT%)*		Exercise	n	Reference
	ΔFEV_1%	**Δ Small Airway			
0 10	+1.8/-0.3 (-2.1)	--	-	20	Ahmed et al. (1983)
0.12	-1.1%/-4.1% (-3%)	-0.6%/+1.5% (+2.2%)	-	10	Koenig et al. (1985)
0.12	-3.1/-3.1 (0)	-3.6/+1.7 (+5.3)	+	10	Koenig et al. (1987)
0.18	-2.8/-1.6 (+1.2)	-5.2/-0.9 (+4.3)	+	10	Koenig et al. (1987)
0.18	-0.5/-0.5 (0)	-0.2/-4.5 (-4.3)	+	9	Kim et al. (1991)
0.30	-0.6/-0.4 (+0.2)	--	+	20	Morrow and Utell (1989)
0.30	-0.5/+0.2 (+0.7)	-0.2/-4.0 (-3.8)	+	9	Kim et al. (1991)
0.30	-1.0/0 (+1.0)	--	+	20	Morrow et al. (1992)
0.30	-0.9/0 (+0.9)	--	+	20 (elderly)	Morrow and Utell (1989)
0.50	+2.2/+1.0 (-1.2)	+6.5/+1.4 (-5.1)	+	10	Kerr et al. (1979)
0.50 (+0.50 SO_2)	+1.2/-0.9 (-2.1)	+3.7/+0.7 (-3.0)	+	24	Linn et al. (1980)
0.60	-0.2/-0.6 (-0.4)	--	+	15	Hazucha et al. (1992)
0.60	+1.2/+1.5 (+0.3)	--	+	9	Frampton et al. (1991)
0.60	+1.4/0 (-1.4)	+2.3/+2.0 (-0.3)	+	16	Drechsler-Parks et al. (1987)
0.60	-1.6/+0.02 (+1.62)	-0.8/-2.1 (-1.3)	++	20	Adams et al. (1987)
0.60	-2.2/-2.0 (+4.2)	-0.2/-0.1 (+0.1)	++	20	Adams et al. (1987)
0.60	+0.1/+0.8 (+0.7)	+0.4/+3.1 (+2.7)	+	32	Drechsler-Parks et al. (1987)
0.60	0/-1.5 (-1.5)	+0.6/+0.5 (-0.1)	+	21	Hazucha et al. (1994)
0.62	+1.2/+0.5 (-0.7)	0/-4.2 (-4.2)	+	15	Folinsbee et al. (1978)
1.5	+1.7/+2.1 (+0.4)	--	+	15	Frampton et al. (1991)
2.0	-3.0/-0.3 (+2.7)	--	-	11	Mohsenin (1987a)
2.0	-2.9/-1.1 (+1.8)	--	-	18	Mohsenin (1988)

* adjusted $\Delta PFT\% = \left(\frac{NO_2 - air}{air}\right) x\ 100$

** small airway = FEF_{25-75}% (forced expiratory flow at 25-75% of FVC) or FEF_{50}% = forced expiratory flow at 50% of FVC)

\+ exercise

\- no exercise

TABLE 3

CHANGES IN LUNG FUNCTION (ΔFEV_1%, Δ SMALL AIRWAY %) AMONG ASTHMATIC AND OTHER PATIENTS WITH OBSTRUCTIVE AIRWAYS DISEASE EXPOSED TO NO_2

NO_2 Concentration (ppm)	Air/NO_2 (adjusted ΔPFT%)*		Exercise	n	Reference
	ΔFEV_1%	Δ Small Airway %**			
Asthmatics					
0.10	-0.7/-2.5 (-1.8)	--	-	20	Ahmed et al. (1983)
0.12	+0.3%/-2.3% (-2.6%)	+7.2%/+3.4% (-3.8%)	-	10	Koenig et al. (1985)
0.12	-6.3/-6.1 (+0.2)	-3.2/-12.7 (-9.5)	+	10	Koenig et al. (1987)
0.15	-2.8/-3.9 (-1.1)	--	+	21	Roger et al. (1990)
0.18	-1.3/-3.3 (-2.8)	-4.7/-7.5 (-2.8)	-	10	Koenig et al. (1987)
0.20	-3.3/-2.6 (+0.7)	--	+	31	Kleinman et al. (1983)
0.30	-10/-17.3 (-7.3)	--	+	6	Bauer et al. (1985) abstract
0.30	-4.1/-10.1 (-6.0)	--	+	15	Bauer et al. (1986a)
0.30	-2.5/-0.3 (+2.2)	-1.8/-3.4 (-1.6)	+	21	Linn et al. (1986)
0.30	-9.9/-12.2 (-2.3)	--	+	59	Avol et al. (1988)
0.30	+1.5/+1.0 (-0.5)	+6.0/+7.2 (+1.2)	+	34	Avol et al. (1989)
0.30	-7.2/-3.7 (+3.5)	--	+	20	Morrow and Utell (1989)
0.30	-2.8/-1.7 (+1.1)	--	+	21	Roger et al. (1990)
0.30	-6/-12 (-6)	--	+	12	Roger et al. (1985) abstract
0.4	-2.2/-6.4 (-4.2)	--	-	10	Devalia et al. (1994)
0.50	-1.0/-3.4 (-2.4)	+5.6/-3.4 (-2.2)	+	13	Kerr et al. (1979)
0.50	-3.2/-4.8 (-1.6)	--	-	10	Mohsenin (1987b)
0.50 (+0.50 SO_2)	-1.9/+0.4 (+2.3)	-0.5/+3.6 (+4.1)	+	19	Linn et al. (1980)
0.60	-9.9/-7.8 (+2.1)	--	+	59	Avol et al. (1988)
0.60	-2.8/-4.0 (-1.2)	--	+	21	Roger et al. (1990)
1.0	-2.5/-1.9 (+0.6)	-1.8/-2.7 (-0.9)	+	21	Linn et al. (1986)
3.0	-2.5/+0.6 (+3.1)	-1.8/-2.8 (-1.0)	+	21	Linn et al. (1986)
Chronic Bronchitis (Kerr et al. 1979) and COPD					
0.30	+4/-10 (-14)	--	+	8	Bauer et al. (1986b) abstract
0.30	+0.3/-4.8 (-5.1)	--	+	20	Morrow and Utell (1989)
0.30	+3.0/-0.9 (-3.9)	--	+	20	Morrow et al. (1992)
0.30	+1.3/+1.3 (0)	--	+	26	Hackney et al. (1992)
0.50	-4.4/-3.6 (+0.8)	-14/-7 (+7.0)	+	7	Kerr et al (1979)
0.50	+0.80/+0.80 (0)	+3.4/+6.9 (+3.5)	+	21	Linn et al. (1985a)
1.0	+0.8/+1.7 (+0.9)	+3.4/+7.3 (+3.9)	+	21	Linn et al. (1985a)
2.0	+0.8/-0.8 (-1.6)	+3.4/0 (-3.4)	+	21	Linn et al. (1985a)

