

A Cohort Study of Indoor Nitrogen Dioxide and House Dust Mite Exposure in Asthmatic Children

Monika Nitschke, PhD

Louis S. Pilotto, MBBS, PhD

Robyn G. Attewell, MSc AStat

Brian J. Smith, FRACP, PhD

Dino Pisaniello, PhD

James Martin, FRACP

Richard E. Ruffin, FRACP, MD

Janet E. Hiller, PhD

Objective: The purpose of this study was to investigate dose-response relationships between asthma symptoms and indoor nitrogen dioxide (NO_2) and house dust mite allergen (HDM) in children. **Methods:** Asthmatic children from 18 primary schools in Adelaide, Australia, kept a daily symptoms diary over 12 weeks. Home and classroom NO_2 levels were measured repeatedly in winter 2000. HDM levels were obtained from beds. Lung function tests were performed at the beginning and at the end of the study period. **Results:** Data on exposure and respiratory outcomes were gathered for 174 children. For school exposure, the estimated relative symptom rate (RR) for a 10-ppb increase in NO_2 for difficulty breathing during the day was 1.09 (95% confidence interval [CI] = 1.03–1.15), at night 1.11 (95% CI = 1.05–1.18), and for chest tightness at night 1.12 (95% CI = 1.07–1.17). Significant symptom rate increases were also found for kitchen NO_2 exposure. This was supported by a negative dose-response relationship between percentage predicted forced expiratory volume in 1 second and NO_2 (–0.39%; 95% CI = –0.76 to –0.02) for kitchen exposure. Significant threshold effects using a 10- $\mu\text{g/g}$ cutoff point for HDM exposure were established in the sensitized children for nighttime wheeze (RR = 3.62, 95% CI = 1.49–8.77), daytime cough (RR = 1.64, 95% CI = 1.14–2.36), and daytime asthma attack (RR = 1.95, 95% CI = 1.06–3.60). **Conclusion:** This study has established reliable risk estimates for exacerbations of asthma symptoms in children based on dose-response investigations of indoor NO_2 and HDM. (J Occup Environ Med. 2006;48:462–469)

From the Departments of Medicine (Dr Nitschke, Dr Smith, Dr Ruffin) and Public Health (Dr Nitschke, Dr Pisaniello, Dr Hiller), University of Adelaide, Adelaide, Australia; the Faculty of Medicine (Dr Pilotto), University of New South Wales, New South Wales, Australia; Covance Pty. Ltd. (Ms Attewell), Canberra, Australia; and Women's and Children's Hospital (Dr Martin), Adelaide, Australia.

Address correspondence to: Dr Monika Nitschke, Department of Health, Level 2, CGU Building, Environmental Health Service, 150 Grenfell St., PO Box 6, Rundle Mall Adelaide SA 5000; E-mail: monika.nitschke@health.sa.gov.au.

Copyright © 2006 by American College of Occupational and Environmental Medicine

DOI: 10.1097/01.jom.0000215802.43229.62

Although the genesis of asthma is still widely debated, it is generally accepted that there are a number of environmental factors that seem to be important as trigger factors for acute asthma.¹ The indoor environment can be a significant source of asthma triggers such as house dust mites (*Dermatophagoides pteronyssinus*; HDM) and nitrogen dioxide (NO_2). HDM are potent sensitizers and an important risk factor in the development of asthma.² Subsequent reexposure to high levels of HDM has been related to current wheeze and bronchial hyperresponsiveness,³ and HDM concentration in beds has been associated with the prevalence of respiratory symptoms in adult participants (asthmatics and nonasthmatics).⁴

NO_2 , a product of gas combustion processes, and its effect on the respiratory system has been of interest in relation to outdoor and indoor air pollution. Several epidemiologic studies have shown an increased risk of acute asthmatic symptoms in children in relation to NO_2 measured outdoors and indoors.^{5–7} A dose-response relationship between NO_2 and upper respiratory symptoms rates was demonstrated in a cohort of primary school children who were exposed to either unflued gas or electrical heating.⁸ A combined effect of these two environmental asthma triggers has also been observed, suggesting an enhanced asthmatic response in airways preexposed to NO_2 .⁹

In a recent randomized, controlled trial in Australia, symptom rates in asthmatic children were significantly

reduced in the intervention group in which unflued gas heaters in classrooms were replaced with flued gas or electrical heaters.¹⁰ This study involves a risk evaluation of indoor pollution sources on an extended sample of children originally recruited for the intervention study. The aims of this study were to determine: 1) independent dose-response effects of indoor NO₂ and HDM exposure on asthma symptoms rates and lung function (percentage predicted forced expiratory volume in 1 second [FEV₁% predicted]), and 2) whether daily exposure to high NO₂ modifies symptoms and FEV₁% predicted in households with high HDM levels.

