ORIGINAL ARTICLE

Exhaled nitric oxide in spray painters exposed to isocyanates: effect modification by atopy and smoking

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ABSTRACT

Background Isocyanate asthma is one of the most frequently identified forms of occupational asthma in industrialised countries. The underlying mechanisms have not been clarified. There is only limited information about the relationship between exhaled nitric oxide (eNO) and occupational exposure to isocyanates and asthma.

Objectives To investigate the association between isocyanate exposure and eNO levels in isocyanate-exposed workers and to elucidate whether eNO acts as a marker of airway inflammation controlling for smoking and atopy in an industry-wide survey.

Methods Information on estimated personal isocyanate exposure, measured eNO levels, health effects and sensitisation were analysed in 229 workers from a cross-sectional study. Univariate and multiple regression analyses were used to explore the exposure–response relationships between isocyanate exposure and eNO, stratified by smoking and atopy.

Results A marginally significant exposure–response relationship was found between isocyanate exposure and eNO in atopic, non-smokers (p=0.054). eNO was significantly associated with atopy and smoking, bronchial hyper-responsiveness (BHR), work-related conjunctivitis and rhinitis after adjustment for age, gender, atopy and smoking (p<0.05). A borderline significant association was found between eNO and asthma-like symptoms after adjustment for age, gender, atopy and current smoking (p=0.055). In a small group of isocyanate-exposed workers with positive serum-specific immunoglobulin E (IgE) antibodies to hexamethylene diisocyanate (HDI), elevated eNO levels were clearly exposure related. eNO was associated with the positive specific IgG antibodies to HDI in non-atopic, non-smokers (p=0.03).

Conclusions Increased eNO levels may indicate increased airway inflammation in atopic, non-smokers exposed to isocyanates especially at higher levels of isocyanate exposure.

What this paper adds

- Increased exhaled nitric oxide levels are suggestive of an increase in airway inflammation in atopic, non-smokers exposed to isocyanates, especially at higher isocyanate exposure levels.
- Elevated exhaled nitric oxide (eNO) levels were clearly exposure related in a small group of isocyanate-exposed workers with positive IgE antibodies to hexamethylene di-isocyanate (HDI).
- Results suggest that eNO levels can only be interpreted correctly if quantitative exposure of a subject is known.

INTRODUCTION

Isocyanates have been well recognised as a major cause of respiratory health effects such as asthma,[1–3] rhinitis,[4] accelerated lung function decline[5] and, less commonly, hypersensitivity pneumonitis.[6 7] An epidemiological study among spray painters demonstrated that isocyanate exposure, mainly consisting of hexamethylene diisocyanate (HDI) oligomers, is associated with work-related as well as non-work-related respiratory symptoms (eg, asthma-like symptoms) and specific sensitisation.[8] Early identification of isocyanate-induced asthma is crucial because symptoms often remain, even after elimination of exposure.[3] In recent years, exhaled nitric oxide (eNO) has been identified as a non-invasive and reproducible exhaled breath marker of allergic airway inflammation, especially in subjects with asthma and/or allergic rhinitis.[9–11] eNO levels are often increased in asthmatic subject[12] and decline after treatment.[13] Consequently, eNO measurements apply as a guide in the treatment of chronic asthma.[14 15]

Small clinical studies explored the role of eNO in isocyanate-induced asthma and allergy.[16–20] No clear relationship was observed between elevated levels of eNO and non-specific bronchial hyper-responsiveness (BHR) as well as specific IgE sensitisation in individuals exposed to 4,4′-diphenylmethane diisocyanate (methylene diphenyl diisocyanate (MDI)).[20] A significantly increased eNO level was found following a challenge test with MDI among most symptomatic isocyanate exposed workers with BHR.[16] The same authors demonstrated that a combination of BHR and elevated eNO level can predict the risk of developing isocyanate-induced asthma.[17] Pronk *et al*[21] showed that subjects with BHR and elevated eNO, as a specific phenotype, had a high isocyanate exposure. No significant association was found between eNO and isocyanate exposure. However, detailed analyses of associations stratifying for smoking and atopy simultaneously were not performed. To extend this study, we hypothesise that eNO levels are associated with specific sensitisation in subgroups and that effect modification is likely to occur by atopy and smoking.

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Thus, the purpose of this study was to explore the influence of isocyanate exposure on eNO levels and to elucidate whether eNO acts as a marker of airway inflammation controlling for smoking and atopy.

