

Different respiratory phenotypes are associated with isocyanate exposure in spray painters

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Short title: Lung function and isocyanate exposure

ABSTRACT

Associations have been observed between exposure to isocyanates consisting mainly of oligomers and respiratory symptoms and isocyanate specific sensitization in spray painters. The aim of the present study was to assess associations between isocyanate exposure and more objective respiratory effect measures such as bronchial hyperresponsiveness (BHR), baseline spirometry and exhaled NO (eNO) in a subset of that population.

Methacholine challenge and eNO measurements were performed in 229 workers. Questionnaires and blood samples were obtained. Specific IgE and IgG to HDI were assessed in serum using various assays. Personal exposure was estimated by combining personal task-based inhalatory exposure measurements and time-activity information.

Workers with higher isocyanate exposure were more often hyperresponsive (prevalence ratio comparing the 75th versus 25th percentile of exposure: 1.8, $p < 0.05$). In addition, significant exposure-related decreased FEV₁, FEV₁/FVC and flow-volume parameters independent of BHR were found. BHR was more prevalent among sensitized workers. This was statistically significant for IgG-ImmunoCAP positives only. eNO was not associated with exposure although slightly elevated eNO levels in specific IgG positive subjects were found.

This study provides evidence that exposure to isocyanate oligomers is related to asthma with BHR as a hallmark, but also shows independent chronic obstructive respiratory effects resulting from isocyanate exposure.

Key words: bronchial hyperresponsiveness, hexamethylene diisocyanate, oligomer, sensitisation, spirometry, spray painter

INTRODUCTION

(Di-)Isocyanates are among the most common causes of occupational asthma (1-3).

Although asthma is considered the major health effect, hypersensitivity pneumonitis and accelerated lung function decline have also been related to isocyanate exposure (4).

Diisocyanate monomers are characterised by two highly reactive NCO-groups and are used as polymerising agents in polyurethane products (5). Most evidence regarding health effects from isocyanates involves these monomers. Lately, isocyanate oligomers or poly-isocyanates, with lower vapour pressure, have been introduced to reduce inhalatory exposure. These are increasingly used and currently many workers are exposed to complex mixtures of isocyanate oligomers. Exposure assessment for these agents is challenging (6) and few epidemiological studies incorporated an exposure assessment component. As a result, exposure-response information for isocyanate oligomers is extremely limited.

We recently demonstrated an association between exposure and (work-related) respiratory symptoms and isocyanate specific sensitization in a population of 581 spray-painters exposed to mainly hexamethylene diisocyanate (HDI) oligomers (7). To further investigate the nature of respiratory health effects caused by isocyanates, a more detailed medical evaluation was performed in a subset of this population. We hypothesize that exposure to isocyanates is associated with bronchial hyperresponsiveness (BHR) as a hallmark of asthma, baseline lung function and exhaled NO (eNO) as a marker of respiratory inflammation.

METHODS

Population and study design

This cross-sectional study was carried out in a subset of a population of workers in the Dutch spray painting industry (7). At the time of selection, results for specific antibodies against diisocyanates measured by ImmunoCAP assay (Phadia, Uppsala, Sweden) were available from the baseline population. All workers from companies with at least one worker with detectable specific diisocyanate IgE or IgG antibodies were invited to participate in the present study. Companies were visited between January and June 2006. All tests were carried out on a working day at the company premises.

The institutional review board for human studies of the University Medical Centre Utrecht, The Netherlands, approved of the protocols and written consent was obtained from all participants.

Questionnaire and exposure estimates

Questionnaire items were previously described (7). For statistical analyses respiratory symptoms suggestive of chronic obstructive pulmonary disease (COPD) and asthma were clustered in: "COPD-like symptoms" (chronic cough, chronic phlegm or shortness of breath); and "asthma-like symptoms" (wheezing or chest tightness). Workers were classified into the following categories based on task information: 'office workers', 'spray-painters' and 'others' (7).

More than 500 personal task-based inhalatory measurements were conducted in a random sample of the participating workers to assess exposure to 23 isocyanate

compounds as described earlier (8). Personal exposure estimates were obtained by combining measurement results and time-activity information (7). Exposure was expressed as the total NCO (isocyanate group) exposure after summing individual monomer and oligomer levels.

