Respiratory Symptoms, Sensitization, and Exposure–Response Relationships in Spray Painters Exposed to Isocyanates

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***Rationale*: Associations between oligomeric isocyanate exposure, sensitization, and respiratory disease have received little attention, despite the extensive use of isocyanate oligomers.**

***Objectives*: To investigate exposure–response relationships of respiratory symptoms and sensitization in a large population occupationally exposed to isocyanate oligomers during spray painting.**

***Methods*: The prevalence of respiratory symptoms and sensitization was assessed in 581 workers in the spray-painting industry. Personal exposure was estimated by combining personal task-based inhalatory exposure measurements and time activity information. Specific IgE and IgG to hexamethylene diisocyanate (HDI) were assessed in serum by ImmunoCAP assay and enzyme immunoassays using vapor and liquid phase HDI–human serum albumin (HDI–HSA) and HSA conjugates prepared with oligomeric HDI.**

***Measurements and Main Results*: Respiratory symptoms were more prevalent in exposed workers than among comparison office workers. Log–linear exposure–response associations were found for asthmalike symptoms, chronic obstructive pulmonary disease–like symptoms, and work-related chest tightness (prevalence ratios for an interquartile range increase in exposure of 1.2, 1.3 and 2.0, respectively; $P \leq 0.05$). The prevalence of specific IgE sensitization was low (up to 4.2% in spray painters). Nevertheless, IgE to N100 (oligomeric HDI)–HSA was associated with exposure and work-related chest tightness. The prevalence of specific IgG was higher (2–50.4%) and strongly associated with exposure.**

***Conclusions*: The results provide evidence of exposure–response relationships for both work-related and non–work-related respiratory symptoms and specific sensitization in a population exposed to oligomers of HDI. Specific IgE was found in only a minority of symptomatic individuals. Specific IgG seems to be merely an indicator of exposure.**

Keywords: oligomer; isocyanate; asthma; spray painter; sensitization

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Isocyanates are among the most common causes of occupational asthma. Oligomeric isocyanates are increasingly used. Associations between oligomeric isocyanate exposure, sensitization, and respiratory disease have received little attention.

What This Study Adds to the Field

There are exposure–response relationships for exposure to oligomeric isocyanates and respiratory symptoms and sensitization. Specific IgE plays a role in a minority of symptomatic individuals. Specific IgG seems merely a marker of exposure.

Isocyanates, low-molecular-weight compounds characterized by highly reactive NCO groups, are one of the most commonly identified causes of occupational asthma (1–3). Besides allergic asthma, isocyanate exposure may also induce irritant asthma, hypersensitivity pneumonitis, and possibly accelerated lung function decline (4). Diisocyanates are used as cross-linking agents in polyurethane (PU) products, such as foams, paints, lacquers, inks, insulating materials, varnishes, rubber modifiers, and bonding and vulcanizing agents (5). The PU industry continues to increase, together with the number of workers at risk for exposure (4). Toluene diisocyanate (TDI), diphenyl-methane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are the most frequently used diisocyanate monomers.

Despite a vast amount of studies on isocyanates, aspects of the association between health effects and isocyanate exposure remain unclear. Isocyanate monomers have been studied relatively well in large TDI manufacturing or foam production units. In the early years of the industry, annual occupational asthma incidence was as high as 5 to 6% (6). The reduction of average TDI concentrations below the 8-hour occupational exposure limit of 5 ppb (17 $\mu g/m^3$ total NCO group mass concentration) led to a decline to an incidence of below 1% (6). Conversely, asthma symptom prevalences of up to 41% have been reported in TDI end-user industries with possibly less controlled exposures (6).

Recently, diisocyanate oligomers, mainly of HDI and MDI with considerably lower vapor pressures, have been increasingly used to reduce inhalation exposure (4). In the present article, all polymeric diisocyanates, which are indicated with different terms (polyisocyanates, oligomers, adducts) in the literature, will be referred to as oligomers. Isocyanate asthma does occur in workers exposed to oligomers and specific inhalation challenge testing of individual patients confirms that oligomers can cause asthma (7). Yet, despite the extensive use of isocyanate oligomers, exposure–response associations have hardly been investigated. Commercial

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products based on oligomeric isocyanates commonly contain a variable mixture of several different chemical structures. The complexity of exposure assessment of these mixtures contributes to the absence of exposure–response studies.

Spray painters, who are exposed to HDI oligomer mixtures, are among the occupational groups with the highest incidence of occupational asthma in industrialized countries (8–10). Figure 1 shows the chemical structures of HDI and two of its oligomers. This article describes a large cross-sectional study of isocyanate exposure and health effects in spray painters. Respiratory symptoms were recorded and specific IgE and IgG antibodies to various isocyanate conjugates were measured. Over 500 task-based exposure measurements to a wide range of isocyanates were used in combination with time activity information to estimate the exposure of each individual. We specifically aimed at establishing quantitative exposure–response relationships for a range of respiratory endpoints.