MATERIALS AND METHODS

Study design and population

This study is a continuation of a large cross-sectional survey[8] exploring the exposure–response relationships between exposure to isocyanates and eNO controlling for smoking and atopy. Briefly, the study population (229 workers) was recruited from several spray painting companies located in the Netherlands in 2006. All workers from companies, with at least one worker per company with detectable HDI-specific IgE or IgG, were invited to participate. Workers were classified as 'spray painters' if they were involved in spray painting. *Controls* were involved in other tasks. The study protocols were approved by the Institutional Review Board for Human Studies of the University Medical Centre, Utrecht, The Netherlands. In addition, all participants gave informed consent.

Questionnaire

The questionnaire has been described elsewhere.[8] For statistical analyses, broader respiratory symptom categories were constructed as follows: 'COPD-like symptoms', defined as chronic cough, chronic productive phlegm or shortness of breath, suggestive of chronic obstructive pulmonary disease (COPD); 'asthma-like symptoms', defined as wheezing or chest tightness, suggestive of asthma. The occurrence of symptoms during or shortly after work was defined as work-related. Work-related rhinitis and conjunctivitis were defined as, respectively, the occurrence of sneezing or runny nose, and itchy or watery eyes during or shortly after work. Current smoking and recent ex-smoking information was obtained by asking about current smoking (cigarettes yes/no) and whether the subject stopped smoking cigarettes last year (yes/no), respectively.

Exposure assessment

Task-based personal air samples were collected using midget impingers to measure the exposure to 23 isocyanate compounds.[22] Task-based personal exposure measurements were combined with self-reported monthly information about time-activity patterns to estimate personal exposure.[8] Total isocyanate (NCO group) exposure was computed by summing individual monomer and oligomer concentrations and expressed as μg NCO/m^3; details about the methodology can be found elsewhere.[8]

Specific IgE and IgG

Serum-specific IgE and IgG against HDI were quantified by ImmunoCAP assay and enzyme immunoassays (EIAs).[8] In EIAs, human serum albumin (HSA)-HDI conjugates were prepared in liquid (HDI_L-HSA) and vapour (HDI_V-HSA) phases and also prepared with commercial products including oligomeric HDI (N3300-HSA and N100-HSA). Atopy was defined as a positive specific IgE against at least one of the common aeroallergens assessed by Phadiatop (Phadia, Uppsala, Sweden).

Spirometry and methacholine challenge

Baseline lung function was assessed by normal spirometry.[8] Briefly, at least two maximal flow-volume manoeuvres were obtained from workers, except in 31 workers for whom only one technically acceptable manoeuvre could be obtained. The largest forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were recorded. Maximum mid-expiratory flow (MMEF) was obtained from the manoeuvre with the largest sum of FEV_1 and FVC. Methacholine challenge test was applied to assess BHR using a dosimeter technique as described elsewhere.[8] In brief, the test was started from 0.019 mg methacholine and was stopped after a reduction of 20% in FEV_1 (PD20) or when the maximum cumulative dose of 2.5 mg was attained. Airway hyper-responsiveness was defined as a provocative dose of methacholine of ≤2.5 mg (~10 μmol) causing at least a 20% fall in FEV_1.

Exhaled NO

eNO measurements were carried out using a NIOX MINO handheld device according to the American Thoracic Society/European Respiratory Society (ERS) recommendations as described elsewhere.[23] NO-free air (containing <5 ppb) was inhaled deeply through the device and then exhaled through the device for 10 s. Throughout the exhalation, the flow rate was maintained at 50 ±5 mL/s. The analysis was performed for the last 3 s of exhaled air using an electrochemical sensor in the device.

Statistical analysis

Statistical analyses were performed with SAS statistical software, V.9.2 (SAS Institute, Cary, North Carolina, USA). eNO and isocyanate exposure were log-normally distributed, and therefore further analyses were carried out using log-transformed data. For statistical analyses, specific IgE and IgG to HDI were dichotomised using cut-off values for human serum albumin (HSA)-corrected optical density values of 0.1 and 0.3, respectively.[8] Univariate and multiple regression analyses (PROC GENMOD) were applied to determine the exposure–response relationships between isocyanate exposure and eNO, as well as the associations between eNO and respiratory health outcomes, BHR, atopy and specific sensitisations.

