Table 3.6 Controlled Human Exposures to NO_2

Reference	Location	Study Design	Number of Subjects	Subject Description	Characteristics of Study	Duration of Exposure	NO_2 Concentration
Orehek, Massari et al., 1976		Single-blind	20	Outpatients with slight to mild asthma (symptom free during study and did not take medication 24 hours prior to study).	Controlled exposure to NO_2 in a chamber.	1 hour	0, 0.1 ppm, 0.2 ppm (4 subjects only)
Goings, Kulle et al., 1989	Baltimore, USA	Placebo-controlled, randomized double-blind	152	Healthy, non-smoking adults who were seronegative to influenza A/Dorea/82 (H3N3) virus. Aged 18-35 years.	Subjects were exposed to NO_2 or ambient air in a environmental chamber.	Exposure in the chamber was for 2 hours on three consecutive days.	Year one: ambient air or 2 ppm NO_2; Year two: ambient air or 3 ppm NO_2; Year three: ambient air, 1 or 2 ppm NO_2
Jorres & Magnussen, 1990	Germany	Crossover	14 (10 male, 4 female)	Mild asthmatics aged 34 (+/-14) years.	Tidal breathing via mouthpiece of filtered air, NO_2, or SO_2 followed by isocapnic hyperventilation of 0.75 ppm SO_2 (on 3 separate days).	30 minutes	0.25 ppm
Rasmussen, Kjaergaard & Petersen, 1990	Denmark	Randomized double-blind	40 (24 male, 16 female)	20 with slight to moderate asthma and 20 healthy. Median age of 33.5 years (20 to 73).	Participants divided into 20 matched asthmatic/healthy teams. Each team exposed via chamber 4 times to randomized concentrations. 10 minutes of exercise between 2nd and 3rd hour.	3 hours (no NO_2 in first hour).	0 ppm; 0.1 ppm; 0.2 ppm; 0.8 ppm
Roger, Horstman et al., 1990	North Carolina, USA	Crossover	34 (all male) in two separate studies	Non-smoking asthmatic Caucasian males (aged 19-35 years). 13 in preliminary experiment and 21 in concentration-response experiment.	**Both:** Exposure via chamber. 20 minutes rest followed by three 10 minute cycles of treadmill exercise followed by 20 minutes of testing and rest. **Concentration-response experiment:** Airway responsiveness measured by methacholine challenge 2 hours after exposure.	75 minutes	0.30 ppm for the preliminary experiment; 0.15 ppm, 0.30 ppm or 0.60 ppm for concentration-response experiment.
Rubinstein, Bigby, et al., 1990		Chamber	9 (4m, 5f)	Non-smoking asthmatics, 23-34 years.	Subjects breathed test air through a mouthpiece at 20 L/min	4 minutes	Doubling doses 0.25-4.0 ppm

Main Comparison	Positive Results	Null or Negative Results	Comments
NO_2 and direct bronchomotor effects (specific airway resistance, with carbachol and without) in asthmatics.	NO_2 induced a slight significant increase in specific airway resistance (initial at 6.0, after NO_2 = 6.9) and enhanced bronchoconstrictor effect of carbachol in 13 subjects (mean dose producing a two-fold increase in initial specific airway resistance decreased from 0.66 mg to 0.36 mg with NO_2).	NO_2 did not modify initially SRaw nor bronchoconstrictor effect of carbachol in 7 subjects, 4 had variable results.	
NO_2 exposure and human susceptibility to respiratory virus infection and lung function (FEV_1, FVC, and FEF_{25-75}).	A significant association was found between FEV_1 decrement and day of observation between exposed and the control group (-2%).	No significant association between NO_2 level and susceptibility to respiratory virus infection was observed. However, people exposed to 1 or 2 ppm in the third year had a higher incidence of infection than controls. No significant difference in lung function was observed between the 2 and 3 ppm exposed groups.	Double blind trial. The authors do state that there was a statistically significantly linear trend of decreasing reactivity over the day of observation ($p<0.01$) for all subjects from day 0 to day 3.