METHODS

Population and Study Design

The population consisted of 581 subjects working in various spray-painting industries in the Netherlands. Car body repair shops, furniture paint shops, and industrial paint shops specializing in ships and harbor equipment or airplanes were contacted by mail or telephone. Companies were visited between 2003 and 2006, and a workplace survey was performed. All workers were asked to complete a self-administered questionnaire and to provide a 20-ml blood sample. All participants were actively working at the time of the study and the study was performed on a working day. The Institutional Medical Ethical review board of University Centre Utrecht approved of the protocols, and written consent was obtained from all participants.

Questionnaire

Items included respiratory symptoms according to the Dutch version of the internationally accepted British Medical Research Council respiratory questionnaire (11), supplemented with questions on work-related symptoms. Symptoms were considered work related when they were reported to occur during or shortly after work. For statistical analyses, respiratory symptoms suggestive of chronic obstructive pulmonary disease (COPD) and asthma were combined: "COPD-like symptoms" included chronic cough, chronic phlegm, and shortness of breath; "asthmalike symptoms" included wheezing and chest tightness.

Additional items included smoking habits, job history, present job title, personal protective equipment use, and monthly task patterns. Workers reporting no tasks outside the office were classified as "office workers." Workers involved in spray painting were classified as "spray painters," and all other workers involved in tasks outside the office were classified as "others." The latter category consisted of mostly mechanics and metal workers. In every company, all workers were working in the same building.

Personal Exposure Estimates

Personal exposure estimates were obtained by combining personal task-based inhalation measurements for 23 different isocyanate compounds (4 monoisocyanates, 5 aminoisocyanates, 6 diisocyanates and 8 diisocyanate oligomers) performed in the population under study (12) and time activity information:

$$\text{Exposure} = \sum_{n=1}^{n} (\text{Time})_n \times (\% > \text{LOD})_n \times (\text{Median NCO Concentration})_n,$$

where Exposure = personal exposure expressed in μg NCO × m^{-3} × hour × $month^{-1}$; n is an arbitrary value from 1 to 6 assigned to the following tasks: (1) spray painting; (2) mixing; (3) cleaning paint equipment; (4) assisting a spray painter; (5) sanding; and (6) welding; $(\text{Time})_n$ = time task n was performed expressed in hours per month (on average, 82 h [SD, 89] out of a 161-h [SD, 26] working month was spent on exposed tasks); $(\% > \text{LOD})_n$ = percentage of samples above the limit of detection (LOD) for task n; $(\text{Median NCO concentration})_n$ = median inhalatory isocyanate concentration during task n expressed in μg NCO/m^3.

Separate task-based airborne exposure measurements were available for each combination of industry and task. The total isocyanate group (NCO) concentration and NCO from HDI and two HDI oligomers (biuret and isocyanurate) concentration were calculated.

More details are provided in the online supplement.

Serologic Analysis

Blood samples were processed within 8 hours and serum aliquots were stored at −20°C until serologic assays. HDI-specific IgE and IgG antibodies were analyzed using the ImmunoCAP assay (Phadia, Uppsala, Sweden) and specific IgE to common aeroallergens using the Phadiatop (Phadia) as a measure of atopy. Cutoff values of 0.35 kU/L for specific IgE and 5 mg/L for specific IgG were used.

Isocyanate-specific IgE and IgG were also assessed by enzyme immunoassay with HDI–HSA conjugates prepared in our own laboratories. HDI–HSA was prepared in liquid phase (HDI_L–HSA) (13) and vapor phase (HDI_V–HSA) (14) reactions essentially as described earlier. HDI oligomer–HSA conjugates were prepared with Desmodur N3300, a commercial product containing a low-viscosity isocyanurate oligomer of HDI, and Desmodur N100, a trimeric biuret structure (Bayer, Pittsburgh, PA). Table 1 gives an overview of immunoassays used. Cutoff values for HSA-corrected optical density values of 0.1 and 0.3 were used for IgE and IgG, respectively.

Details of the enzyme immunoassay procedures and establishment of cutoff values are provided in the online supplement.