To express the associations between eNO and isocyanate exposure, the geometric mean ratio (GMR) for eNO, per unit increase in log-transformed exposure was converted to a GMR per interquartile range (0.27–7.9265 μg $NCO*m^{-3}*h*month^{-1}$). In further analyses, effect modification by current smoking and atopy were explored. Non-parametric regression modelling (smoothing) using generalised additive models (PROC GAM) was applied to explore the relationships between eNO and isocyanate exposure. Smoothing parameter degrees of freedom were selected by generalised cross-validation[24] but restricted to a maximum of three. All analyses were adjusted for age, gender, current smoking and atopy, except for univariate analysis in table 2.

RESULTS

Population characteristics

The study population was restricted to 201 workers for further analyses; 15 had no BHR determined (unacceptable manoeuvres n=6, β-blocker usage n=3, other medical reasons n=3, refusal n=2 and one person stopped during methacholine challenge because of health complaints), 11 for undetectable eNO (problems with exhaling at a constant flow (n=3), health complaints (n=1), refusal (n=3) and device errors (n=4)). Six subjects with missing specific IgE and IgG responses to HDI were also removed.

Characteristics of the study population, including information about the prevalence of self-reported symptoms, BHR_{20}, serological outcomes, eNO levels (ppb), baseline spirometry and exposure levels to isocyanates, are shown in table 1. Overall, spray painters had the highest isocyanate exposure, were somewhat younger and were slightly less often atopic. Current

Table 1 General characteristics, the levels of isocyanate exposure, the prevalence of self-reported symptoms, serological outcomes, BHR_{20}, lung function variables (% reference value) and eNO among spray painters and controls

Characteristics	Spray painters n=80 (39.8%)	Controls n=121 (60.2%)
Gender, male (%)	97.5	95.9
Age, AM (SD)	39.6 (9.6)	40.6 (10.7)
Smoking status		
Current smoker (%)	44.2	37.9
Recent ex-smoker (stopped smoking within last year) (%)	0	5.2
Former smoker (%)	20.8	24.1
Non-smoker (%)	35.0	32.8
Work history, AM (SD)		
Number of years worked	19.0 (9.6)	20.0 (11.0)
Number of years as spray painter	17.4 (9.7)	4.8 (8.8)
Isocyanate exposure (μg NCO*m^{-3}*h*$month^{-1}$)		
Total isocyanate, median (min-max)	3584.4 (182.2–66 464.2)	3.1 (0–3785)
HDI, median (min-max)	33.0 (2.3–472)	0.4 (0–35.1)
Symptoms (%)		
Asthma-like symptoms	36.3	27.3
COPD-like symptoms	27.5	26.4
Work-related chest tightness	9.0	3.4
Work-related rhinitis	18.2	24.2
Work-related conjunctivitis	14.3	11.7
Serological outcomes		
Specific anti-HDI IgE (%)	2.5	4.1
Specific anti-HDI IgG (%)	61.3	52.9
Atopy (%) (Phadiatop)	40.0	43.0
BHR_{20} (%)	21.3	12.4
Lung function variables, AM (SD)		
FEV_1	101.4 (14.2)*	104.0 (13.3)
FVC	106.2 (11.5)	103.5 (13.9)
FEV_1/FVC	79.0 (9.7)†	82.8 (7.9)
ENO, GM (GSD)	17.5 (1.9)	17.0 (1.8)

FEV_1 and FVC are given as a per cent of predicted.
*p<0.10 significantly different from controls after adjustment for age, gender, atopy and current smoking.
†p<0.01 significantly different from controls after adjustment for age, gender, atopy and current smoking.
AM (SD), arithmetic mean (standard deviation); BHR, bronchial hyper-responsiveness; COPD, chronic obstructive pulmonary disease; eNO, exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; FVC: forced vital capacity; FEV_1/FVC: ratio of FEV_1 to FVC; GM, geometric mean; GSD, geometric standard deviation; HDI, hexamethylene diisocyanate; min-max, minimum-maximum.

smokers were more likely to be spray painters than controls. Spray painters more often reported self-reported symptoms (except for work-related rhinitis), had more BHR and had higher serum HDI-specific IgG. Serum HDI-specific IgE sensitisation was not very common among spray painters as well as controls. Work-related rhinitis was more prevalent in controls than spray painters (24.2% vs 18.2%, respectively). FEV_1 was lower in spray painters than among controls (p<0.10), and the FEV_1/FVC ratio was significantly lower in spray painters compared with controls (p<0.01).