Materials and Methods

Study Design

Eighteen primary schools were used as the source for selecting a cohort of asthmatic primary school children (5–13 years of age) with the aim of determining the rate of daily asthma symptoms in relation to exposure to NO₂ and HDM over 12 weeks during winter 2000. These schools had taken part in a randomized, controlled trial of the respiratory effects of unflued gas heaters.¹⁰ In a random selection process, eight of these schools had received heater intervention (installation of electrical or vented gas heaters), whereas the remaining 10 schools continued to operate with unflued gas heaters.

Child Recruitment

In the first instance, all children visiting the selected 18 schools received an information letter and a questionnaire with the purpose to identify the children who had been diagnosed with asthma by a doctor and to establish the use of unflued gas appliances in their homes. Children and parents were contacted at least twice if the questionnaire was not returned within a week. All children with doctor-diagnosed asthma were eligible to take part in the study.

In the second stage of the recruitment process, 118 asthmatic children from households without unflued gas appliances and 155 asthmatic children from households using gas cooking were invited to participate. No unflued gas heating was present in the selected households. Consent was obtained from children and their caregivers for lung function measurements, skin prick testing, home dust collection, and for daily symptoms information. The study was announced to the potential participants in general terms as an indoor pollution study without specific reference to NO₂ and HDM monitoring.

Demographic and Clinical Factors

Information in relation to age, gender, ethnic background, education level of parents, household smoking, serious chest illness before the age of 2, and hay fever in the last 12 months was obtained from the questionnaire and included as potential confounders in the regression models.

Skin Prick Testing

The children were tested for a number of common allergens, including HDM, using the immediate method for skin tests.¹¹ Allergens were supplied by Hollister-Stier (Spokane, WA). The wheal size was measured 10 minutes after the skin prick test to the forearm. A mean wheal size of 3 mm or greater was regarded as positive in the presence of a positive histamine and negative saline control.¹² Before skin prick testing, the children had been asked to stop taking antihistamines. All children responded positive to histamine and negative to saline control.

Outcomes

Symptom and Medication Rates. Data from the daily symptoms diary were collected by a market research company every 2 weeks by telephone interview with the caregivers/parents over a period of 12 weeks from June 1 to August 20, 2000. This diary had been successfully used in

previous studies.^{6,13} Symptom rates were calculated for each child as the number of days of symptoms occurrence divided by the number of days the symptoms diary was maintained.

Lung Function. Lung function measurements were taken at baseline during the first week of the study (beginning of winter heating period in schools) and repeated once in the 2 weeks after the end of the study period. Measurements were taken during school hours (9 AM to 3 PM). Testing was performed using the Jaeger Toennis Master Scope direct reading spirometer with software version 4.34 using a standardized protocol outlined by the Australian Asthma Council.¹⁴ Percentage predicted forced expiratory volume in 1 second (FEV₁) (= measured FEV₁/predicted FEV₁) was used in the analysis.

Exposure Measurements

Nitrogen Dioxide. Nitrogen dioxide was measured using passive diffusion badges according to the Australian standard AS 2365.1.2.¹⁵ Limit of detection calculated over 6 hours was 6 ppb and values below this limit were allocated half this value. Nitrogen dioxide in the classrooms was measured on three occasions, each time over 3 consecutive days (Tuesday, Wednesday, Thursday), resulting in 9 measurement days per classroom. This occurred in three weekly cycles of six schools per week, extending the overall measurement period over 9 weeks, not including 2 weeks of school holidays. Measurements were taken between 9 AM and 3 PM and NO₂ was expressed as time averaged over 6 hours for each of the 9 days for each classroom. Three monitors were placed in different locations within the classrooms to account for spatial differences in NO₂ concentration. Because no spatial differences were observed in classrooms heated electrically or with vented gas heaters, only two monitors were used in these classrooms.

Home NO₂ exposure (gas cooking) was based on 3 consecutive days of monitoring. Parents/caregivers were instructed to place two parallel monitors on the kitchen bench and to record hours of exposure from the time children arrived back from school until bedtime. For each of the 3 days, average NO₂ kitchen levels were calculated for each household. School support officers and parents/caregivers were trained in handling the monitors, time recording, and in storing the badges, including unexposed controls, in the provided sealed containers in the fridge.

To capture the effect of intermittent high NO₂ exposure on symptoms rates and lung function, the maximum of the daily time averaged NO₂ values across the measurement days was selected as representative exposures to be tested in the dose–response models. Separate maxima were used for the school and home environments of each child. In addition, a single composite NO₂ estimate was also defined for each child using the NO₂ source (school or kitchen) representing the highest exposure level for that child during the study period.