Physiological Testing

Bronchial hyperresponsiveness (BHR) was assessed in a subset of 229 workers. Selection of this subset is described in the online supplement. At least two maximal expiratory flow–volume maneuvers were obtained to assess baseline lung function. The largest FEV_1 and FVC were recorded. Maximum midexpiratory flow (MMEF) was obtained from the maneuver with the largest sum of FEV_1 + FVC as described by Miller and colleagues (15). BHR was assessed by methacholine challenge according to the European Respiratory Society guidelines (16). Methacholine was administered using a controlled tidal volume breathing dosimeter technique using the Aerosol Provocation System with a Medic-Aid nebulizer (Jaeger GmbH and Co KG, Wurzburg, Germany), starting with 0.019 mg methacholine after three quadrupling doses and one doubling dose up to a cumulative dose of 2.5 mg (short schedule). FEV_1 was measured 30 and 90 seconds after challenge and the lowest FEV_1 from a technically acceptable maneuver was used. After a fall in FEV_1 of 5%, doubling doses were used (long schedule). The test was stopped when a fall of 20% in FEV_1 was observed (BHR_{20}) or the maximum cumulative dose was reached. Airway hyperresponsiveness was defined

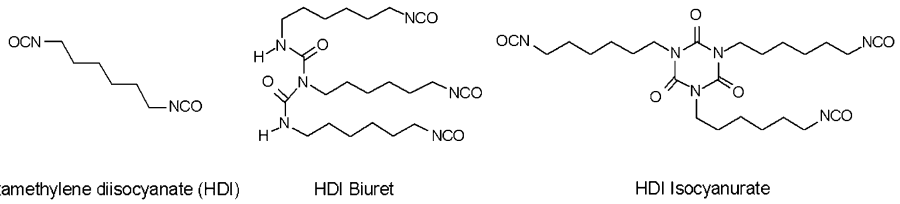

Figure 1. Chemical structures of hexamethylene diisocyanate (HDI) and two HDI oligomers.

TABLE 1. OVERVIEW OF CHARACTERISTICS OF ASSAYS USED IN SPECIFIC IgE AND IgG ANTI–HEXAMETHYLENE DIISOCYANATE ANALYSES

Conjugate	Source*	Carrier	Phase Isocyanate†	Test System
HDI–ImmunoCAP	Phadia	ImmunoCAP (as solid phase)	Done by manufacturers	ImmunoCAP assay
HDI_L–HSA	IRAS	HSA	Liquid	EIA
HDI_V–HSA	Yale	HSA	Vapor	EIA
N3300–HSA	Yale	HSA	Liquid	EIA
N100–HSA	Yale	HSA	Liquid	EIA

Definition of abbreviations: EIA = enzyme immunoassay; HDI = hexamethylene diisocyanate; HDI_L–HSA = HDI–HSA liquid phase; HDI_V–HSA = HDI–HSA vapor phase; HSA = human serum albumin; IRAS = Institute for Risk Assessment Sciences.

Source where the conjugate was prepared, technical details, and system in which the conjugate was used are shown.

* Sources: Phadia, Sweden; IRAS, The Netherlands; Yale School of Medicine, Connecticut.

† Phase of the isocyanate mixture during reaction.

as a provocative dose of methacholine required to cause a 20% fall in FEV_1 of 2.5 mg or less (~10 μmol).

Statistical Analysis

SAS version 9.1 statistical software was used (SAS Institute, Cary, NC). Correlations between the exposure variables were assessed using Pearson correlation coefficients for log-transformed data. In cross-sectional studies, the prevalence ratio (PR) is often a more easily interpretable and meaningful measure of association than the odds ratio (17). Therefore, PRs and 95% confidence intervals (95% CI) were calculated by log-binomial regression (SAS GENMOD procedure) to describe associations for binary health outcomes. Log-transformed exposure data were used and PRs per unit increase were converted to PRs per interquartile range. Associations with exposure were further explored by nonparametric regression modeling (smoothing) using generalized additive models (SAS GAM procedure). Smoothing parameter degrees of freedom were selected by generalized cross-validation (18) but limited to three. Unless stated otherwise, all associations were adjusted for current smoking, age, sex, and atopy. Possible effect modification by atopy was explored as well.

RESULTS

Population Characteristics and Exposure

The 581 participating workers came from 128 companies: 88 car body repair shops; 33 furniture paint shops; and 7 industrial paint shops, of which 6 specialize in ships and harbor equipment and 1 in airplanes. Of all companies contacted through surface mail and telephone, 10 to 30% responded; the average worker participation rate per company was 67%. General characteristics of the study population are shown in Table 2.

Estimated median total NCO exposure levels were higher in the "spray painters" category than among "others," with a wide range in both categories. Exposure to HDI monomer represented only a very small fraction of total NCO. Of the HDI oligomers, which represented a larger fraction of total NCO, isocyanurate exposure was higher than biuret exposure.