The provocative ventilation necessary to increase SRaw by 100% (PV_{100}SRaw) was compared following exposure to filtered air, NO_2, or SO_2.	Mean $\pm$ SEM PV_{100}SRaw was 'significantly' ($p<0.01$) lower after NO_2 (37.7$\pm$3.5) as compared to filtered air (46.5$\pm$5.1) or SO_2 (45.4$\pm$4.2).		
Difference between SRaw and FEV_1 in first hour (no exposure) and 3rd hour (after two hours of exposure). Asthmatic versus controls.		No significant effects among asthmatics at 0.1 ppm, as reported by Orehek. With respect to symptom scores it appears that short-term exposure to NO_2 in concentrations up to 0.8 ppm is unlikely to elicit subjective symptoms of mucous membrane irritation.	
FEV_1 following exercise in NO_2 versus clean air.	**Preliminary experiment:** Decreases in FEV_1 and FVC and increases in SRaw were significantly greater in 0.30 ppm than in clean air. 11% decrease in FEV_1 in 0.30 ppm NO_2 versus 7% in air after first set of exercises.	**Concentration-response experiment:** No overall group-averaged indication of a concentration-related effect of NO2 on pulmonary function. Symptoms were not significantly different than those reported in clean air, which included decreased lung function and increased airway resistance after exercise.	Results from the first experiment (preliminary) were not duplicated in the second experiment (concentration-response).
NO2 exposure and enhanced airway responsiveness from increasing SO2 exposure.		Exposure to NO2 was not associated with any change in the reported symptoms or in measured pulmonary tests.	Subjects exercised for 20 min during NO2/placebo exposure. Exposure to NO2 and ambient air was done in a double-blind randomized fashion.

Reference	Location	Study Design	Number of Subjects	Subject Description	Characteristics of Study	Duration of Exposure	NO_2 Concentration
Frampton, Morrow et al., 1991	Rochester, New York	Experimental	**Group 1** 9 (7 male, 2 female); **Group 2** 15 (11 male, 4 female); **Group 3** 15 (12 male, 3 female)	Healthy, non-smoking adults aged 19-37 years with no pulmonary disease history.	Subjects were exposed to ambient air and/or NO_2 in an environmental chamber while exercising (10 minutes out of every 30 minutes).	3 hour exposures, with one week between NO_2 exposure and ambient air.	**Group 1:** 0.6 ppm; **Group 2:** 1.5 ppm; **Group 3:** 0.05 ppm with three intermittent peak exposures of 2 ppm.
Huang, Wang & Hsieh, 1991	Taipei, Taiwan	Crossover	6 (5 male, 1 female)	Mite-sensitive asthmatic children with mean age 12 years. Moderate severity, given no asthmatic medications for at least 7 days.	Taipei road tunnel air or ambient air administered via mouthpiece.	5 minutes	70-120 ppb SO_2 and 450-500 ppb NO_X (NO_2 and NO) combined.