House Dust Mite Exposure. For assessment of HDM exposure, the bed environment was chosen because it provides the best conditions for house dust mite proliferation, and it is also the site where people come into closest contact with HDM.^{4,16} Sampling in households was only conducted once, because previous studies in Australia and in Germany had shown that mite concentrations in households were highly reproducible with no major seasonal differences.^{18,19} HDM level assessment in classrooms before the study had shown only negligible levels, and this has been substantiated by similar investigations in the school environment.^{20,21}

Dust was collected by vacuuming the mattress, sheets, and bedcover(s) over a 1-minute period with an Electrolux Royal vacuum cleaner, which was equipped with a viewer scope filter holder. Cellulose filters were

folded, secured with clips, and stored in zip-lock bags at –20°C until analysis. Hose nozzle and viewer scope were brushed and cleaned with acetone in between house visits.

Der p 1 concentration, the HDM-specific protein responsible for the allergic effect, was measured in the sieved fine dust extracts (pore size 425 µm) by enzyme-linked immunosorbent assays using commercially available test kits of monoclonal antibodies.¹⁷ The sample was extracted using PBS-T 0.1 Tween20 at pH 7.4. The plates were coated with 5H8 monoclonal mouse antibodies for Der p 1. The limit of detection of Der p 1 was 0.06 µg/g.

Statistical Analysis

Descriptive statistics were tabulated for outcome variables, potential confounders, and exposure levels of nitrogen dioxide and house dust mites. Regression models were fitted to investigate potential dose–response relationships between the NO₂ levels at school and in the kitchen, bed Der p 1 levels, and respiratory symptoms and lung function. Separate models were fitted for each respiratory outcome. For NO₂ exposures, all participating children were considered, whereas only mite-sensitive children were included in the assessment of HDM exposure. Zero-inflated negative binomial regression models were fitted for the symptom counts (with offset as the log number of days in the study).²² Negative binomial models were preferred over Poisson regression models due to the overdispersion indicated in Poisson goodness-of-fit statistics. Zero-inflated models were preferred due to the high proportion of children with no symptoms recorded during the study. For simplicity, a constant term was included for the zero-inflation part of the model. Examination of the presence/absence models of the symptoms confirmed no specific confounders were consistently related to the presence of symptoms.

For dose–response analysis of lung function in relation to exposures, a

multiple regression model was fitted after normality function was observed to be valid using end-of-study lung function values adjusted by baseline levels.

In all models, standard errors were adjusted for potential clustering by school, and the models included the set of predetermined potential confounders. All modeling was conducted in the statistical package STATA Version 7 using the “xtgee” command for the multiple regression and the “zinb” command with the cluster option for the zero-inflated negative binomial regression.²²

Although NO₂ was fitted as linear exposure, Der p 1 levels were investigated as linear and threshold (dichotomized) terms in relation to symptoms and lung function. The threshold value of 10 µg of Der p 1 per 1 g of dust was chosen, because current guidelines identify this level as the threshold for increased risk of wheeze.²³

Potential mediating effects of HDM exposure were investigated in the subset of mite-sensitive children by fitting additional models with interaction terms between nitrogen dioxide levels and Der p 1 levels.

Results

Study Population and Descriptive Data

Of the 354 eligible children who were invited to participate, data on symptoms, exposure, and potential confounders were obtained for 174 (49%) children. Fifty-one percent of the parents declined the invitation. Table 1 presents summary statistics on health outcomes and exposure for the study population. The children ranged in age from 5 to 12 years (mean, 8 years). Forty-six percent were girls, 83% were Australian born, and 80% of children had at least one parent who had completed high school. Twenty-two percent of children had at least one smoker living at home. A total of 36% reported hay fever in the last year and 42% reported a serious respiratory illness before the age of 2. Of the daily asthma symp-

TABLE 1

Descriptive Statistics for Symptom and Medication Rates, Lung Function, Nitrogen Dioxide Levels at School and at Home, and House Dust Mite Levels at Home