Serology

Specific IgE and IgG to HDI were assessed in serum by ImmunoCAP assay and enzyme immunoassays (EIAs) (7). In EIAs, human serum albumin (HSA)-conjugates were used prepared with HDI in the vapour (HDI_V-HSA) and liquid (HDI_L-HSA) phase and prepared with commercial products containing oligomeric HDI (N3300-HSA and N100-HSA). Specific IgE to common aeroallergens was assessed using the Phadiatop (Phadia, Uppsala, Sweden) as a measure of atopy. Cut-off values were used to dichotomise serological outcomes (7).

Spirometry and Methacholine challenge

Baseline lung function parameters were obtained (7). From 31 workers only one technically acceptable manoeuvre could be obtained, while for all other workers at least 2 maximal expiratory flow-volume manoeuvres were obtained. BHR was assessed by a dosimeter method for methacholine challenge (7). Airway hyperresponsiveness was defined as a provocative cumulative dose of methacholine of ≤ 2.5 mg (~ 10 μ moles) required to cause a 20% fall in FEV₁.

Exhaled NO (eNO)

A NIOX MINO[®] hand held device was used to measure eNO according to American Thoracic Society/European Respiratory Society recommendations and manufacturer (Aerocrine AB, Solna, Sweden) guidelines (9). NO free air was inhaled to total lung capacity through the device and then exhaled through the device for 10 sec. During exhalation the flow rate was kept constant at 50 ± 5 ml/sec by a built in flow control unit. The last 3 sec portion of exhaled air was analyzed by an electrochemical sensor in the device. One instrument and sensor was used, which were calibrated and validated at production using a known reference gas source of NO and several validated sensors, respectively. The performance of the electrochemical hand-held NIOX MINO has been compared with standard stationary chemiluminescence units, showing strong correlation and a high degree of agreement (10-12).

Statistical analyses

SAS v9.1 statistical software was used for statistical analysis. For BHR, symptoms, and serology the prevalence ratio (PR) with 95% confidence intervals (95%CI) was calculated as a measure of association (13) using log-binomial regression (PROC GENMOD). Log-transformed exposure data were used and PRs were expressed per inter-quartile range (IQR) increase in exposure: $0.3 - 2799 \mu\text{g NCO} \cdot \text{m}^{-3} \cdot \text{hr} \cdot \text{month}^{-1}$ (factor 9330). The shape of the associations between exposure and BHR was further explored by nonparametric regression modelling (smoothing) using generalized additive models (PROC GAM). Smoothing parameter degrees of freedom were selected by

generalized cross-validation (14) but limited to a maximum of three. Associations for continuous health outcomes (lung function parameters, log transformed eNO) were assessed by linear regression (PROC GENMOD). All associations were adjusted for current smoking, age, gender and atopy. For eNO we also stratified on two major determinants, atopy and smoking. Associations for lung function parameters were additionally adjusted for height and race. When, due to limited power, adjustments could not be made for all of these potential confounders together, the adjustments were made sequentially in separate models. This is mentioned explicitly in the text.

RESULTS

Population characteristics

In total 229 workers from 38 companies participated in this study. Of all companies invited, 90% participated and the worker participation rate within these companies was 66%. Of the workers who did not participate, 27% refused, 22% were not present at the time of the study and 51% had left the company (50% left for another company in the same industry, 25% left the industry, and for 25% their new employer was not known). General characteristics of the study population and the prevalence of symptoms and serology are shown in Table 1. Exposure mainly consisted of HDI oligomers, and HDI monomer contributed for a small fraction of total NCO. Spray-painters had the highest exposure but the exposure distribution clearly overlapped with other workers. Spray-painters tended to be somewhat younger and were more often smokers.