Jorres & Magnussen, 1991	Hamburg, Germany	Crossover	11	Asymptomatic asthmatics aged 17-55 (mean 29).	Subjects breathed test gas through a mouthpiece in a sitting position.	20 minutes of rest followed by 10 minutes of exercise.	0.25 ppm
Kim, Koenig et al., 1991	Seattle, Washington, USA	Crossover	9	Healthy men from 19-23 years of age who were actively involved in intercollegiate cross-country track or a comparable level.	Test atmospheres were inhaled via a rubber mouthpiece with a nose clip in place for 30 minutes including 16 minutes of heavy exercise.	30 minutes	0.18 or 0.30 ppm
Sandstrom, Stjernberg et al., 1991	Sweden	Experimental (not crossover)	18	Healthy, non-smoking males aged 22-32 years.	NO_2 with continuous bicycle activity (ergometer) with a work load of 75 W for the last 15 minutes of exposure in an environmental chamber.	20 minutes	4 mg/m^3
Avol, Linn et al., 1992	Downey, California, USA	Crossover	34	Asthmatics aged 8-16 years.	Exposures were to clean air, 0.30 ppm NO_2, or polluted Los Angeles air on summer mornings when relatively high NO_2 was expected. Alternating 10 minute periods of exposure and rest. Exposures were done in an chamber and separated by one week (not completely randomized).	3 hours	0.30 ppm (controlled); 0.09 ppm mean with range 0.01-0.26 ppm (ambient)
Hackney, Linn et al., 1992	Los Angeles, CA, USA	Chamber	26 (15 male, 11 female)	Residents with physician diagnosed COPD, aged 45-70 years. Subjects all had heavy smoking history and low FEV_1.	Subjects were exposed to NO_2 or ambient air in a chamber with four exercise periods.	4 hour exposures. Exercise periods lasted 7 minutes.	Ambient air or 0.3 ppm NO_2
Morrow, Utell et al., 1992	Rochester, NY, USA	Double-blind Crossover	40 (COPD = 13 male, 7 female; Normal = 10 male, 10 female)	Elderly normal and COPD patients with mean age of 61 and 60 years, respectively.	Exposed to air or NO_2 in chamber; Randomized at least 5 days apart; Intermittent exercise.	4 hours	0.3 ppm
Rasmussen, Kjaergaard, et al., 1992	Denmark	Double-blind Crossover	14 (10 male, 4 female)	Healthy, non-smoking adults with mean age of 34.4 years (22-66).	Subjects exposed via chamber in two groups (2 females, 5 males) to air and to NO_2. Exposures were 1 week apart.	5 hours	2.3 ppm

Main Comparison	Positive Results	Null or Negative Results	Comments
NO_2 exposure and pulmonary function (SGaw, PEFR, MEFR, FVC and FEV_1) and airway reactivity.	A greater decrease in FVC and FEV_1 was observed in response to carbachol in the 1.5 ppm exposed group compared to ambient air (FVC = 1.5% air, 3.9% NO_2, $p<0.01$). This was not observed for the other two groups.	No direct association was found between NO_2 level and pulmonary response for any of the exposure groups.	
Methacholine and allergen sensitivities and pulmonary function after breathing polluted or ambient air were compared.		No difference in pulmonary function was noted, and methacholine and allergen sensitivities of airways were not increased after polluted air was inhaled.	
LUNG FUNCTION: Specific airway resistance during exposure to NO_2 versus exposure to filtered air.		Mean and SD values for specific airway resistance (SRaw) were comparable for NO_2 and filtered air during both rest and exercise.	
Pulmonary function parameters (FEV_1, PEFR (peak expiratory flow rate), R_t (total respiratory resistance), and FVC (forced vital capacity) were compared before and after exposures.		No statistically significant changes were observed in FEV_1, R_t, PEFR, or $Vmax_{50\%}$ after exposure to 0.18 or 0.30 ppm NO_2 (small decreases did occur).	
Results of FEV_1, FVC and BAL after exposure were compared to those prior to exposure.	An inflammatory cell response was found after exposure to all concentrations (mast cells, lymphocytes, lysozome positive alveolar macrophages).	There was no significant change in lung function after exposure. The inflammatory mediators fibronectin, hyaluronan, angiotensin converting enzyme (ACE) and beta-microglobulin were unchanged by exposure.	There were a total of 18 subjects (prior), but only 8 participated in each exposure group.
Questionnaire-reported symptoms and lung function measured just prior to and after 1, 2, and 3 hours of exposure, as well as bronchial reactivity to cold dry air measured 1 hour after exposure were compared for the three different exposures.	Lung function declined slightly during the first hour at 0.3ppm, but improved over the remaining 2 hours. Compared to other conditions, symptoms were increased during 1-week periods following 0.3ppm NO2 exposure.	Ambient exposures did not significantly affect lung function, symptoms, or bronchial reactivity to cold air, relative to the control condition. Compared to other conditions, symptoms were not increased during 0.3 ppm exposures.	Effects of 0.3 ppm exposure may be confounded by decreases in lung function immediately before and severe asthma symptoms during 1 week periods before 0.3 ppm exposures.