See Table 1 for explanation of footnotes

TABLE 4

PROPORTION OF NO_2 EXPOSED SUBJECTS WITH INCREASED AIRWAY RESPONSIVENESS (FROM FOLINSBEE, 1992)

ppm NO_2	% with Increase in Responsiveness (n exposed)		
	All Exposures % (n)	Exposure with Exercise % (n)	Exposure at Rest % (n)
Asthmatics			
<0.20	*64% (105)	59% (17)	*65% (88)
0.20 to 0.30	57% (169)	52% (136)	*76% (33)
>0.30	*59% (81)	49% (48)	+73% (33)
All	*59% (355)	51% (201)	+69% (154)
Healthy Subjects			
<1.0	47% (36)		47% (36)
>1.0	*79% (29)	73% (15)	86% (14)

* $p < 0.01$ that NO_2 increased airway responsiveness to PD
+ $p < 0.05$

TABLE 5

EFFECT OF NO_2 ON AIRWAY RESPONSE TO PROVOCATIVE CHALLENGE OF BRONCHOCONSTRICTING AGENT AS MEASURED BY ΔFEV_1%

NO_2 Concentration (ppm)	n Subjects	ΔFEV_1% Air/NO_2 (adjusted)	Provocative Dose	Reference
0.3 (rest) 0.3 (30 L/min exercise) 0.3 (60 L/min exercise)	14 asthmatics 14 asthmatics 10 asthmatics	-3.2%/-5.1% (-1.9%) -7.4/-12.9 (-5.5) -20.4/-18.9 (+1.5)	cold air cold air cold air	Bauer et al. (1986)
0.30 ppm	21 mild asthmatics	-16.7/-16.1 (+0.6)	cold air	Linn et al. (1986)
0.30 ppm	37 moderate/severe asthmatics	-11.9/13.6 (-1.7)	cold air	Avol et al. (1988)
0.30 ppm	34 asthmatics	-5/-5 (0)	cold air	Avol et al. 1989)
0.30	8 asthmatics	+3.3/-7.1 (-3.9)	carbachol	Morrow and Utell (1989)
0.30	20 normal	0/-0.4 (-0.4)	carbachol	Morrow and Utell (1989)
0.30	20 normal elderly	-1.9/-2.6 (-0.7)	carbachol	Morrow and Utell (1989)
0.30	20 normal elderly	-2/-2.8 (-0.8)	carbachol	Morrow et al. (1992)
0.60	37 moderate/severe asthmatics	-11.9/-11.9 (0)	cold air	Avol et al. (1988)
0.60	9 normal	-4.7/-5 (-0.30)	carbachol	Frampton et al. (1991)
0.60	21 normal	-10.8/-12.8 (-2.0)	ozone	Hazucha et al. (1994)
1.0	21 mild asthmatics	-16.7/-15.5 (+1.2)	cold air	Linn et al. (1986)
1.5	15 normal	-6.6/-6.4 (+0.2)	carbachol	Frampton et al. (1991)
0.5 + 2 ppm peaks	15 normal	-4.9/-6.6 (-1.7)	carbachol	Frampton et al. (1991)
3.0	21 mild asthmatics	-16.7/-17.2 (-0.5)	cold air	Linn et al. (1986)

APPENDIX

NO_2 CHAMBER STUDIES

References	Subjects	Exposures	ΔPFT%/PD	Comments
Abe (1967)	5 healthy subjects age 21-40 yrs	10 min via mouthpiece to 4-5 ppm NO_2; post-exposure PFT at 0 and 30 min	ΔPFT%/PD ΔFEV$_1$% 30 min; MMF 30 min 4-5 ppm NO_2: -7.1%; +4.1%	No measurements of effects of air alone; not included in tables
Beil and Ulmer (1976)	16 healthy persons 8 healthy persons	2 hrs exposure to 0, 1.0, 2.5, 5, and 7.5 ppm NO_2; 16 hrs to 5 ppm; PD = acetylcholine	ΔAWR% AWR decreased when NO_2 <2.5 ppm, significant increase when NO_2 >2.5 ppm. No significant increase in airway responsiveness (AR) to acetylcholine after exposure to 7.5 ppm/2 hrs and 5 ppm/14 hrs	No significant changes in PaO_2 or $PaCO_2$ (arterial oxygen or CO_2); not included in tables
Orehek et al (1976)	20 asthmatics age 15-44 yrs, 6 smokers	1 hr to air and 0.1 ppm NO_2; (in some cases up to 0.2 ppm); PD_{100} = carbachol dose causing 100% increase in specific airway resistance (SRAW)	PD_{100} air: 0.555 0.1 ppm NO_2: 0.357 % change: -36%	7 nonresponders (no change in airway responsiveness or PD, 13 with increased responsiveness and 45% decrease in PD_{100}. SRAW measures changes in central airways; not included in tables
Folinsbee et al. (1978)	15 nonsmoking healthy males age 20-25 yrs	0.62 ppm NO_2 for 2 hrs (exercise at 45% of maximum aerobic capacity for 15 min (A), 30 min (B), and 60 min (C).	ΔFEV$_1$% ΔFEF$_{25-75}$% ΔAWR% Air: +1.2% 0% +1.7% 0.62 ppm NO_2: +0.5 -4.2 +8.7	Groups A-C combined since no profound effect on PFT following any exposures. No reported symptoms. No effects on cardiovascular or PFT, including small airways (e.g., FEF_{75})
Hackney et al. (1978)	15 healthy males age 23-41 yrs, 4 with some history of allergy	2 hrs exposure to 1 ppm NO_2; exercise (2 x resting ventilation) 15 min in every 30. Second and third day = exposure to NO_2; average of 2 days is reported	ΔFEF50% ΔFEV1% ΔFEF50% ΔAWR% ΔSGAW% 1 ppm NO_2: -0.6% -1.8% -7.1% +1.5%	Pre-exposure values not reported, so NO_2 exposure values compared to control PFT values. Not a correct ΔPFT. No statistically significant difference between control and exposed days; not included in tables