Univariate and adjusted analyses

Isocyanate exposure above the median level (176.3 μg NCO*m^{-3}*h*$month^{-1}$) was not associated with eNO levels in both univariate and analyses adjusted for age, gender, smoking and atopy (table 2). A positive significant association between eNO and atopy was observed. As expected, current smoking was strongly negatively associated with eNO levels either before or after adjustment for age, gender and atopy. No significant association was found between eNO levels and specific IgE antibodies to HDI in both univariate and adjusted analyses. Specific IgG antibodies to HDI were associated with elevated eNO levels (unadjusted GMR (95% CI) 1.21 (1.03 to 1.42), p=0.02) in the univariate, but not in the adjusted, analysis (p=0.13).

A marginally significant relationship was also detected between a specific IgG response to HDI and exposure to HDI (GMR (95% CI) 1.52 (0.98 to 2.36), p=0.05). The positive, non-significant relationship between eNO levels and BHR in the univariate analysis became statistically significant after adjustment for confounders. eNO was significantly associated with work-related conjunctivitis and rhinitis both in the univariate and the adjusted analyses. A borderline significant association was found between eNO and asthma-like symptoms only in the adjusted analysis. Elevated eNO levels were not associated with chest tightness and COPD-like symptoms. A significant association was found between eNO and FEV_1 (GMR (95% CI) 1.10 (1.0 to 1.21), p<0.05) and a marginally significant association was observed between eNO and FVC (GMR (95% CI) 1.07 (0.99 to 1.10), p=0.06).

Stratified analyses

A marginally significant association was found between eNO and exposure, only in atopic, non-smoking subjects (p=0.05) (table 3). The smoothed spline showed a marginally significant log-linear relation between eNO and isocyanate exposure in this subgroup

Table 2 Geometric mean of exhaled NO level (ppb), crude and adjusted GMR

Predictor	GM eNO (ppb) (95% CI)	Crude GMR (95% CI)	p Value	Adjusted GMR (95% CI)	p Value
Isocyanate exposure (μg NCO*m^{-3}*h*month^{-1})		0.95 (0.8 to 1.12)	0.59	0.96 (0.83 to 1.11)	0.63
<176.3 (median)	17.8 (15.9 to 20.0)				
≥176.3	17.8 (12.7 to 22.0)				
Atopy		1.21 (1.03 to 1.43)	0.02	1.26 (1.08 to 1.46)*	0.002
No	16.1 (14.4 to 17.9)				
Yes	19.3 (14.4 to 25.0)				
Specific anti-HDI IgE		1.38 (0.88 to 2.16)	0.15	1.19 (0.80 to 1.77)	0.38
No	17.2 (15.8 to 18.7)				
Yes	24.0 (14.2 to 41.1)				
Specific anti-HDI IgG		1.21 (1.03 to 1.42)	0.02	1.11 (0.96 to 1.29)	0.13
No	15.6 (13.8 to 17.7)				
Yes	18.7 (13.8 to 24.8)				
BHR_{20}		1.08 (0.86 to 1.35)	0.49	1.23 (1.01 to 1.51)	0.03
No	17.2 (15.7 to 18.8)				
Yes	18.9 (12.7 to 26.3)				
Smoking		0.59 (0.51 to 0.69)	<0.0001	0.58 (0.50 to 0.67)†	<.0001
No	21.3 (19.4 to 23.4)				
Yes	12.8 (9.7 to 16.4)				
Asthma-like symptoms		1.07 (0.89 to 1.27)	0.45	1.16 (0.99 to 1.36)	0.055
No	17.1 (15.4 to 18.8)				
Yes	18.8 (13.9 to 24.4)				
COPD-like symptoms		0.88 (0.73 to 1.06)	0.18	1.01 (0.85 to 1.20)	0.85
No	18.0 (16.4 to 19.8)				
Yes	16.2 (11.5 to 21.8)				
Work-related chest tightness		0.91 (0.63 to 1.31)	0.64	0.87 (0.64 to 1.20)	0.41
No	17.5 (16.0 to 19.0)				
Yes	15.8 (9.6 to 24.7)				
Work-related conjunctivitis		1.29 (1.0 to 1.65)	0.04	1.27 (1.02 to 1.58)	0.02
No	16.9 (15.5 to 18.5)				
Yes	22.0 (15.5 to 31.5)				
Work-related rhinitis		1.37 (1.13 to 1.67)	0.001	1.32 (1.10 to 1.57)	0.002
No	16.3 (14.9 to 17.9)				
Yes	22.8 (16.4 to 30.4)				