Table 1: General population characteristics, isocyanate exposure levels and prevalence of reported symptoms (n=229) and serological outcomes (n=223)

	Office workers	Spray painters	Others
Characteristics			
N	20	91	118
Gender: % male	80	98	99
Age: AM (SD)	44.3 (11.9)	39.6 (9.7)	40.2 (10.4)
Smoker %	29.4	45.5	38.8
Isocyanate exposure ($\mu\text{g NCO}\cdot\text{m}^{-3}\cdot\text{hr}\cdot\text{month}^{-1}$)			
Total isocyanate, Median (min-max)	0	4,530 (15.4-66,464)	5.6 (0-3,785)
HDI, Median (min-max)	0	36.2 (1.3-472)	0.7 (0-354)
Symptoms %			
COPD-like symptoms [†]	25.0	26.4	27.4
Asthma-like symptoms ^{††}	26.3	35.6	29.1
Work-related rhinitis	35.0	15.9 [^]	22.2
Work-related chest tightness	5.0	8.0	4.3
Work-related conjunctivitis	20.0	13.6	10.3
Serology			
Atopy (Phadiatop) %	55.0	39.3 ^{^§}	39.5 ^{^§}
Specific anti-HDI IgE %			
HDI-ImmunoCAP	0	2.3	1.8
HDI _L -HSA	0	2.3	4.4
HDI _V -HSA	0	0	1.8
N3300-HSA	0	1.1	1.8
N100-HSA	0	3.4	3.5
Specific anti-HDI IgG %			
HDI-ImmunoCAP	5.0	11.2	10.5
HDI _L -HSA	40.0	47.2	43.0
HDI _V -HSA	5.0	19.1	14.0
N3300-HSA	10.0	22.5	17.5
N100-HSA	0	38.2	23.7

AM (SD): Arithmetic mean (standard deviation), min-max: minimum - maximum

[†] chronic cough, chronic phlegm or shortness of breath, ^{††} wheezing or chest tightness

Significance tests for differences in the prevalence of symptoms and serology compared to 'office workers': * p<0.05, ^ p<0.10 after correction for atopy, current smoking, age and gender

§adjusted for age

Symptoms, serology and associations with exposure

Asthma-like symptoms, COPD-like symptoms and work-related chest tightness were more prevalent among workers with higher exposure but associations were not statistically significant (adjusted PR per IQR increase in exposure (95% CI): 1.3 (0.9-1.7), 1.1 (0.6-1.5) and 1.7 (0.7-4.2)) respectively). Specific IgE sensitization was uncommon (below ~4.4%) while the prevalence of specific IgG sensitisation was higher (up to 47% among spray painters) (Table 1). Specific IgG sensitisation assessed by

HDI_L-HSA, HDI_V-HSA and N100-HSA was more common in highly exposed individuals (adjusted PR's IQR: 1.2, 1.7 and 1.9 respectively, $p < 0.1$). Fewer atopics were observed among individuals with high exposure (adjusted PR IQR: (95% CI): 0.7 (0.6-0.9)).

Bronchial hyperresponsiveness, spirometry and eNO

BHR and baseline spirometry were not available for 14 workers because they could not produce an acceptable manoeuvre (6), β -blocker usage (3), other medical reasons (3) and refusal (2). One person stopped during challenge because of health complaints. In 11 workers, eNO could not be determined because of problems with exhaling at a constant flow (3), health complaints (1), refusal (3) and device errors (4).

Table 2: Bronchial hyperresponsiveness (BHR₂₀, n=214), baseline spirometry (% reference value, n=215), and exhaled NO (ppb, n=218)

	Office workers	Spray painters	Others
BHR ₂₀ (%)	0	20.0	14.7
Lung function parameters, AM (SD)			
FEV ₁	103.6 (14.8)	101.6 (14.1)	104.6 (13.3)
FVC	103.6 (16.5)	106.02 (11.4)*	103.9 (13.9)
FEV ₁ /FVC	103.6 (8.5)	98.7 (11.3)*†	103.6 (10.0)
MMEF	92.9 (22.7)	83.3 (29.8)†	94.8 (28.7)
PEF	126.0 (25.8)	114.9 (20.2)*†	121.8 (21.7)
MEF ₇₅	112.7 (23.3)	100.5 (26.7)†	110.5 (26.7)
MEF ₅₀	98.4 (26.0)	87.4 (31.5)†	96.5 (29.2)
MEF ₂₅	77.7 (25.7)	72.6 (31.5)†	87.6 (35.5)
Exhaled NO, GM (GSD)	19.9 (1.8)	17.0 (1.9)	17.3 (1.8)

* Significantly different from 'office workers', † significantly different from 'others' ($P < 0.05$) after adjustment for current smoking, age, gender, height and atopy.