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Kerr et al. (1979) Kulle (1982)	10 normal males age 35 yrs (22-63); 3/10 smokers - - - - - - - - - - - - - - - - - - 7 chronic bronchitis age 30 yrs (24-73); 5/7 smokers - - - - - - - - - - - - - - - - - - 13 asthmatics age 27 yrs (19-50); 3/13 smokers	2 hrs exposure to 0.5 ppm NO_2; light-moderate exercise (60-100 workload) for 15 min of first hour	ΔFEV$_1$% / ΔMMEF% / SGAW% air: +2.2% / +6.5% / +12.8% 0.5 ppm NO_2: +1.0 / +1.4 / +8.7 - - - - - - - - - - - - - - - - - - air: -4.4% / -14.0% / +1.0% 0.5 ppm NO_2: -3.6 / -7.0 / -2.7 - - - - - - - - - - - - - - - - - - air: -1.0% / +5.6% / 0% 0.5 ppm NO_2: -3.4 / -4.6 / +4.4	Symptoms not reported for air exposure and authors conclude were minimal and of doubtful significance, and not correlated with ΔPFT; 1/10 with symptom of nasal discharge - - - - - - - - - - - - - - - - - - 1/7 with nasal discharge - - - - - - - - - - - - - - - - - - 7/13 with symptoms (chest tightness, slight burning of eyes, dyspnea with exercise, slight headache)
von Nieding and Wagner (1979)	116 hospital patients with chronic nonspecific lung disease age 25-74 yrs; smaller groups exposed to various NO_2 concentration	15 to 60 min exposure, although results reported for 15 min. NO_2 ranged from 0.5 to 8 ppm NO_2	ppm NO_2 / n / ΔAWR% / p <1: 14 / -0.2% / >0.1 1.1-1.5: 10 / -3.6 / >0.1 1.6-2: 15 / +14.4 / >0.05 2.1-2.5: 10 / +29.1 / 0.01 >2.5: 14 / +25 / 0.01	15 min at 4 and 5 ppm NO_2 decreased alveolar of PO_2 but 2 ppm NO_2 had no effect. Exposure for 1 hr to 5 ppm NO_2 had no further effect on gas exchange
Linn et al. (1980)	24 normal adults age 26 (±4) yrs; 5/24 with history of allergy - - - - - - - - - - - - - - - - - - 19 asthmatics age 33 (±11) 19/19 with history of allergy	2 hrs exposure to mixture of NO_2 + SO_2; exercise 2 x resting ventilation for 15 min of every 30 min; heat stress via 88 F	ΔFEV$_1$% / ΔFEF50% / ΔFEF$_{75}$% / ΔAWR% air: +1.2% / +3.7% / +4.3% / +2.3% 0.5 ppm NO_2 + 0.5 ppm SO_2: -0.9 / +0.7 / +2.1 / -4.3 - - - - - - - - - - - - - - - - - - air: -1.9% / -0.5% / +6.7% / +6.7% 0.5 ppm NO_2 + 0.5 ppm SO_2: +0.4 / +3.6 / +6.0 / +1.2	Nonsignificant increases in respiratory symptoms but unclear if attributable to exposure - - - - - - - - - - - - - - - - - - No overall increase in symptoms during exposure No significant effect of NO2 on PFT, and no apparent interaction of SO_2 and NO_2
Sackner et al. (1980) (abstract)	6 normal adults	4 hrs by face mask at 0, 0.1, 0.3, 0.5, and 1 ppm NO_2; double-blind	No significant or consistent changes in spirometry, AWR, frequency dependence of compliance (small airways effects), heart rate, BP, arterial oxygen saturation, distribution of ventilation either immediately or up to 2 weeks later	Not included in tables
Orehek et al. (1981)	7 allergic patients	1 hr to air and 0.11 ppm NO_2; single-blind; response to PD of grass pollen measured via AWR	ΔAWR% / ΔAWR% (after PD) air: +1.9% / +126%* o.11 ppm NO_2: +3.7 / +139* *p <0.001	Airway responsiveness (AR) significantly increased, but no apparent difference between air and NO_2 exposure
Sackner et al. (1981) (abstract)	6 asthmatics	4 hours to air; 0.1, 0.3, 0.5, and 1.0 ppm NO_2 via facemask	No significant bronchoconstriction as measured by AWR, FEV_1, and no significant change in arterial O_2 saturation, heart rate, blood pressure, lung volumes, distribution of ventilation, either immediately after exposure or up to 2 weeks later	No subjective complaints; not included in tables

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Hazucha et al. (1982, 1983)	15 normal males age 27 yrs (23-39) and nonresponsive to 5 mg/mL methacholine	1 hr exposure to air and 0.1 ppm NO_2 double-blind design; bronchial challenge of methacholine 20 min post-exposure	ΔSRAW% / PD / ΔAWR%/PD air: -0.8% / 16 / +1.0% 0.1 ppm NO_2: +4.8 / 18 / +4.8 AR after NO_2: 7 = no change; 5 = reduced AR; 3 = increased AR	Symptoms evenly distributed between air and NO_2 exposure. No significant effect of NO_2 on either normal or asthmatic subjects; including a test of frequency dependence of effective resistance (RR) for peripheral airways
	15 atopic mild asthmatics, 100% increase in AWR response to 2.5 mg/mL methacholine, not using bronchodilators in last month, age 29 yrs (21-46)		air: +2.0% / 1.9 / +1.7% 0.1 ppm NO_2: +6.6 / 2.0 / +5.6 AR after NO_2: 9 = reduced AR; 6 = increased AR	3 asthmatic and 1 normal responder, where responder shows >20% reduction in PD. No post-NO_2 exposure effects observed on AR
Ahmed et al. (1983)	20 nonsmoking normal subjects age 18-39; 20 nonsmoking asthmatics age 18-39; 10/20 with allergic asthma and hypersensitivity to ragweed	1-hr exposure to air and 0.1 ppm NO_2; PD of carbachol immediately post-exposure	ΔFEV₁% / ΔSGAW% / %ΔSGAW (PD) Normals air: +1.8 / 0 / -21.6 0.1 ppm NO_2: -0.3 / +3.6 / -49.8 Asthmatics air: -0.7 / -4.6 / -45.4 0.1 ppmNO_2: -2.5 / -15.2 / -45.4	Authors conclude no effect of 0.1 ppm NO_2 on normal or asthmatics in central or peripheral airways. AR not related to baseline PFT, baseline AR, or allergic/nonallergic nature of asthma
Kleinman et al. (1983)	31 asthmatics ((16 = extrinsic allergic; 13 = mixed; 2 = intrinsic) age 31 yrs (18-55); 8/31 not usually on medication; medication stopped 4 hrs prior to exposure	2 hrs to air and 0.2 ppm NO_2; exercise to 2 x resting ventilation first 15 min in each 30; metha-choline challenge immediately post-exposure measured as PD_{10} (>10% reduction in FEV_1	ΔFEV_1% / ΔFVC% / ΔAWR% / PD_{10}% air: -3.3%* / -2.8% / -1.1% / 8.6% 0.2 ppm NO_2: -2.6* / -1.4 / +8.6 / 3.0 *$p < 0.05$	NO concentrations were 20 and 40 ppb in air and NO_2, respectively; slightly more symptoms in air than NO_2 exposures; $p < 0.05$ for PD_{10} difference between exposed and control; saline challenge produced -5.3% on control day and -5.6% ΔFEV_1% on NO_2 exposure; 20/27 subjects showed increased reactivity; AR could not be measured for 4 subjects; 7 had reduced AR