Lung function (FEV_1, FVC, PEF and FEF_{25-75}) was compared between exposure to ambient air and to 0.3 ppm NO_2.		No significant correlation between NO_2 exposure and decreased lung function was observed.	
Pulmonary function following exposure to air and NO_2 for COPD patients was compared to that of normal elderly subjects.	COPD subjects demonstrated progressive decrements in FVC and FEV_1 compared with baseline with 0.3 ppm NO_2 but not with air. NO_2-induced FEV_1 reduction was greater among smokers than never-smokers in normal subjects.	Analyses suggested that responsiveness to NO_2 decreased with COPD severity.	
Lung function (FVC, FEV_1, FEV_1/FVC, $MEF_{25,50\&75}$), glutathione/glutathione perioxidase, and alveolar permeability during and after exposure.	Significant decrease (at the 5% level) of alveolar permeability 6 hours after NO_2 exposure. Significant decrease (5% level) of glutatione peroxidase in serum 18 hours after exposure.	No indication of mucous membrane irritation or decreased lung function during or after exposure.	Background concentration of NO_2 (in air) did not exceed 0.03 ppm.

Reference	Location	Study Design	Number of Subjects	Subject Description	Characteristics of Study	Duration of Exposure	NO_2 Concentration
Devalia, Rusznak, et al., 1994		Experimental	8 (4 male, 4 female)	Non-smoking, mild asthmatic adults aged 18-45 years (mean age 27.6 years), with a minimum of 70% predicted FEV_1 for age and height.	Subjects were exposed to one or more of the gases in a random order, in an environmental chamber.	6 hour exposures, one week apart	Ambient air, 400 ppb NO_2 or 400 ppb NO_2 with 200 ppb SO_2.
Hazucha, Folinsbee et al., 1994	Chapel Hill, NC, USA	Double-blind Crossover	21 (all female)	Aged 18-35 (many were college students).	Exposure to air or NO_2 via chamber for 2 hours followed three hours later by a 2 hour exposure to O_3 (with intermittent exercise) on two separate days.	2 hours	0.6 ppm
Tunnicliffe, Burge & Ayers, 1994		Double-blind	10 (4 male, 6 female)	Non-smoking asthmatic adults aged 16-60 years.	Subjects were exposed to NO_2 or ambient air from a Douglas bag via mouthpiece, which was attached to a Rudolph valve.	1 hour (exposures were spaced at least one week apart).	Ambient air, 100 ppb and 400 ppb NO_2.
Drechsler-Parks, 1995	Santa Barbara, CA, USA	Crossover	8 (6 male, 2 female)	Healthy adults aged 56-85 years.	Subjects were exposed to air, NO_2, or NO_2 & O_3 via chamber on separate days more than 1 week apart. Alternating 20 minute periods of exercise and rest.	2 hours	0.60 ppm NO_2 or 0.60 ppm NO_2 and 0.45 ppm O_3
Jorres, Nowak et al., 1995	Germany	Single-blind Crossover	20 (11 male, 9 female)	12 asthmatic (8m, 4f) aged 21-37 (mean 27) and 8 healthy subjects (3m, 5f) aged 21-33 (mean 27).	Exposures to air or NO_2 via mouthpiece; Alternate exercise and rest; Randomized (>1week apart).	3 hours	1 ppm
Wang, Duddle et al., 1995	UK	Single-blind Crossover	16 (6 male, 10 female)	Asymptomatic adults with history of seasonal allergic rhinitis aged 18 to 55 years (mean = 26.4).	Subjects were exposed to ambient air and/or NO_2 in an environmental chamber during the pollen season.	6 hours	Ambient air or 400 ppb NO_2.