Description	N	Minimum	Maximum	Mean	Median	Standard Deviation	Nonzero (%)
Symptom rate per week							
Day wheeze	174	0	7.0	0.4	0.0	0.8	47
Night wheeze	174	0	2.8	0.2	0.0	0.4	38
Day difficulty breathing	174	0	6.8	0.3	0.0	0.8	43
Night difficulty breathing	174	0	6.8	0.2	0.0	0.6	29
Day chest tightness	174	0	3.1	0.3	0.0	0.5	44
Night chest tightness	174	0	2.5	0.2	0.0	0.4	34
Day cough	174	0	7.0	1.2	0.8	1.4	85
Night cough	174	0	7.0	0.9	0.5	1.2	82
Difficulty breathing after exercise	174	0	6.8	0.4	0.0	0.9	51
Day asthma attack	174	0	2.7	0.2	0.0	0.4	34
Night asthma attack	174	0	1.4	0.1	0.0	0.3	31
Miss school due to asthma	174	0	1.3	0.1	0.0	0.2	37
Healthcare facility visits due to asthma	174	0	0.3	0.1	0.0	0.1	35
Using any asthma medication	174	0	7.0	2.2	0.8	2.6	75
Using reliever medication	174	0	7.0	1.4	0.4	2.0	74
Using preventer medication	174	0	7.0	2.1	0.0	2.9	44
Lung function, percentage predicted forced expiratory volume in 1 second							
Baseline	146	69	146	104	104	13	100
Poststudy	151	61	153	109	110	16	100
Nitrogen dioxide levels at school (ppb)							
Average classroom level	174	7	117	34	23	28	100
Maximum classroom level	174	9	307	69	39	63	100
Nitrogen dioxide levels at home (ppb)							
Average kitchen level	174	3	147	20	13	22	100
Maximum kitchen level	174	3	423	38	22	53	100
Nitrogen dioxide levels at school/home (ppb)							
Maximum (classroom, kitchen)	174	9	423	82	58	71	100
House dust mite levels (bed) ($\mu\text{g/g}$)							
All children	174	0	171	10	1	24	99
Mite-sensitive	88	0	171	12	3	28	100
Not mite-sensitive	70	0	87	8	1	17	99
Age (yr)	174	5	12	8	9	2	100

toms, day and night cough were the most frequently recorded and 75% of the children took some form of asthma medication at least once during the study period. Of the 158 children from whom permission was obtained to conduct skin prick tests, 88 (56%) reacted positively to HDM skin prick testing.

NO₂ Exposure and Relationship to Asthma Symptoms and Lung Function

Maximum time-averaged daily NO₂ concentration (6 hours) in classrooms ranged from 9 to 307 ppb (median, 39 ppb). Maximum kitchen levels ranged from 3 to 423 ppb

(median, 22 ppb). For 71% of children, the maximum exposure level at school was higher than the maximum home exposure. Over half of the children were exposed to NO₂ levels of 40 ppb and greater (Table 2) when both school and home exposure were considered.

Dose-response relationships between asthma symptoms and NO₂ are expressed as adjusted relative symptom rates (RR) associated with 10-ppb increments in NO₂ (Table 3). Statistically significant increasing relative symptom rates were observed with classroom levels of NO₂ for difficulty breathing during the day (RR = 1.09, 95% confidence

interval [CI] = 1.03–1.15) and night (RR 1.11, 95% CI = 1.05–1.18), chest tightness at night (RR = 1.12, 95% CI = 1.07–1.17), and difficulty breathing after exercise (RR = 1.04, 95% CI = 1.01–1.09). In addition, statistically significant increasing relative symptom rates were observed with kitchen levels of NO₂ for difficulty breathing at night (RR = 1.03, 95% CI = 1.01–1.05) and asthma attacks at night (RR = 1.04, 95% CI = 1.00–1.07). Statistically significant increasing dose-response relationships were also seen for difficulty breathing during the day and night and chest tightness at night when the separate school and home

TABLE 2

Distribution of Nitrogen Dioxide Levels at School and at Home, and House Dust Mite (HDM) Levels at Home (by mite sensitivity)

Nitrogen Dioxide (ppb)	School (maximum)		Home (kitchen) (maximum)		Composite* (maximum)	
	N	Percent	N	Percent	N	Percent
<10	4	2.3	23	13.2	1	0.6
10–19.9	25	14.4	56	32.2	11	6.3
20–39.9	59	33.9	50	28.7	51	29.3
40–79.9	30	17.2	25	14.4	43	24.7
80+	56	32.2	20	11.5	68	39.1
Total	174	100.0	174	100.0	174	100.0

HDM (μg/g)	Mite-Sensitive		Not Mite-Sensitive		All Children	
	N	Percent	N	Percent	N	Percent
<2	41	46.6	44	62.9	93	53.5
2–9.9	21	23.9	12	17.1	36	20.7
10–19.9	17	19.3	7	10.0	27	15.5
20+	9	10.2	7	10.0	18	10.3
Total	88	100.0	70	100.0	174	100.0

*Based on the higher nitrogen dioxide exposure whether that be at school or at home.