Within the group of spray painters, those working in airplane paint shops were, on average, more highly exposed than those in furniture paint shops, ship and harbor equipment paint shops, and car body repair shops (median: 16,600 vs. 4,900, 4,700, and 3,300 $\mu g\ NCO \times m^{-3} \times h \times mo^{-1}$, respectively). The minimum, 25th percentile, median, 75th percentile, and maximum of the total exposure distribution were 0, 1.7, 165, 33,821, and 66,464 $\mu g\ NCO \times m^{-3} \times hour \times month^{-1}$, respectively. Pearson correlation coefficients among the exposure estimates for total NCO, HDI, biuret, and isocyanurate were very high ($\geq$0.95).

Prevalence of Symptoms and Positive Serology

Exposed workers more often reported respiratory symptoms than office workers (Table 3). Asthmalike symptoms were significantly ($P \leq 0.05$) more prevalent in both spray painters and other workers (adjusted PR [95% CI]: 2.8 [1.3–5.9] and 2.2 [1.0–4.8], respectively). Spray painters also reported more COPD-like symptoms (adjusted PR [95% CI], 2.9 [1.1–8.0]). No significant differences were found for any of the work-related symptoms.

TABLE 2. GENERAL POPULATION CHARACTERISTICS, WORK HISTORY AND ISOCYANATE EXPOSURE OF 581 WORKERS IN SPRAY-PAINTING COMPANIES

	Office Workers	Spray Painters	Others
No.	50	241	290
Sex, % male	58	99	97
Age, yr, AM (SD)	40.1 (10.1)	36.9 (10.4)	39.0 (12.0)
Smoking status			
Smoker, %	23.4	42.8	35.6
Stopped smoking within last year, %	2.1	4.7	5.3
Former smoker, %	40.4	19.2	23.2
Never smoked, %	34.0	33.3	35.9
Total pack-years, AM (SD)	7.7 (10.4)	8.2 (11.9)	8.5 (13.4)
Branch type, %			
Car body repair shop	72	66	85
Furniture paint shop	6	16	11
Boat/harbor equipment paint shop	2	6	2
Airplane paint shop	20	12	2
Work history, AM (SD)			
No. of years worked	15.6 (10.1)	16.3 (9.7)	19.2 (12.4)
No. of years in branch	11.9 (10.7)	15.7 (9.6)	18.1 (12.4)
No. of years as spray painter	2.0 (4.7)	14.9 (9.6)	3.4 (7.5)
Isocyanate exposure, $\mu g\ NCO \times m^{-3} \times h \times mo^{-1}$			
Total isocyanate, median (min–max)	0	3,682 (4–66,464)	8 (0–13,473)
HDI, median (min–max)	0	27 (0.2–1,427)	0.3 (0–1,920)
Biuret, median (min–max)	0	269 (0.2–13,568)	2 (0–1,587)
Isocyanurate, median (min–max)	0	2,250 (6–87,623)	6 (0–30,0006)

Definition of abbreviations: AM = arithmetic mean; HDI = hexamethylene diisocyanate.

TABLE 3. PREVALENCE OF RESPIRATORY AND ALLERGIC SYMPTOMS AND SEROLOGIC OUTCOMES*

	Office Workers (*n* = 50)	Spray Painters (*n* = 241)	Others (*n* = 290)
Respiratory symptoms, %			
Chronic cough	2.0	15.4†	13.6†
Chronic phlegm	4.0	13.3	10.8
Shortness of breath	4.0	8.8	8.4
Wheezing	12.0	29.1‡	22.6†
Frequent wheezing (>1 wk)	4.0	12.5‡§	4.9
Shortness of breath during wheezing	4.0	16.2†	10.5
Chest tightness	14.0	18.3	14.4
Chest tightness before start work	10.0	8.8	7.1
Clusters of symptoms, %			
COPD-like symptoms	8.0	26.1‡	20.6†
Asthmalike symptoms	14.0	33.6‡	28.0‡
Work-related symptoms, %			
Work-related rhinitis	14.3	19.8	15.0
Work-related chest tightness	2.0	8.3	4.0
Work-related conjunctivitis	12.0	16.0	10.4
Positive serology, %			
Atopy (Phadiatop)	44.0	33.6‡‖	37.6
Specific IgE			
HDI–ImmunoCAP	0	2.1	1.0
HDI_L–HSA	0	2.9	3.5
HDI_V–HSA	0	0.4	0.7
N3300–HSA	0	2.1	1.0
N100–HSA	0	4.2	2.1
Specific IgG			
HDI–ImmunoCAP	4.0	9.5	7.2
HDI_L–HSA	32.0	50.4‡	41.5
HDI_V–HSA	2.0	20.0‡¶	9.3
N3300–HSA	10.0	23.3	15.1
N100–HSA	4.0	34.6‡	21.5‡

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HDI = hexamethylene diisocyanate; HDI_L–HSA = HDI–HSA liquid phase; HDI_V–HSA = HDI–HSA vapor phase; HSA = human serum albumin.