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Bauer et al. (1984) (abstract)	10 asthmatics age 20-44 yrs	30 min (20 min rest + 10 min exercise) at 3 X resting ventilation) to air and 0.3 ppm NO_2 via mouthpiece; PD (cold air) 1 hr post-exposure at resting, 30 L/min, and 60 L/min	ΔFEV$_1$% air: -3 0.3 ppm NO_2: -10* *p <0.05	NO_2 deposition estimated at 73% during rest and 88% during exercise; no significant change in airways after rest; NO_2 exposure increased AR at 30 L/min, but not at rest or 60 L/min; same protocol, similar results to Bauer et al. (1986). Since may include same subjects this report is not included in tables
Stacy et al. (1984)	Normal males with different subjects (n = 9-15) in each exposure group, so individual is not his own control; age ~24 yrs	2 hrs exposure to air and 0.5 ppm NO_2; 15 min exercise at 1 hr 45 min	ΔFEV$_1$% / ΔFVC% / ΔFEF$_{50}$% air: -1.2% / -1.4% / -0.8% 0.5 ppm NO_2: +0.4 / -1.2 / +3.5	No statistically significant effect of NO_2; not included in tables as subject is not his own control
Bauer et al. (1985) (abstract)	6 asthmatics aged 26-44 yrs	4 hrs to air and 0.30 ppm NO_2 and 10 min exercise (3 X resting ventilation) at 30 min, 120 min, and 210 min; unencumbered breathing	ΔFEV$_1$% (30 min / 120 min) — ΔSGAW%* (30 min / 60 min) air: -10.1% / -10% — -29.7% / -29.3% 0.30 ppm NO_2: -13.5 / -17.3* — -36.2% / -49.3* *p <0.05	Both oral and oral-nasal inhalation of 0.30 ppm NO_2 "may potentiate bronchospasm in asthmatic subjects". No report on ΔFEV$_1$ after 4 hrs
Bylin et al. (1985)	8 normal subjects age 20-36 yrs - - - - - - - - - - - - - - - - - - - 8 asthmatics age 17-45 yrs (4 with allergic extrinsic); all hyperreactive to histamine	20 min to air, 0.12, 0.24, and 0.48 ppm NO_2; bronchial reactivity to histamine measured after air and 0.48 ppm NO_2 exposure. AR started with saline, and then increased dose of histamine to produce 100% increased SRAW (PD$_{100}$)	ΔSRAW% / ΔAWR% Normal air: -1.0% / -10.8% 0.12 ppm NO_2: +0.3 / 0 0.24: +9.2 / +13.1 0.48: -24.5 / -26.9 - - - - - - - - - - - - - - - - - - - Asthmatic air: +1.5% / +2.1% 0.12 ppm NO_2: -6.6 / -1.9 0.24: -5.1 / -7.3 0.48: -5.2 / +1.4	AR: no measurable change in 5/8, decreased in 2/8, and increased in 1/8 (air vs. 0.48 ppm NO_2); authors conclude no significant effects of NO_2 exposure - - - - - - - - - - - - - - - - - - - AR: 3/8 = no measurable changes, 5/8 increased (air vs. 0.48 ppm NO_2); despite selection for AR to histamine, 1 of 5 had increased AR of "clinical significance" (167% increase), others had increases of 0-33% Authors suggest a nonmonotonic E-R of increased AWR at low concentrations and decreased AWR at moderately high NO_2 concentrations

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Koenig et al. (1985)	10 healthy nonatopic adolescents aged 13-18 yrs and negative response to exercise test and methacholine challenge 10 atopic adolescent asthmatics aged 11-18 yrs with reversible reactions (airway obstruction) to irritants (dust, mites, mold, pollen), and exercise	60 min to air and 0.12 ppm NO_2 via mouthpiece; measurements at 30 and 60 min; double-blind	ΔFEV$_1$% / ΔFEF$_{50}$% / ΔAWR% 30 min / 60 min / 30 min / 60 min / 30 min / 60 min Normal air: 0% / -1.1% / +3.3% / -0.6% / -0.3% / +7.9% 0.12 ppm NO_2: -3.3 / -4.1 / -1.3 / +1.6 / +0.3 / +5.1 *p <0.05 Asthmatics air: -2.1% / +0.3% / -0.9% / +7.2% / +0.4% / -5.3% 0.12 ppm NO_2: -1.7 / -2.3 / -4.2 / +3.4 / -1.9 / -4.5	No change in blood oxygen or symptoms attributable to NO_2, and no measurable difference between healthy and asthmatic adolescent responses to NO_2
Linn et al. (1985a)	21 volunteers with COPD age 48-69 yrs and able to exercise without supplemental O_2	1 hr to air, 0.5, 1.0, and 2.0 ppm NO_2; exercise to typical level at 0-15 and 30-45 min; mean concentration of PM = 40 µg/m^3	ΔFEV$_1$% / ΔSRAW% / ΔMMFR% 30 min / 60 min / 30 min / 60 min / 60 min air: -1.7% / +0.8% / +6.5% / -3.6% / +3.4% 0.5 ppm NO_2: -1.7 / +0.8 / +4.6 / +10.5 / +6.9 1 ppm: -0.8 / +1.7 / +1.8 / 0 / +7.3 2 ppm: -1.7 / -0.8 / -0.6 / -2.4 / 0	No effect of NO_2 on symptoms, PFT, or arterial oxygen saturation; 20/21 had asthma; other diagnoses were bronchitis (4) and asthma (4); 15/21 on bronchodilators withheld 4 hrs prior to exposure
Linn et al. (1985b)	25 normal subjects age 20-36 yrs; 23 mild asthmatics age 18-34 yrs with hyperactive airways responsive to 0.75 ppm SO_2 during exercise	75 min to air and 4 ppm NO_2; 15 min light exercise (25 L/min) and 15 min heavy exercise (~50 L/min); mean of 2 exposures to air and 2 exposures to NO_2	ΔSRAW% (estimate from graph) Post-Light Exercise / Heavy Exercise Normal air: +18% / +27% 4 ppm NO_2: +16 / +20 Asthmatics air: +24% / +76% 4 ppm NO_2: +7 / +32	Symptoms unrelated to NO_2; despite high statistical power and replication of exposures were unable to detect significant differences in respiratory response between 4 ppm NO_2 and purified air either at rest or following exercise." Previous "conclusions that short-term NO_2 exposure increases airway resistance need to be re-examined."
Roger et al. (1985) (abstract)	12 mild asthmatics age 18-35 yrs; hypersensitive to methacholine	110 min to air and 0.3 ppm NO_2; 20 min rest and 10 min exercise (42 L/min)	ΔFEV$_1$% (30 min) / ΔFVC% (30 min) air: -6 / -3 0.3 ppm NO_2: -12* / -6 *p <0.02	At longer exposures FEV_1 returned to baseline values
Smeglin et al. (1985) (abstract)	20 nonsmoking healthy adults aged 21-48 yrs, without response to carbachol	4 hrs to air and 0.30 ppm NO_2; exercise (3 x resting ventilation) for 10 min at end of 1, 2, and 3 exposure hrs; double-blind	No significant change in FEV_1%, FVC, AWR, maximal and partial expiratory flow rates diffusing capacity or symptoms attributable to NO_2. No increased AR (PD = carbachol) immediately after exposure or after 24-hrs post-exposure	Not included in tables