Adjusted for age, gender and smoking and atopy.
*Adjusted for age, gender and smoking.
†Adjusted for age, gender and atopy.
BHR, bronchial hyper-responsiveness; COPD, chronic obstructive pulmonary disease; eNO, exhaled nitric oxide; HDI, hexamethylene diisocyanate; GM, geometric mean; GMR, geometric mean ratio.

(p linear=0.06; p spline=0.47) (figure 1A). A higher adjusted GMR was found in atopic, non-smokers with positive specific IgE antibodies to HDI (GMR (95% CI) 1.54 (0.85 to 2.80), p>0.10), as well as in non-atopic, non-smokers (GMR (95% CI) 1.39 (0.57 to 3.42), p=0.46). A small group of 7 subjects out of the 201 workers had specific IgE antibodies to HDI, and 5 of these subjects were also atopic. eNO levels were significantly elevated with increasing exposure to isocyanates especially at higher exposure levels in this small subgroup (p spline <0.0001) (figure 1B).

The positive significant association between eNO and BHR persisted when the analysis was limited to atopic non-smoking subjects. This group showed an increase in average eNO levels (GMR (95% CI) 1.58 (1.03 to 12.43), p=0.03). Moreover, no significant relationship was found between eNO and exposure in subjects with BHR (p spline>0.10) (figure 1C). Similarly, when specific IgE positives were removed from the group with BHR, the relations did not change (data not shown).

Table 3 Adjusted GMR (95% CI) (p value) for eNO, stratified by atopy and smoking

	Atopic smokers (n=35)	Atopic non-smokers (n=49)	Non-atopic smokers (n=43)	Non-atopic non-smokers (n=74)
Isocyanate exposure+	0.84 (0.63 to 1.12) (0.25)	1.24 (0.99 to 1. 54)* (0.05)	0.83 (0.67 to 1.02) (0.09)	0.95 (0.80 to 1.12) (0.57)
Specific anti-HDI IgE	0.97 (0.29 to 3.26) (0.96)	1.54 (0.85 to 2.80) (0.15)	0.58 (0.24 to 1.39) (0.22)	1.39 (0.57 to 3.42) (0.46)
Specific anti-HDI IgG	0.99 (0.65 to 1.50) (0.98)	1.14 (0.82 to 1.59) (0.41)	1.05 (0.79 to 1.40) (0.70)	1.25 (1.01 to 1.54) (0.03)

+Per IQR ($exp^{7.66}$=factor 2121.8) increase in isocyanate exposure (μg NCO*m^{-3}*h*month^{-1}).
All other analyses, adjusted for age and gender.
*Adjusted for age.
eNO, exhaled nitric oxide; GMR, geometric mean ratio; HDI, hexamethylene diisocyanate.