* Atopy and specific IgE and IgG sensitization against HDI.

† $P < 0.10$; significantly different from "office workers" category after adjustment for atopy, current smoking, age, and sex.

‡ $P < 0.05$; significantly different from "office workers" category after adjustment for atopy, current smoking, age, and sex.

§ Adjusted for atopy, current smoking, and sex.

‖ Adjusted for current smoking, age, and sex.

¶ Adjusted for for atopy, current smoking, and age.

Despite the high symptom prevalence, specific IgE antibodies to isocyanates were found only in a small proportion of exposed workers (0.4–4.2% in spray painters, 0.7–3.5% in other workers, and none in the office group) (Table 3). The prevalence of elevated specific IgG antibody concentrations was much higher. Among spray painters, prevalences up to 50% were found. Antibodies to N100–HSA and HDI_L–HSA were found most frequently, both for specific IgE and IgG.

Specific IgG to HDI_L–HSA, HDI_V–HSA, and N100–HSA was significantly ($P \leq 0.05$) more prevalent among spray painters compared with office workers (adjusted PR [95% CI]: 1.6 [1.0–2.6], 10.6 [1.5–75.2], and 7.8 [1.9–32.5], respectively). IgG antibodies to N100–HSA were also more often found in other workers than in office workers (adjusted PR [95% CI], 4.7 [1.1–19.4]).

Atopy was significantly ($P \leq 0.05$) less common among spray painters than office workers (adjusted PR [95% CI], 0.7 [0.5–1.0]).

Association between Symptoms and Serology

Table 4 shows the associations between symptoms and the presence of isocyanate-specific antibodies. A consistent pattern of significant positive associations was found for work-related rhinitis and specific IgE to each of the conjugates, with PRs between 1.8 and 2.8. All PRs for work-related chest tightness and specific IgE were positive, but showed much more variation, and only the association with IgE to N100–HSA was significant. Overall PRs for asthmalike and COPD-like symptoms were lower and, for most conjugates, were close to 1.0.

Statistically significant associations were found for COPD-like symptoms and work-related rhinitis and conjunctivitis with IgG to N100–HSA. However, for the other conjugates, PRs for the association between specific IgG and symptoms were close to 1. Exclusion of workers with a high IgG background reaction to HSA did not alter the associations (data not shown).

Associations with Exposure

PRs were calculated based on log-transformed exposure data and expressed for an interquartile range increase in exposure (1.7–3,382 $\mu g\ NCO \times m^{-3} \times h \times mo^{-1}$, or an approximate difference in exposure of a factor of 2,000) (Table 5). Significant positive log-linear associations with exposure were found for asthmalike symptoms, COPD-like symptoms, work-related chest tightness, and work-related conjunctivitis (Table 5). Only the association between work-related conjunctivitis and exposure differed between atopic and nonatopic individuals (P interaction term $\leq$ 0.1). Surprisingly, the association was stronger in nonatopic than in atopic subjects (adjusted PR [95% CI]: 2.1 [1.2–3.9] and 1.1 [0.7–1.8], respectively). For asthmalike symptoms (Figure 2A) and COPD-like symptoms (plot not shown) the smoothed plots corroborate log-linear relations. For work-related chest tightness (Figure 2B), the smoothed plot suggests a steeper increase at high exposure levels (P spline $\leq$ 0.05). No statistically significant association between rhinitis and exposure was found (Figure 2C).

Interestingly, the prevalence of atopy was lower at high exposure levels. Figure 2D shows a sharp reduction for the prevalence of atopy at isocyanate exposures above approximately 1,000 $\mu g\ NCO \times m^{-3} \times hour \times month^{-1}$ (P spline $\leq$ 0.05). Atopic subjects were significantly less exposed than nonatopic subjects (geometric mean: 24.1 and 57.9 $\mu g\ NCO \times m^{-3} \times h \times mo^{-1}$, respectively; $P \leq 0.05$).

Exposure was also associated with N100–HSA-specific IgE. The smoothed plot shows a very slight increase (Figure 2E). Specific IgG antibodies to all conjugates except HDI–ImmunoCAP were positively associated with exposure. Especially strong associations were found for IgG to HDI_V–HSA and N100–HSA.

For IgG measured by ImmunoCAP (P interaction term ≤ 0.1), IgG to N3300–HSA (P interaction term $\leq$ 0.05) and to N100–HSA (P interaction term ≤ 0.05), stronger associations were seen in atopic subjects (adjusted PR [95% CI]: 2.5 [0.99–6.4], 2.8 [1.6–4.8], and 3.5 [2.1–5.8], respectively) than in nonatopic subjects for whom none of the associations was significant. Exclusion of workers with a high IgG background reaction to HSA did not alter any of these associations (data not shown).