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Bauer et al. (1986a)	15 nonsmoking asthmatics age 33 yrs (20-45) requiring either intermittent or daily bronchodilators; medication withheld 12 hrs prior to exposure; abnormal response to PD of cold air. Selected for hyperreactivity by screening for responsiveness to cold air	30 min to air and 0.30 ppm NO_2 via mouthpiece; 10 min exercise at 3 x increased ventilation; response to PD of cold air was measured 1 hr post-exposure at resting ventilation, 30 L/min ventilation, and 60 L/min ventilation	ΔFEV_1% Post-Rest (20 min) / Post-Exercise (30 min) / Post-Exposure (1 hr) / ΔFVC% (30 min) air: +2.0% / -4.1% / +2.8% / 3.7% 0.30 ppm NO_2: +0.6 / -10.1* / -3.3 / -5.7 *p <0.05 Effect of Cold air on Airway Reactivity (n = 14) ΔFEV_1% (n = 10) / ΔSGAW% Rest / 30 L/min / 60 L/min / Rest / 30 L/min / 60 L/min air: -3.2% / -7.3% / -20.4% / -8.8% / -36.4% / - 50.9% 0.30 ppm NO_2: -5.1 / -12.9* / -18.9 / -17.8 / -47.5* / -60.3 *p <0.05 that % changes from baseline after air and cold air compared to % change from baseline after NO_2 and cold air	Change in SGAW after NO_2 exposure and exercise did not differ from air exposure. No response at rest. "...[E]xercising mild asthmatics develop transient airway dysfunction after inhalation of [0.3 ppm] NO_2 and ...airway hyperreactivity persists after lung function returns to normal."
Bauer et al. (1986b) (abstract)	8 COPD patients age 47-63 yrs; FEV_1/FVC = 0.60	4 hrs to air and 0.30 ppm NO_2; 7 min exercise (4 X resting ventilation) at 20 min, 1 hr and 2 hrs exposure	ΔFEV_1% (1 hr) / (2 hrs) air: +4 / 0 0.3 ppm NO_2: -10 / -9	No effect on airway conductance
Linn et al. (1986)	21 mild asthmatics age 20-34 yrs hyperreactive to cold air and/or exercise; most had allergic extrinsic asthma, and required infrequent bronchodilators	1 hr to air, 0.3, 1, and 3 ppm NO_2 with 3 10-min exercise periods (41 L/min). AR assessed by PD of cold air immediately post-exposure. ΔPFT measured after initial exercise (early = 10 min) and at end of exposure (late)	ΔFEV1% / ΔMMFR% / ΔSRAW% Early / Late / Early / Late / Early / Late air: -3.2% / -2.5% / -6.0% / -1.8% / +44% / +63% 0.3 ppm NO_2: -1.6 / -0.3 / -6.2 / -3.4 / +44 / +64 1 ppm NO_2: -3.1 / -1.9 / -6.7 / -2.7 / +32 / +35 3 ppm NO_2: -2.2 / -0.6 / -6.8 / -2.8 / +31 / +29 Effect of Post-Exposure PD of Cold Air (ΔFEV_1%) 1 min Post-Exposure / 4 min Post-Exposure air: -11.4% / -16.7% 0.3 ppm NO_2: -12.1 / -16.1 1 ppm NO_2: -11.2 / -15.5 3 ppm NO_2: -14.2 / -17.2	No change in PFT and symptoms attributable to NO_2, and no increase in AR. Results inconsistent with other findings of NO_2 effect, perhaps because subjects had mild disease. Three subjects with most severe disease also showed no consistent response to NO_2
Mohsenin et al. (1986) (abstract)	11 nonsmoking normal subjects, mean age 25 yrs; 9 asthmatics mean age 30 yrs	1 hour to air and 2 ppm NO_2 1 hour to air and 0.5 ppm NO_2	No significant change in flow rates, lung volumes, or airway conductance for either normal or asthmatic subjects	Not included in tables; similar protocol as Mohsenin (1987, 1988)
Adams et al. (1987)	20 healthy female subjects aged 21 yrs (19-25); 20 healthy male subjects aged 23 yrs (18-30) All were aerobically trained	1 hr to air and 0.60 ppm NO_2 via mouthpiece while undergoing continuous heavy exercise (70 L/min for males, 50 L/min for females)	ΔFEV_1% / ΔFEF_{25-75}% / ΔSRAW% Female air: -2.2% / -0.2% / +0.9% 0.60 ppm NO_2: -2.0 / -0.1 / -0.7 Males air: -1.6% / -0.8% / +2.4% 0.60 ppm NO_2: +0.02 / -2.1 / -3.8	No effect on PFT or symptoms in either sex

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Bauer et al. (1987) (abstract)	20 COPD patients age 47-68 yrs; FEV_1/FVC = 0.58; 8/20 = mild COPD with FEV_1/FVC = 0.72; 12/20 = moderate/severe COPD with FEV_1/FVC = 0.40	4 hrs to air and 0.30 ppm NO_2 with 7 min exercise (3 X resting ventilation) at 20 min, 1 hr, and 2 hrs	ΔFEV$_1$% 20 min 1.5 hrs 2.0 hrs 4 hrs air → → → no change → → → 0.3 ppm NO_2 → little change → -3 -5 ΔFVC% 20 min 1.5 hrs 2.0 hrs 4 hrs air → → → no change → → → 0.3 ppm NO_2 -1 -6 -9 -8	No included in tables as actual air values not provided
Drechsler-Parks (1987a)	32 healthy nonsmokers; 16 "young" subjects age 18-26; 16 "old" subject age 51-76	2 hrs to air and 0.6 ppm NO_2; alternate 20 min rest, 10 min exercise (25 L/min)	ΔFEV$_1$%(SE) ΔFEF$_{25-75}$% ΔFEF$_{75}$% All air +0.11% (1.1) +0.4% (2.1) -5.9% (3.5) 0.6 ppm NO_2 +0.8 (0.7) +3.1 (2.0) -3.4 (3.5) "Young" air +1.6% (1.4) +3.0% (2.1) -5.8% (4.2) 0.6 ppm NO_2 +1.7 (0.7) +4.2 (2.2) -5.9 (5.6) "Old" air -1.4% (1.6) -2.3% (3.7) -5.9% (5.8) 0.6 ppm NO_2 -0.05 (1.1) +2.0 (3.6) -1.0 (4.3)	No significant effect of NO_2 on PFT or symptoms; no interaction of NO_2 and O_3
Drechsler-Parks et al. (1987b)	16 healthy nonsmokers age 63 yrs (51-76)	2 hrs to air and 0.60 ppm NO_2; alternating 20 min of rest and exercise (25 L/min)	ΔFEV$_1$% (SE) ΔFEF$_{25-75}$% (SE) air +1.4% (1.6) +2.3% (3.7) 0.6 ppm NO_2 0 (1.1) +2.0 (3.4) (-14 TO +5.2%) (-38 TO 21%)	No statistically significant differences between men and women, young or old, and no apparent effect of NO_2 on PFT or symptoms