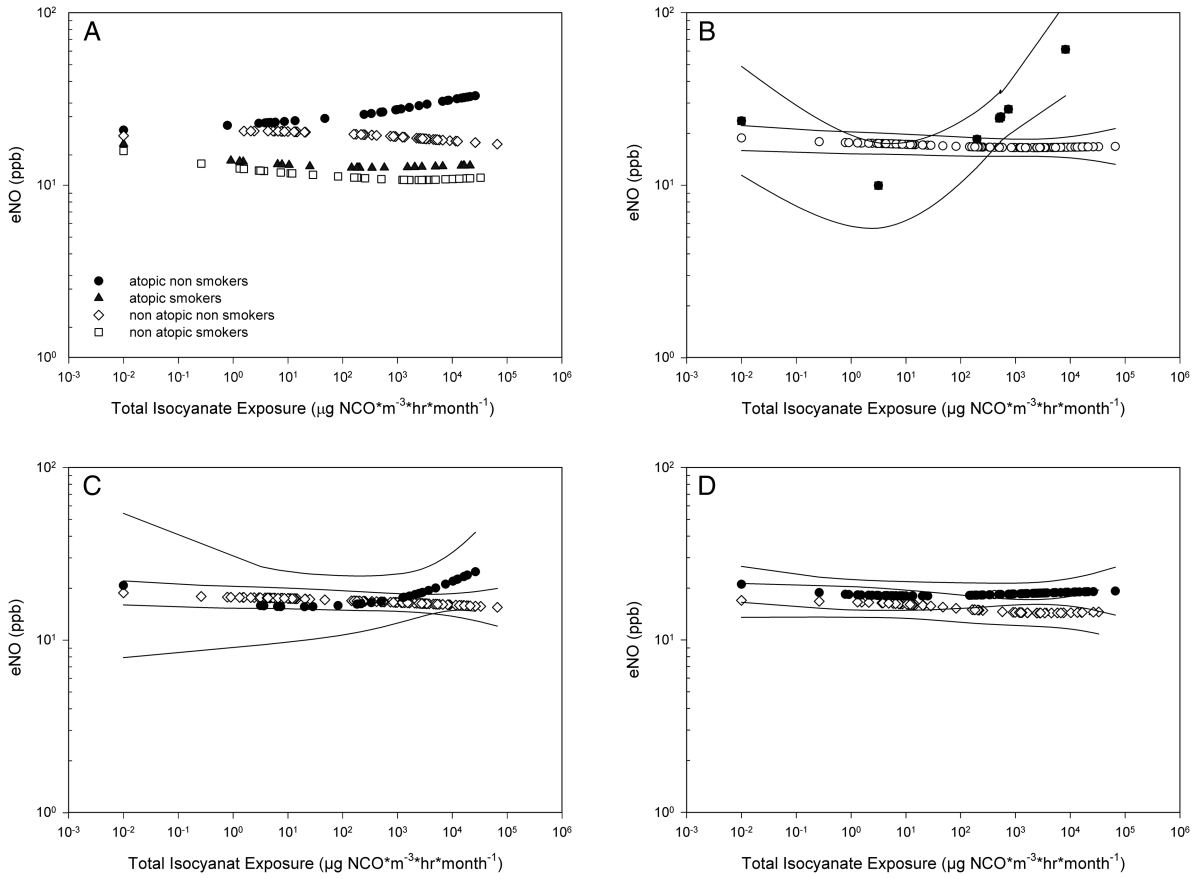

Figure 1 Association between log-transformed isocyanate exposure (µg NCO*m^{-3}*h*$month^{-1}$) and log-transformed exhaled nitric oxide. Penalised smoothed spline plots are presented. Smoothed 95% CIs are given for (B)–(D). (A) By atopy and smoking: 49 atopic non-smokers (circles; linear: p=0.067; spline: p=0.47), 74 non-atopic non-smokers (diamond; relation ns), 35 atopic smokers (triangle; relation ns) and 43 non-atopic smokers (square; spline: ns; linear: p=0.097). (B) By serum-specific IgE, 7 workers with positive specific IgE (square; spline: p<0.0001; linear: ns) and 194 workers with negative specific IgE (circles; relation ns). (C) By bronchial hyper-responsiveness (BHR), 32 workers with BHR (circles; spline: p>0.10; linear: ns) and 169 workers without BHR (diamond; relation ns). (D) By serum-specific IgG, 113 workers with positive specific IgG (circle; relation ns) and 88 workers with negative specific IgG (diamond; relation ns).

The relationship between eNO and specific IgG antibodies to HDI was significant only in non-atopic, non-smokers (GMR (95% CI) 1.25 (1.01 to 1.54), p=0.03). No significant association was shown between eNO and exposure in subjects with a positive specific IgG response to HDI (figure 1D). This association did not change after excluding subjects with positive specific IgE in a separate analysis (data not shown).

In atopic non-smokers, a positive significant association was observed between eNO and asthma-like symptoms (data not shown). eNO levels in atopic smokers were, respectively, negatively and positively associated with work-related chest tightness and rhinitis (data not shown).

We also explored the role of eNO as an intermediate marker of the association between isocyanate exposure and self-reported symptoms (table 4), BHR and specific sensitisations in non-atopic, non-smokers by adding eNO to the exposure-response models. The regression coefficient for exposure and eNO did not change for any of these explored endpoints, and this demonstrates that eNO did not seem to act as an intermediate factor for isocyanate-induced symptoms, BHR and sensitisation.