Kelly, Blomberg et al., 1996		Single-blind	44	Non smoking, asymptomatic male and female volunteers (19-45yrs) randomly separated into 3 groups. **Group 1:** bronchoscopy after 1.5hrs; **Group 2:** bronchoscopy after 6hrs; **Group 3:** bronchoscopy after 24 hrs.	Controlled exposure via chamber. Light exercise alternated with rest in 15 minute intervals.	4 hours	0 or 2 ppm

Main Comparison	Positive Results	Null or Negative Results	Comments
Exposure to NO_2 (alone or in combination with SO2) and airway response (FEV_1, FVC, CBU, $PD_{20}FEV_1$) to allergen inhalation.	A significant association was observed between exposure to both NO_2 and SO_2 and $PD_{20}FEV_1$ (60.5%, SE=8.1%, p=0.015).	No significant association was observed for either NO_2 or the combination of NO_2 and SO_2 and FEV_1 or FVC. No significant association was recorded between NO_2 exposure and $PD_{20}FEV_1$ (41.2%, p=0.125).	CBU = Cumulative breath units of allergen (*D pteronyssinus*). $PD_{20}FEV_1$ = amount of allergen required to cause a 20% fall in FEV_1. NOTE: dose response curves for two subjects are depicted in the report, others available through Lancet.
Spirometry and plethysmography after exposure to air followed by O_3 or NO_2 followed by O_3.	Following NO_2-O_3 exposure, median $PD_{10}FEV_1$ (dose required to reduce FEV_1 by 10%) was reduced from 5.6 mg/ml to 1.7 mg/ml compared with air-O_3 exposure (n=16, p<0.05).	NO_2 exposure alone did not reduce FEV_1. No 'significant' effects were observed in plethysmography.	
NO_2 exposure and airway response (FEV_1 and FVC).	A significant difference in early and late asthmatic response (FEV_1) was observed between ambient air exposure and 400 ppb NO_2 exposure (-4.01%, 95%CI = -1.34 to 6.69%, p<0.009; and -5.28, 95%CI = -0.73 to -9.83%, p<0.02, respectively).	No significant difference in early or late asthmatic response (FEV_1) was observed between ambient air and 100 ppb, and 100 ppb and 400 ppb.	
An electrocardiogram was monitored throughout each exposure, and heart rate was recorded at 5 minute intervals during exercise. Cardiac output, stroke volume and systolic time intervals were measured at rest preceding exposure, and during the last 2 minutes of each period of exercise. Results were compared for air, NO_2, and NO_2 in combination with O_3.	The exercise-induced increase in cardiac output with NO_2/O_3 exposure was significantly smaller (p<0.05) than with air or O_3 alone.	There were no statistically significant differences in heart rate, respiratory frequency or oxygen uptake between exposures. There were no significant differences in stroke volume or systolic time intervals among the four exposures.	Six of the subjects completed all 4 exposures, one completed 2 exposures, and one completed 3 exposures.
Results of bronchoscopy with BAL 1 hour after exposure, and results of lung function 2, 10, 20, and 30 minutes after exposure were compared for healthy and asthmatic subjects.	In the asthmatic subjects, NO_2 induced a small mean drop in FEV_1. In subjects with asthma, NO_2 was capable of inducing an activation of cells.	Differential cell counts in BAL fluid did not reveal significant effects of NO_2.	Activation of cells induced by NO_2 is compatible with enhancement of airway inflammation.
NO_2 exposure and nasal airway resistance (NAR) and changes in inflammatory mediators (eosinophil cationic protein, mast cell tryptase, myeloperoxidase and interleukin-8).	A significant increase in eosinophil cationic protein was observed in subjects when exposed to NO_2 compared to ambient air during allergen challenge.	There was no significant association observed with NAR and NO2 exposure.	