TABLE 3Adjusted* Relative Symptom and Medication Rates (RR) and 95% Confidence Intervals (CIs) for Dose–Response by Maximum Nitrogen Dioxide (NO₂) Level at School and at Home Based on 16 Zero-Inflated Negative Binomial Models on 174 Asthmatic Children

Symptom	School Maximum NO ₂			Home Maximum NO ₂		
	RR†	95% CI	P	RR†	95% CI	P
Wheeze						
Day	1.01	0.97–1.06	0.6	0.98	0.92–1.04	0.5
Night	0.99	0.93–1.06	0.8	1.00	0.90–1.11	1.0
Difficulty breathing						
Day	1.09	1.03–1.15	0.003	1.00	0.98–1.03	0.8
Night	1.11	1.05–1.18	<0.001	1.03	1.01–1.05	0.008
Chest tightness						
Day	1.08	0.99–1.19	0.09	0.97	0.89–1.06	0.5
Night	1.12	1.07–1.17	<0.001	1.02	0.95–1.09	0.6
Cough						
Day	1.01	0.99–1.03	0.5	1.01	0.97–1.05	0.7
Night	1.01	0.98–1.04	0.6	0.99	0.96–1.02	0.7
Difficulty breathing after exercise	1.04	1.01–1.09	0.03	0.97	0.92–1.02	0.2
Asthma attacks						
Day	1.03	0.98–1.08	0.2	1.00	0.95–1.05	0.9
Night	1.00	0.93–1.08	1.0	1.04	1.00–1.07	0.03
Missed school	1.01	0.97–1.06	0.6	0.93	0.87–1.00	0.05
Visited health care	1.02	0.90–1.16	0.8	1.01	0.77–1.33	0.9
Used asthma medication	0.99	0.95–1.03	0.6	1.01	0.99–1.03	0.4
Used reliever	0.99	0.96–1.03	0.6	1.03	1.00–1.06	0.07
Used preventer	0.99	0.96–1.03	0.8	1.0	0.98–1.02	0.8

*Adjusted for potential confounding by age, gender, household smoking, ethnic background, education level of parents, hay fever, and serious respiratory illness before the age of 2 and adjusted for clustering by school.

†RR per 10 ppb NO₂.

exposure terms were replaced with a single composite term representing the maximum exposure across both school and home (not tabulated). These dose–response relationships are reflected in plots of the crude mean symptom rates versus mean maximum NO₂ levels (school and home) within the following five exposure levels <20, 20–39, 40–59, 60–79, and 80+ ppb (Fig. 1). Translating the symptom rates to a winter heating period lasting 100 days, increasing from a low NO₂ level of 18 ppb up to 130 ppb corresponds to an increase from a mean of 3 to 7 days with difficulty breathing.

A statistically significant decrease was observed in mean FEV1% predicted (−0.39% per 10 ppb NO₂; 95% CI = 0.76 to −0.02; *P* = 0.04) for maximum kitchen NO₂ level with adjustment for potential confounders but not for school exposure (*P* = 0.3). The composite NO₂ term was marginally associated with a decrease in lung function (−0.25 per 10 ppb NO₂, 95% CI = −0.51 to −0.0005; *P* = 0.05).

Der p 1 Exposure and Relationship to Asthma Symptoms and Lung Function

Over one fourth of the children were exposed to Der p 1 levels of 10 μg/g or greater (Table 2). Mean concentration of Der p 1 was significantly higher (*P* = 0.02) in the beds of children who were sensitized to HDM (12.1 μg/g) compared with the nonsensitized children (7.5 μg/g).

There were no consistent linear dose–response trends in asthma symptoms with Der p 1 levels, but a threshold effect was observed using a 10-μg/g cutoff point. Significantly higher symptom rates were observed for high versus low Der p 1 exposure (≥10 μg/g vs <10 μg/g Der p 1) for night wheeze (RR = 3.62, 95% CI = 1.49–8.77), daytime cough (RR = 1.64, 95% CI = 1.14–2.36), and daytime asthma attacks (RR = 1.95, 95% CI = 1.06–3.60) (Table 4). The differential described by the model is