AM (SD): Arithmetic mean (standard deviation), GM (GSD): Geometric mean (geometric standard deviation)

In Table 2, BHR, baseline spirometry and eNO are presented for each of the job title categories. None of the office workers had bronchial hyperresponsiveness (BHR₂₀) despite that many reported asthma-like symptoms or were atopic. In contrast, 20% of spray-painters were hyperresponsive. When using a fall in FEV₁ of 15% as a cut-off

level (BHR₁₅), a prevalence of 15, 22 and 29% was found for office workers, other workers, and spray-painters respectively. FEV₁/FVC and several flow parameters were significantly lower in spray painters compared to other workers. A total of 18 workers met the GOLD criteria for COPD (FEV₁/FVC < 70%); 5, 4 and 15% of office workers, other workers and spray painters, respectively. Of these, 12 fell in the GOLD1 and 6 in the GOLD2 category (4 spray-painters and 2 other workers). Geometric mean eNO levels ranged between 17.0 and 19.9 ppb without significant differences between job title categories.

Associations with exposure

Hyperresponsiveness was clearly associated with exposure expressed as total NCO (Table 3). Exposure-response relationships explored using smoothed spline plots confirmed this and showed similar log-linear associations for both BHR₂₀ and BHR₁₅. The occurrence of hyperresponsiveness increased gradually with increasing exposure without a clear indication for an exposure threshold (Figure 1A). The association between exposure and BHR remained, but became statistically borderline significant and slightly weaker, when COPD cases according to the GOLD criteria (FEV₁/FVC<70%) were excluded from the analysis (adjusted PR (CI) IQR: 1.8 (0.9-3.8) and 1.4 (0.9-2.4) for BHR₂₀ and BHR₁₅, respectively). GOLD defined COPD (FEV₁/FVC<70%) was also strongly associated with exposure (Table 3). Other determinants of FEV₁/FVC<70% were age and smoking, which were not associated with BHR.

No significant association between exposure and exhaled NO was found for log eNO on a continuous scale or dichotomized with 75th or 90th percentiles as cut-off points, with and without stratification for atopy and smoking.

To explore associations between exposure and potentially different asthma phenotypes, BHR₂₀ was combined in different ways with FEV₁/FVC, eNO or symptoms. BHR together with FEV₁/FVC <70% or eNO resulted in strong and (borderline) significant associations with exposure, respectively (Table 3). Asthma-like symptoms with BHR₂₀ were also strongly associated with exposure in a log-linear fashion (Table 3, Figure 1B).

Table 3: Association between (combined) health end-points and isocyanate exposure (PR (95% CI) for an inter quartile range increase (0.3-2799 NCO*m⁻³*hr*month⁻¹) in exposure adjusted for smoking, age, gender and atopy

	n	PR (95% CI)
BHR ₂₀	33	2.0 (1.1-3.8)
FEV ₁ /FVC < 70%	18	2.7 (1.1-6.8)
eNO (ppb) ≥ 90th percentile	22	0.8 (0.4-1.6)*
Combined parameters		
BHR ₂₀ + FEV ₁ /FVC < 70%	10	6.1 (1.2-31.7)**
BHR ₂₀ + eNO (ppb) ≥ 90th percentile	6	7.0 (0.7-72.1)*
BHR ₂₀ + Asthma-like symptoms	19	2.7 (1.0-6.8)
BHR ₂₀ + COPD-like symptoms	15	1.5 (0.6-3.9)
BHR ₂₀ + Work related chest tightness	3	0.9 (0.1-8.3)***
BHR ₂₀ + Work related rhinitis	10	2.2 (0.6-8.0)****
BHR ₂₀ + Work related conjunctivitis	7	4.3 (0.7-28.0)****