Exposure to NO_2 results in oxidative depletion of antioxidants from the respiratory tract lining (measured reduced glutathione, uric acid, ascorbic acid and malondialdehyde as a marker for lipid peroxidation in bronchial and bronchoalveolar lavage fluid).	Significant decrease in uric acid (after 24 hours returned to control levels) and ascorbic acid (returned to control levels at 6 hours) within 1.5 hours of exposure to NO_2 (both bronchial and bronchoalveolar lavage fluid). Significant increase in GSH at 1.5 and 6 hours in bronchial lavage fluid which returned to control levels at 24 hours.	No change in GSH or malondialdehyde concentrations seen after NO_2 exposure in bronchoalveolar lavage fluid.	Antioxidants in lung fluids react and modulate NO_2 impact on lung.

Reference	Location	Study Design	Number of Subjects	Subject Description	Characteristics of Study	Duration of Exposure	NO_2 Concentration
Salome, Brown et al., 1996	Sydney, Australia	Crossover	20	9 adults (19-65) and 11 children (7-15) with diagnosed asthma requiring daily medication.	Ambient air, NO_2, or NO_2 and combustion by-products.	60 minutes	0.3 or 0.6 ppm
Strand, Salomonsson et al., 1996	Huddinge, Sweden	Crossover	19 (9 male, 10 female)	Subjects with mild asthma aged 20-48 years.	Subjects breathed either clean air or NO_2 during intermittent exercise in chamber. On two randomized days separated by 3 to 4 weeks.	30 minutes	0.26 ppm (488 $\pm$ 13 ug/m^3)
Vagaggini, Paggiaro et al., 1996		Single-blind	22	Three groups were enrolled: **Group 1:** 7 healthy non-smoking adults (mean age 34 +/-5 years); **Group 2:** 8 mild asthmatics (mean age 29+/-14 years); **Group 3:** 7 COPD patients (mean age 58+/-12 years).	Subjects were exposed to NO_2 and/or ambient filtered air in an exposure chamber, at least one week apart.	One hour exposure with moderate intermittent exercise (10 minutes every 15 minutes).	Ambient air and/or 0.3 ppm NO_2
Blomberg, Krishna et al., 1997	Umea, Sweden	Crossover	30 (18 male, 12 female)	Healthy adults aged 20-30 years (mean 35).	Randomized exposure to air or NO_2 via chamber. Alternate 15-minute periods of rest and exercise. Exposures separated by $\geq$ 3 weeks.	4 hours	2.0 ppm
Strand, Rak et al., 1997	Huddinge, Sweden	Crossover	18	Mild seasonal asthmatics aged 18-50 years.	Exposure at rest to either air or NO_2 via chamber; Randomized and separated by more than 2 weeks.	30 minutes	490 ug/m^3
Azadniv, Utell, et al., 1998	Rochester, NY, USA	Crossover	15 (11 male, 4 female)	12 subjects aged 22-35 years participated in each phase. 15 subjects total.	Exposure to air or NO_2 via chamber; Intermittent exercise; Randomized; Alternate exposure $\geq$ 3 weeks after the first. (Both NO_2 and air administered separately on the same subject in each PHASE).	6 hours	2.0 ppm
Strand, Svartengren et al., 1998		Experimental	16 (10 male, 6 female)	Non-smoking, mild asthmatic adults aged 21-52 years with allergy to pollen.	Subjects were exposed to NO_2 and/or ambient filtered air in an exposure chamber, at least four weeks apart.	30 minute exposures over four subsequent days.	Ambient air and/or 500 ug/m^3 NO_2

Main Comparison	Positive Results	Null or Negative Results	Comments
Difference in airway hyperresponsiveness (AHR) and peak expiratory flow during and 1 hour after exposure were compared to baseline.	There was a small but statistically significant increase in AHR after exposure to 0.6 ppm NO_2 in ambient air.	Exposure to NO_2 either in ambient air or mixed with combustion by-products from a gas heater had no significant effect on symptoms or lung function in adults or children. There was no effect of 0.6 ppm NO_2 on AHR when combustion by-products were included in the test atmosphere nor of 0.3 ppm NO_2 under either exposure condition.	