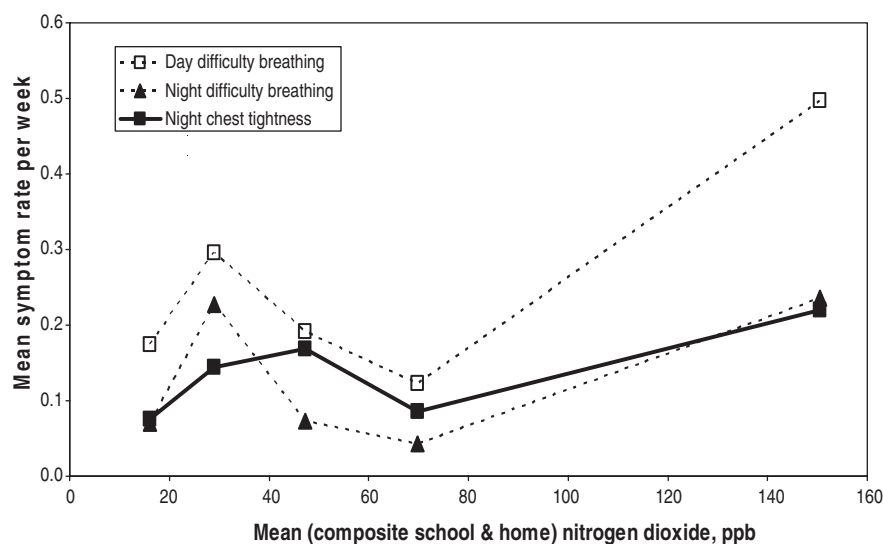


Fig. 1. Mean symptom rates per week (difficulty breathing during the day and night, and chest tightness at night) plotted against mean maximum nitrogen dioxide levels (composite of school and home exposure) grouped as <20 ppb ($n = 12$), 20–39 ppb ($n = 51$), 40–59 ppb ($n = 25$), 60–79 ppb ($n = 18$), and 80+ ppb ($n = 68$).

TABLE 4

Adjusted* Relative Symptom and Medication Rates (RR) and 95% Confidence Intervals (CIs) for a Threshold Level of House Dust Mites From 16 zero-Inflated Negative Binomial Models on Asthmatic Children Sensitive to Der p 1

Symptom	Der p 1 (bed) ($N = 88$ mite-sensitized children)		
	RR†	95% CI	P
Wheeze			
Day	2.11	0.91–4.92	0.08
Night	3.62	1.49–8.77	0.004
Difficulty breathing			
Day	0.68	0.25–1.85	0.4
Night	0.83	0.32–2.11	0.7
Chest tightness			
Day	0.67	0.42–1.07	0.09
Night	0.63	0.31–1.27	0.2
Cough			
Day	1.64	1.14–2.36	0.008
Night	1.61	0.91–2.82	0.1
Difficulty breathing after exercise	1.04	0.41–2.63	0.9
Asthma attacks			
Day	1.95	1.06–3.60	0.03
Night	0.80	0.20–3.13	0.7
Missed school	0.66	0.33–1.29	0.2
Visited health care	1.25	0.79–1.98	0.3
Used asthma medication	0.90	0.63–1.28	0.6
Used reliever	1.57	0.89–2.76	0.1
Used preventer	1.03	0.60–1.77	0.9

*Adjusted for potential confounding by maximum nitrogen dioxide level at school, maximum nitrogen dioxide level at home, age, gender, household smoking, ethnic background, education level of parents, hay fever, and serious respiratory illness before the age of 2 and adjusted for clustering by school.

†RR Der p 1 ≥ 10 $\mu\text{g/g}$ vs <10 $\mu\text{g/g}$.

also evident in the mean symptoms days, which translate to 4 days per 100 with wheeze at night for low HDM exposure compared with 9 days for high HDM exposure. Der p 1 concentration in the bed was not related to FEV1% predicted for the subset of mite-sensitive children.

Nitrogen Dioxide/House Dust Mite Interaction

When effect modification between Der p 1 and maximum NO_2 (school and home) was tested in the sensitized children, a statistically significant interaction ($P < 0.001$) was only found for wheeze at night. Although an increasing dose-response relationship with NO_2 was seen for households with low levels of Der p 1 (RR = 1.08, 95% CI = 1.00–1.17), a statistically significant inverse relationship with NO_2 was seen for households with high levels of Der p 1 (RR = 0.85, 95% CI = 0.79–0.92).

Discussion

Nitrogen Dioxide

Exposure levels reflecting the major indoor NO_2 sources were tested with linear dose-response models to evaluate the relationship between exposure and rates of symptoms of asthma in children over a 12-week study period. Significant dose-response relationships with increases of 4% to 12% were demonstrated for difficulty breathing (day and night and after exercise) and chest tightness (night) for an increment in NO_2 of 10 ppb in maximum classroom levels. This is consistent with the findings from the randomized, controlled trial showing a risk reduction of approximately 60% for these symptoms after removal of unflued gas heaters in schools accompanied on average by a reduction in NO_2 of 30 ppb NO_2 .¹⁰ The results also compare favorably with an average asthma symptom increase of 20% to 30% established in a previous panel study (NO_2 increase of 44 ppb), which was based on unflued gas

appliances generally found in the home environment.⁶ Further evidence comes from two recent studies showing significant dose–response evidence between NO₂ and asthma symptoms in children exposed to unflued gas heaters²⁴ and in infants exposed to gas cooking.²⁵ These results are supported by the 20% increase in respiratory symptoms established in a meta-analysis of some of the earlier indoor studies (NO₂ increase of 15 ppb).²⁶ Despite lower levels of NO₂ exposure within homes, we also found statistically significant dose–response relationships with increases of 3% to 4% in nighttime symptoms (difficulty breathing and asthma attacks) with NO₂ levels measured in the children's homes. The participants in this study were allocated a home and a school NO₂ level, which was to reflect an exposure scenario for the whole winter period during which the children had kept the symptoms diary. This procedure may have led to some nondifferential exposure misclassification and a potential underestimation of the risk estimates.