* adjusted for smoking and age; adjustment for atopy resulted in similar PR, ** adjusted for smoking and atopy

*** adjusted for smoking, age and atopy, **** adjusted for smoking, gender and atopy

Highly exposed individuals had lower FEV₁, FEV₁/FVC, and flow-volume parameters (Table 4). For all parameters but MEF₂₅, associations differed between atopic and non-atopic individuals (p<0.05). Surprisingly, after stratification these associations between lung function and exposure were especially found in non-atopic workers. Smoothed spline plots showed that at medium to high exposure levels exposure-response relationships in atopic individuals run parallel to those observed in non-atopic

individuals. However, low exposed atopic individuals showed a systematically lower lung function.

Consequently, associations with exposure within atopic individuals were not statistically significant (data not shown). When hyperresponsive individuals were excluded from the analyses, similar associations between exposure and lung function were found.

Excluding the workers with only one technically acceptable baseline manoeuvre also did not affect the association between lung function parameters or BHR and exposure.

Table 4: Association between lung function parameters and exposure (β log exposure (p-value)) adjusted for current smoking, age, gender, height and atopy

	All	Non-atopics	Atopics
FEV ₁	-0.004 (0.60)	-0.017 (0.08)	0.012 (0.27)*
FVC	0.015 (0.08)	0.010 (0.37)	0.020 (0.10)
FEV ₁ /FVC	-0.321 (0.004)	-0.543 (0.0002)	-0.088 (0.61)*
PEF	-0.026 (0.31)	-0.088 (0.01)	0.045 (0.22)*
MMEF	-0.037 (0.03)	-0.071 (0.003)	0.0014 (0.95)*
MEF ₇₅	-0.043 (0.13)	-0.109 (0.01)	0.032 (0.42)*
MEF ₅₀	-0.037 (0.07)	-0.076 (0.01)	0.010 (0.72)*
MEF ₂₅	-0.024 (0.02)	-0.038 (0.002)	-0.010 (0.51)

* Atopics and non atopics significantly different (p<0.05)

Associations with serology and symptoms

Overall, sensitized (specific IgE and IgG) workers were more often hyperresponsive (Table 5). This was statistically significant for IgG measured with the ImmunoCAP assay only. Exhaled NO was not associated with a specific IgE antibody response to isocyanates, but was increased in workers with specific IgG against N3300-HSA or N100-HSA (Table 5). When restricting this analysis to non-smoking subjects without atopy these associations were much stronger (β (p): 0.303 (0.02) and 0.357 (0.001) for IgG against N3300-HSA and N100-HSA respectively) and was also statistically significant for IgG against HDI_V-HSA (β (p): 0.329 (0.02)).

Workers with asthma-like symptoms had significantly more BHR and significantly lower baseline FEV₁, FEV₁/FVC and MMEF. COPD-like symptoms and work-related rhinitis were (borderline) significantly associated with BHR as reported earlier (7). Statistically significant increased exhaled NO levels were found in workers with reported asthma-like symptoms and work-related rhinitis and conjunctivitis (adjusted PR (95% CI): 1.7 (1.1-2.5), 1.6 (1.1-2.4) and 1.9 (1.1-3.6) respectively).

Table 5: Associations between BHR and specific sensitization (Prevalence ratio (95% Confidence Interval)) and between exhaled NO and specific sensitization (β log eNO (ppb) (p-value)) adjusted for current smoking, age, gender and atopy.

	BHR20	Log eNO (ppb)
<i>Specific IgE</i>		
HDI-ImmunoCAP	1.62 (0.30-8.85)*	0.312 (0.24)
HDI _L -HSA	1.69 (0.53-5.45)*	0.182 (0.37)
HDI _V -HSA	2.32 (0.52-10.31)*	-0.043 (0.91)
N3300-HSA	1.96 (0.39-9.93)*	0.320 (0.30)
N100-HSA	2.38 (0.75-7.57)*	0.190 (0.35)
<i>Specific IgG</i>		
HDI-ImmunoCAP	3.07 (1.14-8.22)	0.153 (0.20)
HDI _L -HSA	0.76 (0.40-1.45)	0.040 (0.58)
HDI _V -HSA	1.06 (0.45-2.49)	0.064 (0.52)
N3300-HSA	1.28 (0.61-2.69)	0.169 (0.07)
N100-HSA	1.03 (0.52-2.05)	0.156 (0.05)