Airway responsiveness to histamine, SRaw, and thoracic gas volume 30 minutes, 5 hours, 27 hours and 7 days after exposure, and peripheral blood inflammatory mediators and the expression of an adhesion molecule (Mac-1) on granulocytes 30 mins and 27 hours after exposure were compared for air and NO_2.	Bronchial responsiveness to histamine was significantly increased 5 hours after NO_2 exposure when compared to air ($PDSRaw_{100}$ of 110 ug for NO_2 vs 203 ug for air). A nonsignificant increase (153 vs 100 ug) was seen 30 minutes after NO_2 exposure. TDV was significantly reduced after NO_2 exposure. Expression of Mac-1 on granulocytes was increased 30 minutess after NO_2 exposure when compared to pre-exposure values.	NO_2 exposure did not affect SRaw. No effect was seen on tryptase, eosinophil cationic protein (ECP) or myeloperoxidase (MPO).	
Short term NO_2 exposure and airway inflammation.	COPD subjects showed a slight decrease in FEV_1 after exposure to NO_2 compared to ambient air.	No significant association between NO_2 exposure and pulmonary function tests in normal subjects and mild asthmatics was observed.	Single blind trial. Note: the authors did record symptoms before and after exposure. This showed a slight increase in symptom score after NO_2 exposure versus ambient air exposure for all groups.
Flexible fiberoptic bronchoscopy with BW and BAL performed either 1.5 or 6 hours after exposure was compared for air and NO_2.	In BW, exposure to NO_2 induced a 1.5-fold increase in IL-8 ($p<.05$) at 1.5 hours and a 2.5-fold increase in neutrophils ($p<.01$) at 6 hours. In BALF, small increases were observed in $CD_{45}RO+$ lymphocytes, B-cells, and natural killer (NK) cells only.	Examination of bronchial biopsy specimens showed no signs of upregulation of adhesion molecules, and failed to reveal significant changes in inflammatory cells at either time point after NO_2 exposure.	
Allergen inhalation challenge 4 hours after exposure. Response to histamine 1 day after exposure. Lung function during and after exposure. Peripheral blood cell counts and serum levels of eosinophil cationic protein (ECP) before and after NO_2/allergen.	PEF after allergen challenge was on average 6.6% lower after NO_2 exposure than after air exposure. The number of subjects with a fall in FEV_1 >15% was 7 after air, 10 after NO_2.	NO_2 did not affect lung function before allergen challenge. NO_2 was neither associated with an increase in eosinophil numbers nor with ECP levels.	Results indicate that "short exposure to an ambient level of NO_2 followed several hours later by allergen inhalation enhances allergen-induced late asthmatic reaction."
BAL results compared following air or NO_2 exposure. (PHASE 1: BAL performed 18 hours after exposure. PHASE 2: BAL performed immediately after exposure).	PHASE 1: Exposure to NO_2 'caused airway inflammation'. Polymorphonuclear leukocytes increased from 2.2 ± 0.3 to 3.1 ± 0.4% ($p=0.05$). Small decreases in percentage of blood CD8T lymphocytes ($p=0.01$) and in blood T lymphocytes expressing neither CD4 nor CD8 ($p=0.03$).	These variables were not 'significantly' different in PHASE 2.	
NO_2 exposure and lung function (Raw, TGV, FEV_1) in combination with allergen exposure.	A significant decrease in FEV_1 was observed following NO_2 exposure and allergen compared to allergen alone (early phase:- 2.5 versus -0.4%, $p=0.02$; late phase: -4.4 versus -1.9%, $p=0.01$).		Note: the authors state that there was an increase in early phase response after a single NO_2 exposure ($p=0.03$).