The decrease in FEV1% predicted of 0.4% for an increment in NO₂ of 10 ppb at home is comparable in magnitude to that experienced in a previous study of children exposed to gas heating in Australia (–0.12%; 95% CI = –0.23 to –0.01 per 1 ppb NO₂).²⁷ Lung function measurements in this study were conducted at the end of the heating season and that may have minimized the opportunity to capture the full effect of NO₂ on lung function, which is of particularly reversible nature in children.²⁸

House Dust Mites

In this study, Der p 1-sensitized children were exposed to significantly higher allergen levels than the nonsensitized children. Although it cannot be implied from this study design that high Der p 1 levels in the households had led to sensitization, it is likely that these relatively young children may have spent their entire

lives in these households. Several other studies had similar findings.^{29–32} Children exposed to high levels of Der p 1 at 2 years of age also have a higher risk of developing asthma.³³ Previous studies have reported the prevalence of symptoms in relation to Der p 1 levels in children^{16,21} and in adults.^{4,34} The current study demonstrates an increased risk in rates of wheeze, cough, and asthma attacks in relation to Der p 1 levels measured in beds of children. In this study, and in accordance with the study by Gehring,⁴ a threshold level with the cutoff point at 10 µg/g Der p 1 best describes the relationship after other cutoff points below 10 µg/g had also been explored. Although other studies have reported significant correlations between lung function measurements and Der p 1 levels,^{16,21} in this study, a dose–response relationship between Der p 1 levels and FEV1% predicted could not be established.

The type of symptom responses differed distinctly between NO₂ and Der p 1. This observation may indicate separate mechanisms of airway inflammation as has been discussed recently.³⁵ Wheeze and cough may indicate an allergic response, whereas breathlessness and chest tightness may be due to a neutrophilic or “asthma-like” pathway.

Interaction

In this study, increasing levels of NO₂ did not potentiate the allergic effect of Der p 1 in households with HDM levels above threshold in the subgroup of mite-sensitized children. This study does not corroborate the evidence gathered from clinical studies, but interpretation has to be cautious because the study was underpowered to properly examine the relationship with only few participants exposed to above-threshold levels of Der p 1 levels (*N* = 26). Further epidemiologic interaction investigations should be considered.

Conclusion

It has been demonstrated that two of the most prominent indoor pollutants have the potential to exacerbate asthma symptoms in children. NO₂ and Der p 1 trigger distinct symptoms that may indicate separate underlying asthma disease processes. In light of the strong evidence that the extent of health effects is dose-dependent, exposure should be minimized. Although a successful reduction of HDM associated with health improvements has as yet to be demonstrated, the effectiveness of a reduction of NO₂ exposure has recently been demonstrated in a heater replacement trial.

Acknowledgements

We thank the following people for advice and assistance: Mr. Rob Mitchell and Mr. Matthew Hartley (both EPA, South Australia) for NO₂ assessment in schools, Associate Professor John Edwards (Flinders University) for HDM measurements, and Mr. Paul Rogers (Pulmonary Medicine, Women's and Children's Hospital) for skin prick testing and lung function measurements.

References

1. Becklake MR, Ernst P. Environmental factors. *Lancet*. 1997;350:10–13.
2. Nelson HS. The importance of allergens in the development of asthma and the persistence of symptoms. *J Allergy Clin Immunol*. 2000;105:S628–S632.
3. Peat JK, Tovey E, Toelle BG, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med*. 1996;153:141–146.
4. Gehring U, Heinrich J, Jacob B et al. Respiratory symptoms in relation to indoor exposure to mite and cat allergens and endotoxins. Indoor Factors and Genetics in Asthma (INGA) Study Group. *Eur Respir J*. 2001;18:555–563.
5. Boezen MH, van der Zee SC, Postma DS, et al. Effects of ambient air pollution on upper and lower respiratory symptoms and peak expiratory flow in children. *Lancet*. 1999;353:874–878.
6. Smith BJ, Nitschke M, Pilotto LS, Ruffin RE, Pisaniello DL, Willson KJ. Health effects of daily indoor nitrogen dioxide exposure in people with asthma. *Eur Respir J*. 2000;16:879–885.
7. Ng TP, Seet CS, Tan WC, Foo SC. Nitrogen dioxide exposure from domestic gas cooking and airway response in asth-