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Koenig et al. (1987)	10 Healthy adolescent subjects aged 13-18 without hyperreactivity to exercise, methacholine, or skin tests for inhalant pollen factors 10 asthmatic atopic adolescent subjects with reversible COPD, elevated IGE, and AR to exercise and methacholine challenge	1 hr to air and 0.12 ppm NO_2 via mouthpiece; Phase 1: at rest Phase 2: 10 min exercise at 5-6 X resting ventilation in last 30 min. Phase 3: 0.18 ppm NO_2 with 10 min exercise in last 30 min	Healthy Phase 1 air: -1.1% / -0.6% / -2.0% / +7.9% 0.12 ppm NO_2: -4.1 / +1.6 / +3.1 / +5.1 Phase 2 air: -3.1% / -3.6% / -2.6% / -3.2% 0.12 ppm NO_2: -3.1 / +1.7 / -6.5 / +5.3 Phase 3 air: -2.8% / -5.2% / -8.5% / +5.3% 0.18 ppm NO_2: -1.6 / -0.9 / -6.1 / -0.2 Asthmatics Phase 1 air: +0.3% / +7.2% / +5.8% / -5.3% 0.12 ppm NO_2: -2.3 / +3.4 / +2.2 / -4.5 Phase 2 air: -6.3% / -3.2% / -6.1% / -4.3% 0.12 ppm NO_2: -6.1 / -12.7 / -10.5 / +10.3 Phase 3 air: -1.3% / -4.7% / -3.5% / +5.2% 0.18 ppm NO_2: -3.3 / -7.5 / -9.7 / +11.6 (ΔFEV$_1$% / ΔFEF$_{50}$% / ΔFEV$_{75}$% / AWR%)	Asthmatics were no more responsive to NO_2 than healthy subjects; statistically significant increases in AWR in Phase 2, both normal and asthmatics. No significant changes in Phases 1 and 3
Mohsenin (1987a)	11 healthy subjects aged 18-36 years	1 hr to air and 2 ppm NO_2; AR to methacholine ≤45 min post-exposure; double-blind	ΔFEV$_1$% / ΔSGAW% / PD$_{40}$ air: -3.0% / -2.4% / 64 2 ppm NO_2: -0.3 / +1.0 / 51* (20% decreased air vs. NO_2) *p <0.04	No significant effect of NO_2 . PD$_{40}$ = dose of methacholine to cause 40% reduction SGAW. Change in AR is "small compared with spontaneous variation as a result of external stimuli in patients with asthma"
Mohsenin (1987b)	10 mild asthmatics aged 30 yrs (22-40) with airway reactivity to methacholine; FEV_1 >60% predicted; medication stopped 24 hrs prior to exposure (8/10 on medication)	1 hr to air and 0.5 ppm NO_2; AR determined immediately post-exposure using methacholine; PD$_{40}$ = cumulative methacholine dose producing ≥40% reduction in Vp$_{40}$ double-blind	ΔFEV$_1$% / ΔSGAW% / ΔVp$_{40}$% / PD$_{40}$ (S.D.) air: -3.2% / +6.0% / -13.9% / 9.2 (15) 0.5 ppm NO_2: -4.8 / -6.4 / -17.8 / 4.6 (8.2)* *p 0.04 AR: 7/10 increased 2/10 decreased 1/10 no change Vp$_{40}$ = partial expiratory flow at 40% of vital capacity, a sensitive test for small airways abnormality	No significant change in lung function and symptoms following exposure. Measurement of AR a "more sensitive test on detecting airway effect due to airway irritants" and occurs without exercise or broncho-constriction in asthmatics

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Avol et al. (1988)	59 moderate to severe asthmatics aged 30 yrs (18-48) regularly using bronchodilators, and $FEV_1/FVC \geq 43$ to 98%; medication withheld 8 hrs prior to exposure	2 hrs at air, 0.3, and 0.6 ppm NO_2; alternate 10 min rest/10 min exercise (40 L/min); AR to cold air tested 1 hr and 24 hrs (n = 37) post-exposure measured as ΔFEV_1	ΔFEV_1% / ΔSRAW% 1 Hour / 2 Hours / 1 Hour / 2 Hours air: -14.2% / -9.9% / +83.5% / +72.9% 0.3 ppm NO_2: -14.8 / -12.2 / +85.9 / +48.9 0.6 ppm: -11.2 / -7.8 / +71.7 / +65.2 AR (ΔFEV_1% due to PD cold air) (n = 37) 1 Hour / 24 Hours air: -11.9% (10.9) / -14.2% (14.3) 0.3 ppm NO_2: -13.6 (12.1) / -12.9 (12.3) 0.6 ppm: -11.9 (13.6) / -10.6 (12.7)	No significant effect of NO_2 on PFT, symptoms (either by respiratory symptom subgroup or severity), or bronchial reactivity (AR). No effects of NO_2 among more severe asthmatics (FEV_1/FVC <0.65). AR not tested in 22 subjects because of already compromised lung function
Bylin et al. (1988)	20 mild asthmatics aged 33 yrs (17-56) (12 = allergic extrinsic); mean AWR and SRAW similar to predicted values for nonasthmatic (1.13 ± 0.08 and 3.76 ± 0.33)	30 min to air, 0.14, 0.27, and 0.54 ppm NO_2; AR tested 25 min post-exposure with PD histamine to produce 2-fold increased SRAW	ΔAWR% at PD (SE) / 0.2 mg/mL PD / % Increased AR air: 0.39 (0.07) / +47% 0.14 ppm NO_2: 0.28 (0.05) / +58 / 14/20 0.27: 0.24 (0.04)* / +81 / 14/20 0.54: 0.34 (0.08)* / +64 / 15/20 *p <0.05 ΔSRAW% (estimate from graph) air: 12% 0.14 ppm NO_2: 0 0.27: +2 0.54: -8	There were no increases in SRAW during any air or NO_2 exposure, and tendency for SRAW to decrease as time of exposure increased. An increase of 15% or more was assumed to be of clinical significance and never occurred with or without adjustment for air. PD for histamine threshold defined as 100% increase in SRAW. Increase in AR at 0.27 ppm similar to spontaneous variability in AR among asthmatic patients, and therefore of "limited clinical importance"
Mohsenin (1988)	18 nonsmoking healthy subjects age 25 yrs (18-33)	1 hr to air and 2.0 ppm NO_2; methacholine challenge 10 min post-exposure; PD_{40} = concentration to decrease SGAW by 40%	PD_{40} / % Increased AR air: 101 (44) / 12/18 increased AR 2.0 ppm NO_2: 81 (45)* / 4/18 no change 8/18 reduced AR *p 0.003 ΔFVC (L) / ΔFEV_1 (L) / Vp_{40} (L/Sec) / ΔSGAW (L/sec cm H_2O/L) air: -0.12 (0.18) / -0.11 (0.15) / -0.12 (0.53) / +0.016 (0.087) 2 ppm NO_2: -0.06 (0.14) / -0.04 (0.11) / -0.18 (0.44) / +0.009 (0.042) Approximate ΔPFT% (using baseline absolute values) ΔFVC% / ΔFEV_1% / ΔSGAW% air: -2.6% / -2.9% / +5.5% 2 ppm NO_2: -1.3 / -1.1 / +3.1	No significant change in spirometry, SGAW, flow at low lung volumes, or symptoms as result of NO_2. For 5 subjects whose PD_{40} could not be obtained, PD methacholine produced reduction SGAW of 31% (air) and 40% (NO_2)