Glove use during paint-related tasks, which varies among workers, did not affect exposure–response associations in this study. The use of respiratory protection during spray painting is compulsory and was always observed during the fieldwork. Therefore, the effect of respiratory protection could not be investigated.

Physiological Testing

Individuals with asthmalike symptoms were more likely to have BHR (adjusted PR [95% CI], 2.2 [1.5–3.2]). These individuals also had lower baseline FEV_1, FEV_1/FVC, and MMEF between 90 and 96% compared with symptom-free workers. For COPD-like symptoms, the association with BHR was less strong than for asthmalike symptoms and only borderline statistically ($P = 0.07$) significant (adjusted PR [95% CI], 1.6 [1.0–2.5]). In addition,

TABLE 4. ASSOCIATION BETWEEN RESPIRATORY SYMPTOMS AND POSITIVE IgE AND IgG SENSITIZATION*

	COPD-like Symptoms	Asthmalike Symptoms	Work-related Chest Tightness	Work-related Rhinitis	Work-related Conjunctivitis
IgE					
HDI–ImmunoCAP	1.1 (0.3-3.6)	0.8 (0.2-2.5)	1.6 (0.2-10.3)	2.6 (1.4-4.8) †	1.6 (0.5-5.6)
HDI_L–HSA	1.2 (0.6-2.6)	0.9 (0.4-1.9)	1.8 (0.5-6.9)	2.0 (1.1-3.6) †	1.3 (0.4-3.7)
HDI_V–HSA	2.3 (0.9-5.5)	1.6 (0.7-3.7)	4.3 (0.8-23.1)	2.8 (1.1-6.7) †	—‡
N3300–HSA	1.0 (0.3-3.4)	0.7 (0.2-2.4)	1.5 (0.2-10.2)	2.1 (1.0-4.4) †	0.8 (0.1-5.3)
N100–HSA	1.6 (0.9-3.2)	1.1 (0.6-2.2)	3.7 (1.4-9.8) †	1.8 (1.0-3.4) †	1.2 (0.4-3.4)
IgG					
HDI–ImmunoCAP	1.4 (0.8-2.4)	1.2 (0.8-1.9)	0.8 (0.2-3.2)	1.5 (0.8-2.6)	1.0 (0.5-2.2)
HDI_L–HSA	0.9 (0.6-1.2)	1.0 (0.8-1.3)	1.4 (0.7-3.0)	1.4 (0.9-2.0)	1.3 (0.8-2.0)
HDI_V–HSA	0.8 (0.5-1.4)	1.2 (0.9-1.7)	1.2 (0.5-3.0)	1.2 (0.8-2.0)	1.3 (0.7-2.3)
N3300–HSA	1.0 (0.7-1.5)	0.9 (0.7-1.3)	1.0 (0.4-2.3)	1.3 (0.3-1.9)	1.1 (0.7-2.0)
N100–HSA	1.4 (1.0-1.9)†	1.1 (0.8-1.5)	1.7 (0.8-3.5)	1.5 (1.1-2.2)†	1.6 (1.0-2.5)†

For definition of abbreviations, *see* Table 3.

* Prevalence ratio (95% confidence interval) adjusted for age, sex, current smoking, and atopy.

† $P \leq 0.05$.

‡ Too few positives to calculate a prevalence ratio.

none of the lung function parameters was significantly associated with COPD-like symptoms. Individuals with work-related symptoms were more likely to be hyperresponsive, but this was statistically significant only in those with rhinitis symptoms (PRs ≥ 1.8). No clear associations between work-related symptoms and lung function were found.

DISCUSSION

The results of this study provide evidence for exposure–response relationships for exposure to complex mixtures of isocyanates and both work-related and non–work-related respiratory symptoms and specific sensitization.

Exposure to diisocyanate monomers has been assessed in various epidemiologic studies. In the majority of these studies, mean or maximum exposure levels are reported for a population in which a measure of disease frequency is investigated (19–25). However, few studies have considered the issue of quantitative exposure response in isocyanate asthma (26). Two case-control studies demonstrated that higher exposure levels were more likely to be found in companies at which there were workers with a successful claim for occupational asthma (27) or in doctor-diagnosed asthma cases (26) than in control companies or matched control subjects from the same company, respectively. Differences in study design complicate the comparison of these studies with the present study.