- matic women. *Thorax*. 2001;56:596–601.
8. Pilotto LS, Douglas RM, Attewell R, Wilson SR. Respiratory effects associated with indoor nitrogen dioxide exposure in children. *Int J Epidemiol*. 1997;26:788–795.
 9. Tunnicliffe WS, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet*. 1994;344:1733–1736.
 10. Pilotto LS, Nitschke M, Smith B, et al. Randomized controlled trial of unflued gas heater replacement on respiratory health of asthmatic schoolchildren. *Int J Epidemiol*. 2004;33:208–214.
 11. Pepys J. Skin testing. *British Journal of Hospital Medicine*. 1975;412–417.
 12. Peat JK, Woolcock AJ. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyperresponsiveness in children from three different climatic areas in Australia. *Clin Exp Allergy*. 1991;21:573–581.
 13. Creer TL, Kotses H, Reynolds RVC. Living with asthma: part II. Beyond CARIH. *J Asthma*. 1989;26:31–51.
 14. Pierce RJ, Johns DP. *Spirometry: The Measurement and Interpretation of Ventilatory Function in Clinical Practice*. National Asthma Campaign; 1995.
 15. Australian Standard (AS 2365.1.2.). *Methods for Sampling and Analysis of Indoor Air. Method 1.2: Determination of Nitrogen Dioxide Spectrophotometric Method Treated Filter/Passive Badge Sampling Procedure*. Standard Australia; 1990.
 16. Chan-Yeung M, Manfreda J, Dimich-Ward H, et al. Mite and cat allergen levels in homes and severity of asthma. *Am J Respir Crit Care Med*. 1995;152:1805–1811.
 17. Luczynska C, Aruda LK, Platts-Mills TA. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p 1 and der f 1. *J Immunol Methods*. 1989;118:227–235.
 18. Marks GB, Tovey E, Toelle BG, Wachinger S, Peat JK, Woolcock AJ. Mite allergen (Der p 1) concentration in houses and its relation to the presence and severity of asthma in a population of Sydney schoolchildren. *J Allergy Clin Immunol*. 1995;96:441–448.
 19. Heinrich J, Holscher B, Douwes J, et al. Reproducibility of allergen, endotoxin, and fungi measurements in the indoor environment. *Journal of Exposure Analysis & Environmental Epidemiology*. 2003;13:152–160.
 20. Wardrope C, Rutherford S, Simpson RW. Air pollution and allergens in two South East Queensland schools. *Clean Air*. 2001;35:39–44.
 21. Zock JP, Brunekreef B, Hazebroek-Kampschreur AAJM, Roosjen CW. House dust mite allergen in bedroom floor dust and respiratory health of children with asthmatic symptoms. *Eur Respir J*. 1994;7:1254–1259.
 22. *Stata Statistical Software*, release 7.0(7). College Station, TX: Stata Corp; 2001.
 23. Platts-Mills TA, Tovey E, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Dust mite allergens and asthma: report of a second international workshop. *Allergy Clin Immunol*. 1992;89:1046–1060.
 24. Shima M, Adachi M. Effect of outdoor and indoor nitrogen dioxide on respiratory symptoms in schoolchildren. *Int J Epidemiol*. 2000;29:862–870.
 25. van Strien RT, Gent J, Belanger K, Triche E, Bracken MB, Leaderer B. Exposure to nitrogen dioxide and nitrous acid and respiratory symptoms in the first year of life. *Epidemiology*. 2004;15:471–478.
 26. Hasselblad V, Eddy DM, Kotchmar DJ. Synthesis of environmental evidence: nitrogen dioxide epidemiology studies. *J Air Waste Manag Assoc*. 1992;42:662–671.
 27. Ponsonby AL, Glasgow N, Gatenby P, et al. The relationship between low level nitrogen dioxide exposure and child lung function after cold air challenge. *Clin Exp Allergy*. 2001;31:1205–1212.
 28. The Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med*. 2000;343:1054–1063.
 29. Sporik R, Squillace SP, Ingram JM, Rakes G, Honsinger RW, Platts-Mills TA. Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. *Thorax*. 1999;54:675–680.
 30. Thomas KD, Dyer SM, Ciuk J, Volkmer RE, Edwards J. Domestic exposure of asthmatic and non-asthmatic children to house dust mite allergen (Der p 1) and cat allergen (Fel d 1) in Adelaide, South Australia. *Environmental Health*. 2001;1:52–59.
 31. Tunnicliffe WS, Fletcher TJ, Hammond K, et al. Sensitivity and exposure to indoor allergens in adults with differing asthma severity. *Eur Respir J*. 1999;13:654–659.
 32. Custovic A, Taggart SCO, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol*. 1996;98:64–72.
 33. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house dust mite allergen (Der P I) and the development of asthma in childhood. *N Engl J Med*. 1990;323:502–507.
 34. Alvarez MJ, Olaguibel JM, Acero S, Garcia BE, Tabar AI, Urbiola E. Effect of current exposure to Der p 1 on asthma symptoms, airway inflammation, and bronchial hyperresponsiveness in mite-allergic asthmatics. *Allergy*. 2000;55:185–190.
 35. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax*. 2002;57:643–648.