DISCUSSION

This study describes additional analyses for a large cross-sectional survey that demonstrates exposure–response relations between isocyanate exposure and eNO with a special focus on atopy and smoking. As expected, eNO levels were higher among atopics and lower among smokers, two well-known modifying variables.[17 25] A marginally significant exposure–response relationship was found between exposure to isocyanate and eNO in atopic non-smokers. In a small group of isocyanate-exposed workers with a positive specific IgE response to HDI, elevated eNO levels were clearly exposure related. Positive specific IgG antibodies to HDI were associated with eNO in non-atopic, non-smokers as well. No significant association was found between eNO and isocyanate exposure in subjects with positive specific IgG to HDI. eNO levels were also statistically significantly higher among individuals with BHR, work-related rhinitis and conjunctivitis, and a borderline significant association was observed with asthma-like symptoms.

The main strength of this study is that eNO-level changes could be explored in relation to quantitative personal exposure levels to isocyanates. eNO levels are potentially influenced by many non-occupational factors such as atopy and smoking;[17] thus to estimate the associations between eNO and occupational exposure to isocyanates accurately, exposure–response relationships between isocyanate exposure and eNO were stratified by atopy and current smoking. Effect modification was clearly present. Spray painters were slightly less often atopic. This might be explained by more exposure avoidance of atopic workers in relation to high isocyanate exposure (healthy worker

Table 4 Association between isocyanate exposure and self-reported symptoms, specific sensitisation and BHR in non-smoking, non-atopic isocyanate-exposed workers (n=79), with and without adjustment for eNO

Predictor	n	PRs (95% CI)*, Model 1†	p Value	PRs (95% CI)*, model 2‡	p Value
BHR_{20}	75	4.65 (0.54 to 40)§	0.15	5.14 (0.51 to 51.8)	0.16
Serology					
Specific IgE	78	3.37 (0.04 to 268)	0.58	4.77 (0.03 to 591)	0.52
Specific IgG	79	1.52 (1.13 to 2.05)	0.005	1.57 (1.12 to 2.19)	0.007
Symptoms					
Asthma like symptoms	79	1.44 (0.70 to 2.96)	0.30	1.51 (0.69 to 3.31)	0.29
COPD-like symptoms	79	1.05 (0.63 to 1.76)	0.83	1.04 (0.61 to 1.78)	0.85
Work-related chest tightness	77	1.69 (0.29 to 9.69)	0.55	1.73 (0.28 to 10.5)	0.55
Work-related rhinitis	77	1.19 (0.48 to 2.93)	0.70	1.25 (0.48 to 3.22)	0.64
Work-related conjunctivitis	77	1.18 (0.29 to 4.87)	0.80	1.20 (0.28 to 5.02)¶	0.79

*Prevalence ratio (PRs) for an IQR ($exp^{7.66}$=factor 2121.8) increase in isocyanate exposure (mg NCO/m^3).
†Model 1, adjusted for gender and age.
‡Model 2, adjusted for gender and age and eNO.
§Adjusted for gender.
¶Adjusted for eNO and gender.
BHR, bronchial hyper-responsiveness; COPD, chronic obstructive pulmonary disease; eNO, exhaled nitric oxide.

effect). Increased levels of eNO relevant to occupational exposures have been described previously for a few occupational settings.[26–28] Although a significant association was found between the eNO levels and ozone gassings, adjusted for atopy and smoking, no significant differences in median NO output were found among smokers and non-smokers and also among atopics and non-atopics in a population of bleaching workers.[29] The small population size hampered more refined analyses. Interestingly, there is one study that investigated the relationship between eNO and occupational endotoxin exposure with effect modification by atopy and smoking.[30] Although a significant positive relationship was reported between endotoxin exposure and eNO in non-smoking, non-atopic workers, this cannot easily be explained, especially because endotoxin exposure is associated with non-allergic neutrophil-mediated inflammation. In the present study, a marginally significant exposure–response relationship was observed between isocyanate exposure and eNO only in atopic non-smokers.