TABLE 5. ASSOCIATION BETWEEN RESPIRATORY SYMPTOMS AND SPECIFIC IgE AND IgG SENSITIZATION AND EXPOSURE

	PR (*95% CI*)
Symptoms	
Asthmalike symptoms	1.2 (1.01.5)*
COPD-like symptoms	1.3 (1.0–1.7)*
Work-related chest tightness	2.0 (1.0–3.9)*
Work-related rhinitis	1.3 (0.9–1.7)
Work-related conjunctivitis	1.5 (1.0–2.1)*
Specific IgE	
HDI–ImmunoCAP	2.2 (0.6–8.2)
HDI_L–HSA	1.4 (0.7–3.0)†
HDI_V–HSA	0.6 (0.1–3.9)‡
N3300–HSA	1.8 (0.5–7.2) ‡
N100–HSA	3.0 (1.1–8.4)*§
Specific IgG	
HDI–ImmunoCAP	1.2 (0.71.9)
HDI_L–HSA	1.2 (1.1–1.4)*
HDI_V–HSA	2.2 (1.5–3.4)*
N3300–HSA	1.6 (1.2–2.2)*
N100–HSA	2.0 (1.5–2.6)*

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HDI = hexamethylene diisocyanate; HDI_L–HSA = HDI–HSA liquid phase; HDI_V–HSA = HDI–HSA vapor phase; HSA = human serum albumin.

Shown are prevalence ratios (95% CI) for an interquartile range increase in exposure adjusted for age, sex, smoking, and atopy.

* $P \leq 0.05$.

† Adjusted for age, smoking, and atopy.

‡ Adjusted for age, smoking, and sex.

§ Adjusted for age, sex, and atopy.

The use of product formulations containing complex mixtures of oligomer isocyanates is increasing (4). Currently, oligomers are the major contributor to isocyanate exposure worldwide. Several studies have shown respiratory symptoms or asthma in workers exposed to oligomeric aromatic isocyanates (28–30). Oligomers of aliphatic HDI are widely used in the spray-painting industry. Decreased lung function parameters (31, 32) and high asthma symptom prevalences have been reported in this industry (33–38). Only one study has incorporated exposure assessment. That study demonstrated a relation between peak exposure and reduced lung function in car painters who smoke (32). However, the population size was too small (n = 36) to be conclusive.

This is the first study performed in an end-user industry in which complex exposure patterns of isocyanates were assessed. Over 500 task-based exposure measurements were taken using a state-of-the-art method (12) and used to estimate monthly cumulative personal exposure.

A working day of a spray painter consists of cycles of short tasks, and even exposure during spray painting is highly variable for all workers (12). Therefore, isocyanate exposure in this study consists of a series of peaks, which is highly correlated with average exposure through the duration of the tasks. Consequently, it is not possible to differentiate between cumulative and peak exposure.

Although HDI oligomers were the major exposure factor, product formulations also contained trace amounts of monomeric HDI leading to detectable but very low monomer exposure levels. Personal task-based HDI levels up to 29 $\mu g\ NCO/m^3$ were found, which did not exceed the Dutch short-term exposure limit for HDI (70 $\mu g\ NCO/m^3$). In contrast, HDI oligomer levels ranged up to 3,760 $\mu g\ NCO/m^3$. Therefore, despite the high correlation between oligomer and monomer levels, it seems unlikely that these monomer levels contributed significantly to the observed associations with symptoms.

Animal studies indicate that relative potencies of different isocyanate compounds are variable (39–42). Theoretically, this