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Avol et al. (1989)	34 young asthmatics aged 8-16 yrs with >34% reduction in FEV_1 after exercise test and cold air challenge	3 hrs to air and 0.30 ppm NO_2 with alternating 10 min rest and exercise (30 L/min)	**ΔFEV_1%** 60 min / 120 min / 180 min air: -0.6% / +0.2% / +1.5% 0.30 ppm NO_2: -5.4% / -0.9 / +1.0 **ΔMMFR%** 60 min / 120 min / 180 min air: -3.3% / +4.7% / +6.0% 0.30 ppm NO_2: -4.9 / +2.1 / +7.2 **ΔSRAW** 60 min / 120 min / 180 min air: +9.8% / +9.1% / +4.7% 0.30 ppm NO_2: 16.9 / +8.3 / +5.2	No large or consistent effect of NO_2 on lung function or symptoms. PFT improved in last 2 hrs of exposure
Morrow & Utell (1989)	20 normal nonsmokers aged 31 yrs (20-48) 20 nonsmoking mild/moderate asthmatics age 29.5 yrs (19-54) 20 smoking COPD patients age 60 yrs (47-70) tolerant to exercise 3 x resting ventilation 20 elderly normal subjects aged 60 yrs (49-69)	4 hrs to air and 0.3 ppm NO_2; 30 min exercise 3-4 x resting ventilation; carbachol challenge immediately and 24 hrs post-exposure	ΔFVC% (SD) / ΔFEV_1% / ΔSGAW% / ΔFEV_1% PD Normal air: -2.6 (3.9) / -0.6 (3.3) / +1.9 (6) / -2.7 (3.5) 0.3 ppm NO_2: -2.5 (5.4) / -0.4 (2.7) / +4.4 (6) / -0.4 (1.6) Asthmatics air: -6.7 (10) / -7.2 (11.9) / -20.0 (28.7) / -3.6 (n = 8) 0.3 ppm NO_2: -8.0 (10.3) / -3.7 (9.1) / -12.5 (24.2) / -7.5 COPD air: -3.7 (15.9) / +0.3 (12.3) / -8.6 (18.6) / -- 0.3 ppm NO_2: -8.2 (14.0) / -4.8 (9.4) / +2.1 (8.0) / -- Elderly air: -0.2 (4.6) / -0.9 (4.5) / +0.6 (7.2) / -1.9 (3.8) 0.3 ppm NO_2: -0.7 (5.3) / 0.0 (3.1) / +0.6 (7.1) / -2.6 (2.7)	No significant changes in FEV_1, FVC, SGAW, or symptoms during or 24-hours after exposure in young or elderly control groups, asthmatics, and COPD patients (FVC significantly reduced). No effect on AR was observed in any group either immediately post-exposure or 24 hrs later
Jorres and Magnussen (1990)	14 nonsmoking atopic (12) mild asthmatics aged 34 yrs (20-55 yrs) with AR to histamine and SO_2; FEV_1 = 86% of predicted	30 min to air and 0.25 ppm NO_2 via mouthpiece; AR measured 15 min post-exposure via hyper-ventilation of 0.75 ppm SO_2	ΔSRAW% / PV_{100}* (SEM) air: -11.5% (-49 to +73) / 46.5 (5.1) 0.25 ppm NO_2: -11.4 (-49 to +13) / 37.7 (3.5) *PV_{100} SRAW (SO_2) = provocative ventilation necessary to increase SRAW by 100%	No significant effect of NO_2 on SRAW or symptoms, PV_{100} was significantly less ($p < 0.01$) after NO_2 compared to air
Roger et al. (1990)	21 male mild asthmatics aged 23 yrs (19-30); bronchodilators withheld 12 hrs prior to exposure; experience cold air or exercise induced bronchoconstriction and sensitivity to methacholine; baseline FEV_1/FVC = 0.67	75 min to air and 0.15, 0.30, and 0.60 ppm NO_2; 3 10-min cycles of moderate exercise (45 L/min). AR tested 2 hrs post-exposure; PD = methacholine (n = 19). PD_{100} provocative dose producing doubling of SRAW	ΔFEV / ΔSRAW 1st Exercise / 3rd Exercise / 1st Exercise / 3rd Exercise air: -4.8% / -2.8% / +86% / 52% 0.15 ppm NO_2: -5.9 / -3.9 / +107 / +69 0.30 ppm: -4.4 / -1.7 / +79 / +54 0.60 ppm: -4.7 / -4.0 / +81 / +56 PD_{100} (SE) (n = 19) air: 3.3% (0.7) 0.15 ppm NO_2: 3.1 (0.7) 0.30 ppm: 3.3 (0.8) 0.60 ppm: 3.7 (1.1)	No significant effect of NO_2 on PFT symptoms, or AR

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Rubenstein et al. (1990)	9 nonsmoking asthmatics age 23-34 yrs on regular bronchodilators withheld prior to exposure	30 min to air and 0.30 ppm NO_2, exercise (3 x resting ventilation) for first 20 min of exposure; AR 1 hr post-exposure via SO_2	ΔSRAW% / $PD8USO_2$ air: +1.4% / 1.25 (0.70) (0.53 - 2.5 ppm) 0.30 ppm NO_2: 0 / 1.31 (0.75) (0.29 - 2.80 ppm) $PD8USO_2$ = dose of SO_2 required to increase SRAW 8U above baseline	No change in PFT (SRAW, FEV_1/FVC, SBN_2), symptoms or AR attributable to 0.30 ppm NO_2
Frampton et al. (1991); same data reported in Utell et al. (1991)	Healthy nonsmoking volunteers age 18-40 yrs without AR: 1) n = 9, age 30 yrs 2) n = 15, age 25 yrs 3) n = 15, age 24 yrs	3 hrs to air and NO_2; exercise 10 min of each 30 min (40 L/min); AR to carbachol 30 min post-exposure: 1) continuous to 0.60 ppm 2) baseline 0.05 ppm with intermittent peaks to 2.0 ppm 3) continuous 1.5 ppm	Δ FEV_1% / ΔSGAW% / Response to Carbachol (ΔFEV_1%) 1) air: +1.2% / -4.9% / -4.7% 0.60 ppm NO_2: +1.5 / -2.9 / -5.0 2) air: +1.5% / -5.3% / -4.9% 0.5 ppm with peaks to 2.0 ppm: -0.5 / -4.6 / -6.6 3) air: +1.7% / -4.0% / -2.8% 1.5 ppm NO_2: +2.1 / -6.1 / -6.4* *p 0.03	No significant effect of NO_2 on symptoms or PFT (including MEFR and $PEFV_{60}$%), and no change in AR for exposures to 0.60 ppm and peaks. Slight increase in AR for 1.5 ppm NO_2, with 11/15 showing greater decrease FEV_1 for NO_2 than air; one with 20% decrease FEV_1
Jorres & Magnussen (1991)	11 mild asthmatics age 29 yrs (17-55); % predicted FEV_1 = 91 (8) % (76-104); 7 atopic, all hyperresponsive to methacholine; medication withheld prior to exposure	30 min to air and 0.25 ppm NO_2 via mouthpiece; 10 min exercise (30 L/min)	ΔSRAW% (Resting / Exercise) / PC_{100}* (SEM) air: -9.2% (-37 to +41) / +69.7% (-15 to 177) / 0.41 (1.62) 0.25 ppm NO_2: -2.7 (-28 to +29) / +83.6 (+16 to +216) / 0.41 (1.61) PC_{100} = provocative concentration of methacholine necessary to increase SRAW by 100%	No significant effect of 0.25 ppm NO_2 on airway tone or AR of asthmatics at rest or exercise
Kim et al. (1991)	9 healthy athletes age 18-23 yrs	30 min to air, 0.10 and 0.30 ppm NO_2 via mouthpiece; variable exercise (stand, walk, run = 12 min at 40 L/min). Methacholine challenge for AR immediately post-exposure	ΔFEV1% / ΔFEF50% / ΔAWR% air: -0.5% / -0.2% / -1.8% 0.18 ppm NO_2: -0.5 / -4.5 / -1.3 0.30 ppm: +0.2 / -4.0 / -1.6	No significant effect of NO_2 on PFT, symptoms or AR
Hackney et al. (1992)	26 smoking COPD patients aged 47-69; mean FEV_1/FVC = 0.56; 9 on respiratory medication	4 hrs to air and 0.30 ppm NO_2 with 4-7 min exercise at 25 L/min; double-blind	ΔFEV1% / ΔSRAW% air: +1.3/+1.3 / 0/0 0.3 ppm NO_2: +1.3/+1.3 / 0/0 Estimated from graph	Symptom reports and hourly forced expiratory function tests showed no statistically significant differences between clean air and NO_2. Also, no differences between ΔFEV_1 in field and in exposure chamber
Hazucha et al. (1992)	15 healthy nonsmoking females	2 hrs to air and 0.6 ppm NO_2 during intermittent exercise (40 L/min) alternating 15 min rest/15 min exercise	ΔFEV1% / ΔSRAW% air: -0.2% / -8.8% 0.6 ppm NO_2: -0.6 / -9.2	Not included in tables