Eosinophilic airway inflammation[31] is common among atopics, specifically in asthmatics.[32] Our results showed that eNO was positively associated with atopy irrespective of the presence of asthma. This finding is in agreement with results from previous studies in occupational and non-occupational settings.[25 33–36] Although it has been suggested that eNO is mainly a reflection of allergic airway inflammation in atopics with asthma, both atopy and upper or lower airways disease may cause higher eNO levels in exhaled air.[34] However, the exact mechanism for elevated eNO in atopic individuals has not yet been fully clarified.[37]

The demonstration of a marginally significant relationship between eNO and exposure to isocyanate in a small-sized subgroup of atopic, non-smokers (49 out of 201 workers) in this study should be interpreted carefully and cannot easily be generalised. The higher eNO levels in this subgroup may reflect an increase in airway inflammation, particularly at higher levels of exposure to isocyanates. Further studies are needed to evaluate the usefulness of eNO as a sensitive marker of airway inflammation and the extent to which eNO measurement can be used to predict likely subsequent development of asthma in sensitised individuals.

The prevalence of positive specific IgE antibodies to HDI in our study was low (4.1%), and consequently the power of the analysis was limited. Despite this limitation, we found a strong exposure–response association between eNO and exposure to isocyanate. Others reported a remarkable increased eNO in IgE-sensitised MDI workers.[16] The relationship between eNO and isocyanate exposure in atopic and sensitised individuals indicates that ongoing current exposure seems relevant for the occurrence of inflammation and probably symptoms.

The role of specific IgG antibodies in the development of isocyanate-induced asthma or as a biomarker of exposure has been previously investigated by several studies.[8 38 39] Although in the present study no significant relationship was found between eNO and isocyanate exposure in subjects with a positive specific IgG response to HDI, nonetheless, a positive significant relationship was demonstrated between elevated eNO levels and HDI-specific IgG response in non-atopic, non-smokers, which has not been previously reported. As far as specific IgG is thought to be a marker of exposure, we found, somewhat surprisingly, that in non-atopic non-smokers, increased eNO levels were significantly associated with specific IgG response to HDI. The interpretation of this result cannot be given easily. Only further knowledge about underlying mechanisms can help to interpret the biological relevance of the present observation.

The positive association between eNO level and work-related rhinitis in the present study is in agreement with the results obtained by other publications.[17 26 40] Similarly, the pattern of increased eNO levels in subjects with work-related conjunctivitis is consistent with a finding previously reported.[17] A positive significant association between eNO and asthma-like symptoms in atopic non-smokers in the present study is corroborated with the finding of one study indicating eNO as a marker of airway inflammation only in atopic asthma.[25]

The present data suggested that eNO did not act as an intermediate factor in the associations between self-reported symptoms, BHR, specific sensitisation and exposure because inclusion of eNO in models that described associations between exposure and these endpoints did not change associations in a meaningful way. This has not been investigated earlier.

First, our observation that eNO levels are associated with isocyanate exposure levels in sensitised individuals indicates that ongoing exposure, after an individual becomes sensitised, is

crucial for the development of airway inflammation. However, there is a group of individuals, in the case of isocyanate exposure, who develop asthma, without any sign of specific IgE sensitisation. The fact that adjustment for eNO levels does not change any of the associations (table 4) indicates that eNO does not play an intermediate role. It is possible that early inflammation plays a role in the individuals who are bronchial hyperresponsive, but are not sensitised. Following a separate analysis excluding specific IgE-sensitised individuals, the relationship between eNO and exposure to isocyanates in subjects with BHR did not change, so we believe again that early inflammation is unlikely. We do not have strong indications for eNO-mediated inflammatory responses in subgroups other than atopics or specific IgE sensitised. However, this is a cross-sectional study, and further longitudinal epidemiological studies are needed to evaluate the usefulness of eNO as a sensitive marker of airway inflammation and the extent to which eNO measurement can be used to predict likely subsequent development of asthma.

The fact that eNO levels in the different subpopulations (atopic non-smokers and specifically sensitised workers) are exposure-dependent indicates that eNO levels in populations from environments with potential work-related exposure can only be interpreted correctly if information about quantitative exposure levels is available.

CONCLUSION

The present study suggests that increased eNO levels may represent an increase in airway inflammation in atopic non-smoking isocyanate-exposed workers who are (currently) high exposed to isocyanates. In a small group of isocyanate-exposed workers with positive IgE to HDI, elevated eNO levels were clearly exposure related. Specific IgG sensitisation to HDI was associated with eNO in non-atopic, non-smokers. There is a need for longitudinal studies to assess the clinical function of eNO in isocyanate-exposed workers who are at risk of developing isocyanate asthma in relation to their exposure.

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Competing interest None declared.

Patient consent Obtained.

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