* adjusted for current smoking, gender and atopy

DISCUSSION

In this study a clear and positive association was found between exposure to isocyanate oligomers and bronchial hyperresponsiveness. Exposure-related obstructive lung function changes (FEV₁, FEV₁/FVC and flow parameters) independent of BHR were also found. These results points towards different exposure-related respiratory

phenotypes related to occupational isocyanate exposure. The findings also support our earlier observations (7) that asthma-like and COPD-like symptoms are associated with isocyanate exposure level in workers exposed to mainly HDI oligomers in the spray painting industry.

BHR is closely related to variable airway obstruction and is usually considered a hallmark of asthma in epidemiological studies (15). The direct associations between BHR and exposure, which were independent from chronic obstruction, as well as the association with asthma-like symptoms are suggestive of isocyanate-induced asthma-like respiratory effects. BHR and asthma-like symptoms (wheezing or chest tightness) taken together appeared even more strongly related to exposure. Two previous studies have assessed BHR on the population level in isocyanate exposed workers, which both did not incorporate exposure measurements. A study by Redlich et al. showed a similar pattern of BHR across job title categories in car body repair shop workers, exposed to HDI based paint, which did not reach statistical significance, likely due to the small sample size (16). In a case-control study among furniture workers, exposed to toluene diisocyanate based paint, bronchial hyperresponsiveness was not more prevalent among painters than among assembly and wood workers (17).

Isocyanate exposure was also associated with obstructive respiratory changes. Accelerated lung function decline has been demonstrated in adults with self-reported asthma (18) and a recent study suggests that occupational asthma results in faster rates of decline than non-occupational asthma (19). Although these changes may be

observed in specific subgroups of asthmatics (20), effects on lung function are usually not observed in the population at large in healthy working populations exposed to occupational allergens. BHR in the presence of airway obstruction ($FEV_1/FVC < 70\%$) was strongly associated with exposure to isocyanates in our study. This may reflect asthma-related chronic effects. Yet, the association between lung function parameters and exposure was also found in non-atopic workers without BHR. Since asthma was probably not manifest in these workers, other mechanisms are likely involved. Lung function decline caused by toluene diisocyanate has been previously described, but could not be reproduced in later studies (21). Isocyanate related effects on lung function have also previously been reported for spray painters exposed to HDI. A similarly sized cross-sectional study reported an association between estimated exposure levels and expiratory flow rates in aircraft painters (22). A smaller longitudinal study that included exposure measurements found an association in smokers between FEV_1 , FVC and VC and the frequency of peak exposures but not with mean levels (23). Interestingly, we found significant associations with lung function in non-atopic workers only. Since smoking was not associated with atopic status, it seems unlikely that smoking is responsible for the observed differences between atopic and non-atopic subjects in our study.

Specific IgE was found in only 1 of the 19 workers with both BHR and asthma-like symptoms and in none of the 9 workers with both BHR and $FEV_1/FVC < 70\%$. These results indicate that asthma-like respiratory effects including lower respiratory symptoms and BHR can not be attributed to specific IgE antibodies using the isocyanate antigen

preparations available in this population. Although we used a range of conjugates, the conjugation reactions in the human airways are unknown and it is possible that an IgE mediated response could be demonstrated using a different conjugate. However, alternative explanations also exist. Firstly, cell-mediated allergy or irritant mechanisms may be involved (4, 24). Secondly, exacerbations of pre-existing asthma due to isocyanate exposure may explain the findings to some extent and cannot be excluded. Yet, the clear exposure-related difference in prevalence of BHR seems more suggestive of isocyanate associated asthma than for exacerbations of pre-existing asthma. The association between IgG to HDI-ImmunoCAP and BHR in the absence of an association between HDI-ImmunoCAP and exposure is remarkable and needs further exploration. The presence of specific IgG antibodies also seemed associated with elevated eNO levels.