Reference	Location	Study Design	Number of Subjects	Subject Description	Characteristics of Study	Duration of Exposure	NO_2 Concentration
Blomberg, Krishna et al., 1999	Umea, Sweden	Crossover	12 (8m, 4f)	Mean age of 26 years.	Exposure once to filtered air and on 4 consecutive days to NO_2 via chamber. Intermittent exercise.	4 hours	2.0 ppm
Jenkins, Devalia et al., 1999		Randomized, single-blind	11	Mild atopic asthmatic volunteers, non-smokers, aged 18-45 years.	Controlled exposure via chamber.	6 hours	0, 100 ppb O_3, 200 ppb NO_2, and 100 ppb O_3 + 200 ppb NO_2
			10	Mild atopic asthmatic volunteers, non smokers, aged 18-45 years.		3 hours	200 ppb O_3, 400 ppb NO_2, and 200 ppb O_3 + 400 ppb NO_2
Avissar, Reed et al., 2000		Single-blind	21 (12 male, 9 female)	Aged 18-40 years, non-smokers with normal spirometry and no symptoms of upper respiratory infection at least 6 weeks prior to study.	Controlled exposure via special chamber. Separated by 3 weeks.	3 hours	0, 0.6 ppm and 1.5 ppm
Solomon, Christian et al., 2000		Single-blind	15 (11 male, 4 female)	Healthy non-smokers with no respiratory illness in the three weeks prior to testing. Mean age = 29.3 +/- 4.8 years.	Subjects were exposed to NO_2 or ambient filtered air in an exposure chamber.	4 hour exposures over three consecutive days.	Ambient air and/or 2.0 ppm NO_2.
Chambers & Ayres, 2001	Birmingham, UK	Crossover	10 (3 male, 7 female)	Healthy, non-smoking subjects with mean age 35.1 years (range 23-51).	Exposure to NO_2 or medical air in a perspex head dome.	20 minutes	1.5 ppm

Main Comparison	Positive Results	Null or Negative Results	Comments
Results of bronchoscopy with endobronchial biopsies, bronchial wash (BW), and BAL 1.5 hrs after air exposure were compared to those after the last consecutive NO_2 exposure. Lung function measurements were compared before and after all 5 exposures.	BW following the last NO_2 exposure revealed a two-fold increase in neutrophil content ($p<0.05$) and a 1.5-fold increase in myeloperoxidase ($p<0.01$). 'Significant' decrements in FEV_1 and FVC were found after the first NO_2 exposure.	Antioxidant status of neither BW nor BAL fluids were changed following NO_2 exposure as compared to air. Changes in pulmonary function after the first NO_2 exposure were attenuated with repeated NO_2 exposure.	
Exposure to NO_2 and O_3 on response to inhaled allergen in exercising mild atopics.		No significant increase in airway response to inhaled allergen when compared to exposure with air.	Pollutant induced changes in airway response of mild atopic asthmatics to allergen may be dependent on threshold concentration rather than the total amount of pollutant inhaled over a period of time.
	Significant decrease in the dose of allergen required to decrease FEV_1 by 20% compared with exposure to air.		
Effect of exposure to NO_2 on glutathione peroxidase and extracellular glutathione peroxidase concentrations, polymorphonuclear cells and epithelial permeability markers (albumin) in the epithelial lining fluid of the lung (by bronchoalveolar lavage).		NO_2 had no effect on glutathione peroxidase or extracellular peroxidase concentration, NO_2 had no effect on lung function or in polymorphonuclear cells or epithelial permeability markers.	
NO_2 level and leukocyte level in bronchoalveolar lavage.	An increase in the percentage of neutrophils were observed in those exposed to NO_2 compared to those exposed to filtered air (10.6, 4.8-17.2% versus 5.3, 2.5-8.3%; $p=0.005$). A decrease in the percentage of T-helper cells was observed when exposed to NO_2 versus filtered air (55.9, 40.8-62.7% versus 61.6, 52.6-65.2%; $p=0.022$).		
Change in expired NO (ppb) and FEV_1 were compared before and for 3 hours after exposure.	NO_2 induced a decrease in mean post-exposure exhaled NO. This was not observed after exposure to medical air.	No 'statistically significant' change in FEV_1 was observed post exposure to NO_2 compared to placebo exposure.	Post-exposure FEV_1 results were not shown.