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Jorres et al. (1992) abstract	8 normal subjects aged 27 yrs; 10 subjects with mild extrinsic asthma aged 27 yrs; mean FEV_1 = 90% predicted	3 hrs to air and 1 ppm NO_2 during intermittent exercise; measured during and up to 1 hr after exposure	No significant effects of exposure on VC, FEV_1, and AWR on normal and asthmatic subjects	Not included in tables
Morrow et al. (1992)	20 normal elderly subjects aged 61 yrs (49-69) matched to COPD group. 20 COPD smoking/ exsmoking patients aged 60 yrs (47-70) FEV_1 <80% predicted; FEV_1/FVC <75 but >0.45; <15% increases in FEV_1 after but not oral inhalation of broncho-dilator; inhaled (4/20) medication (12/20) withheld prior to exposure	4 hrs to air and 0.30 ppm NO_2 with 21 min exercise at 4 x resting ventilation; carbachol AR challenges at 4 hrs and 24 hrs for normals; isoproterenol challenge to COPD patients to ascertain and relieve exposure-related broncho-constriction	ΔFEV1% 1 Hour / 4 Hours / Carbachol / 24 Hours / Carbachol Normal Elderly air: +0.8% / -1% / -2% / -0.5% / -3% 0.3 ppm NO_2: +0.8 / 0 / -2.8 / -1 / -1.2 ΔFVC% 1 Hour / 4 Hours / Carbachol / 24 Hours / Carbachol air: +0.8% / -0.3% / -2% / 0% / -3.5% 0.3 ppm NO_2: -1.0 / -0.5 / -4 / 0 / -2 --- ΔFEV1% 1 Hour / 4 Hours / Isoproterenol / 24 Hours air: +3.0% / +0.27% / +4% / +2% 0.3 ppm NO_2: -0.9 / -4.8 / +6 / +2 ΔFVC_1% 1 Hour / 4 Hours / Isoproterenol / 24 Hours air: -1.3% / -3.7% / +5% / +0% 0.3 ppm NO_2: -1.2 / -8.2 / +1 / -2 ΔSGAW% 1 Hour / 4 Hours / Isoproterenol / 24 Hours air: -11.5% / -8.5% / -- / -6% 0.3 ppm NO_2: -11 / -11 / -- / -4.5	No significant change in any measured parameter attributable to NO_2 as responses to NO_2 and air were virtually identical. --- No significant changes in SGAW, DL_{CO}, oxygen saturation, AR, or symptoms could be attributed to NO_2; COPD patients with less severe disease a slightly longer response to NO_2
Devalia et al. (1994)	10 mild asthmatics with allergy to house-dust-mite antigen, age 28 yrs	6 hrs to air and 0.4 ppm NO_2	ΔFEV1% / ΔFVC% air: -2.2% (3.0) / -4.1% 0.6 ppm NO_2: -6.4 (3.2) / -0.1 (4.5) PD_{20} reduced 41% after NO_2 compared to air	"Our finding that exposure of people with mild asthma to 400 ppb nitrogen dioxide does not significantly change FEV_1 or FVC is consistent with other studies that have failed to demonstrate any effects on lung function to concentrations of up to 4000 ppb"

NO_2 CHAMBER STUDIES (cont'd)

References	Subjects	Exposures	ΔPFT%/PD	Comments
Hazucha et al. (1994)	21 healthy nonsmoking women age 18-35 yrs	2-hrs exposure to air and 0.6 ppm NO_2 with intermittent exercise (15 min on/off) at 40 L/min. Spirometry at 1-hr intervals; 2-hrs exposure to 0.3 ppm O_3 3 hrs post-NO_2 exposure; methacholine challenge following O_3 exposure	<u>ΔFEV$_1$%</u> <u>ΔFVC%</u> <u>ΔFEF$_{25-75}$%</u> <u>ΔSRAW%</u> air 0% -1.0% +0.6% -9.4 0.6 ppm NO_2 -1.5 -2.0 +0.5 -8.5 <u>Post-O_3 Challenge</u> <u>ΔFEV$_1$%</u> <u>PD$_{10}$FEV$_1$ (methacholine challenge)</u> air -10.8 5.6 mg/mL 0.6 ppm NO_2 -12.8* 1.7 mg/mL* *p <0.05	NO_2 alone had no significant effect on symptoms or lung function; NO_2 pre-exposure enhanced spirometric effects but not AWR of O_3 and methacholine

SRAW	specific airway resistance
SGAW	specific airway conductance
MMRF	mid maximal respiratory flow (similar to FEF_{50}, FEF_{25-75})
FEV_{50}	forced expiratory flow at 50% of FVC
ΔPFT%	$\left(\frac{\text{after exposure PFT - before exposure PFT}}{\text{before exposure PFT}}\right)$
PFT	pulmonary function test results
PD	provocative dose of bronchoconstriction to measure airway responsiveness
AR	airway responsiveness