eNO is thought to be a direct marker of eosinophilic inflammation immediately after exposure (25, 26), while BHR probably reflects the continuous process of airway wall remodelling (27). A specific inhalation study has reported significantly more eNO increase in asthmatics who were isocyanate-responders and of whom 25% showed diisocyanate specific IgE antibodies (28). We showed that eNO is associated with symptoms and BHR, but found no clear association with isocyanate exposure. Interestingly, BHR in the presence of high eNO levels was strongly associated with exposure. Combining BHR and eNO may have resulted in a distinct subgroup of allergic asthmatics (27). Although specific IgE to any of the isocyanate conjugates was not

found in any of the six workers with BHR and high eNO levels, all of these individuals were atopic.

The main strength of this study is the quantitative inhalation exposure assessment component, which contributes considerably to the discriminative power of the study. Some misclassification of exposure may have occurred since the use of personal exposure equipment, previous exposures and dermal exposure were not taken into account. Yet, the incorporation of quantitative exposure estimates based on over 500 inhalation exposure measurements and detailed task activity information is an improvement over most previous epidemiological studies on isocyanates, which generally relied on qualitative exposure assessment. Only one previous epidemiological study included exposure measurements of isocyanate oligomers (23), but this study was of limited size. Another strength is the inclusion of several objective respiratory effect measures, which have not been investigated together in one population before.

A limitation of the study is that it was performed in a complex exposure environment, complicating the establishment of causal relationships. It should be considered that isocyanates have strong irritating properties and are therefore very likely the most relevant irritant in the spray painting environment. However, exposure to other irritants may occur. Solvents, which may be associated with respiratory effects (29), are another major component of paint. We performed some exposure studies focused on solvents in the population under study (not published). Exposure levels were all well below existing occupational exposure limits. Highest exposure levels were found for non spray-painting

tasks, such as tasks involving solvent based fillers and degreasers, and solvent exposure did not correlate with isocyanate exposure. Therefore it seems unlikely that solvents are responsible for the associations found in this study. Other exposures with possibly irritating properties include welding fumes and sanding dust. These exposures are experienced mainly by auto body workers, which are included in the 'other workers' category, and cannot explain the higher risks found for spray painters.

A limitation of the categorization of the self-reported symptoms into asthma-like and COPD-like symptoms is that chronic cough and shortness of breath can be present in both asthma and COPD resulting in misclassification. The distinction between asthma and COPD, which are both phenotypic heterogeneous diseases characterized by obstructive airflow limitation and inflammatory changes in the respiratory tract is difficult and the present study was not directed to establish or confirm a diagnosis of respiratory disease.

Another limitation is that because this is a healthy population, bronchodilators were not administered before baseline spirometry. This may have resulted in overestimation of chronic airflow limitation in this study (30), while variable airflow limitation may have been underestimated.

The participation rate of this study was acceptable. However, the time consuming and more invasive tests in the present study compared to the baseline study may have led to a participation bias. Although overall the prevalence of symptoms and serology was similar to the baseline study, office workers showed a higher prevalence of symptoms and atopy. As a result associations with exposure were less strong than in the baseline study (7). In both the current and the baseline study, atopy was less common among

workers with high exposure, which we interpreted as indicative of a healthy worker effect. Despite these limitations and potential biases, clear associations were found between respiratory effects and isocyanate exposure.

In conclusion, this study provides evidence of the existence of independent exposure-related respiratory phenotypes associated with occupational isocyanate exposure. Besides asthma, with BHR as a hallmark, chronic obstructive effects were found. The different phenotypical subgroups with respect to BHR, airway obstruction, antibodies, and increased eNO may point towards heterogeneity in underlying mechanisms. The results indicate that at exposure levels commonly found in this industry (8) isocyanate oligomers can induce respiratory health effects, stressing the importance of regulation and control of isocyanate oligomer exposure.

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Figure legend:

Figure 1: Association between log-linear exposure to isocyanates and health end points. Penalized smoothed spline plots with smoothed 95% CI. A: BHR20 (black) and BHR15 (grey), splines: non significant (ns), B: BHR20 and asthma-like symptoms, spline: ns.