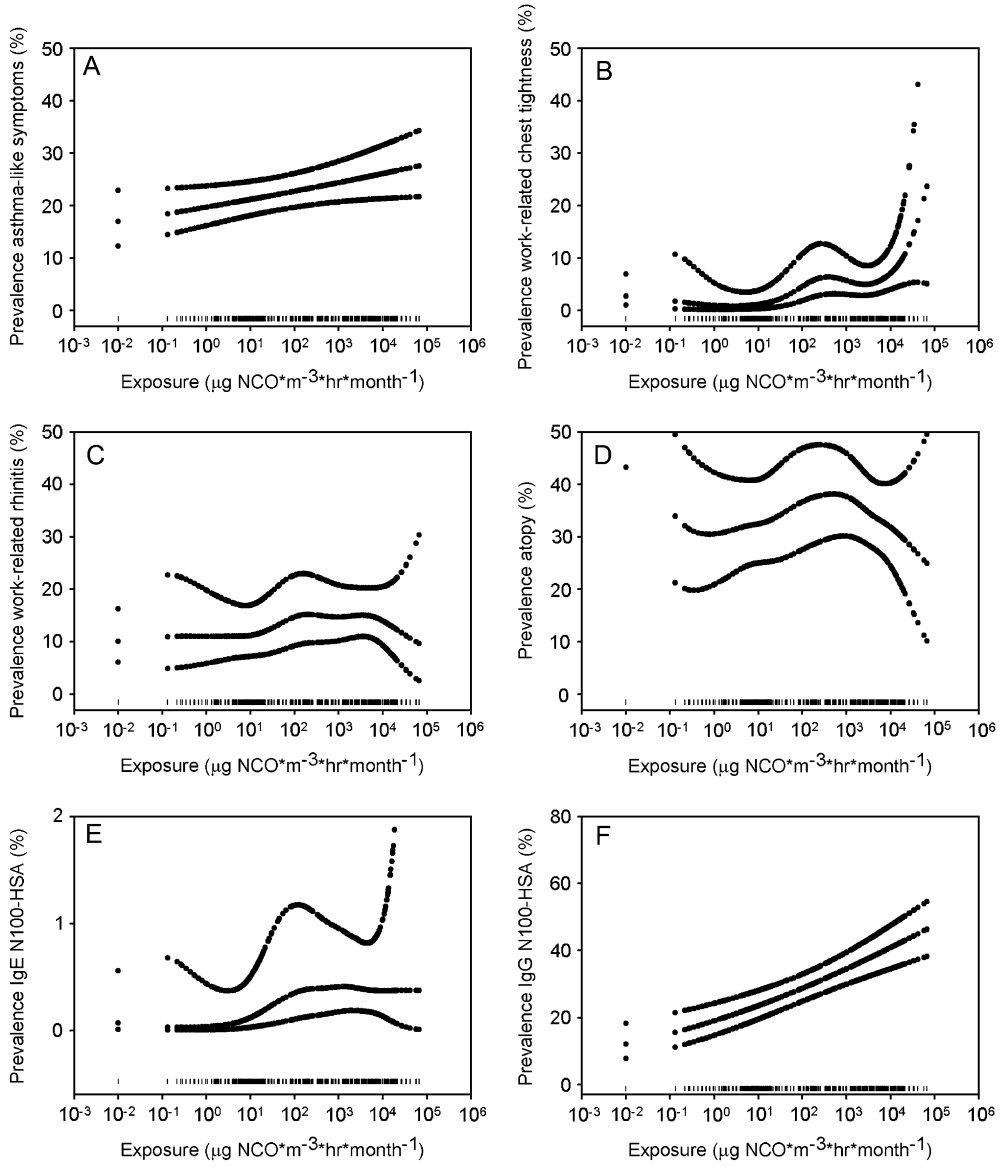

Figure 2. Association between log-transformed exposure to isocyanates (μg NCO × m^{-3} × h × mo^{-1}) and selected health endpoints. Penalized smoothed spline plots are given with smoothed 95% confidence intervals for (*A*) asthmalike symptoms (spline: $P > 0.10$); (*B*) work-related chest tightness (spline: $P \leq 0.05$); (*C*) work-related rhinitis (spline: $P > 0.10$); (*D*) atopy (spline: $P \leq 0.05$); (*E*) IgE N100–HSA (spline: $P > 0.10$); (*F*) IgG N100–HSA (spline: $P > 0.10$). Data rugs at the bottom of each graph indicate the distribution of data points. HSA = human serum albumin.

kind of information might be used to calculate a weighted total NCO concentration. However, for many of the measured isocyanate compounds, this information is not available, which limits the possibilities to use the information on oligomer levels for calculation of overall NCO levels weighted by toxic properties. Moreover, because exposure to HDI and its individual oligomers correlated highly, this would practically only have led to a rescaling of the exposure variable.

The company participation rate of this study was low (10–30%), whereas the mean worker participation rate within the companies of 67% was acceptable. Control measures are very similar among car body repair shops in the Netherlands and spray-booths and ventilation are always present. Yet, working practices may vary, and it cannot be ruled out that more compliant companies were more likely to participate. The negative association between atopy and exposure may point toward another type of selection bias. Possibly, atopic workers are more likely to develop symptoms and leave the industry or atopic workers with preexisting conditions may avoid seeking work as a spray painter. This warrants further attention in follow-up studies because it may result in a healthy worker effect.

Regardless of a possible healthy worker effect, a high prevalence of reported symptoms was noted in spray painters but also in other workers. Positive associations with exposure were found for asthmalike and COPD-like symptoms, work-related chest tightness, and work-related conjunctivitis. Smoothed spline plots corroborated these associations and confirmed that the log-linear models describe the relation with asthmalike symptoms in a satisfactory way. For work-related chest tightness, a steeper increase at high exposure levels was suggested. The surprisingly stronger association for work-related conjunctivitis in nonatopic individuals seems to be explained by the underrepresentation of atopic workers in the highest exposure range.

The significance of asthmalike symptoms found in this study was corroborated by the BHR results and lung function testing. Asthmalike symptoms were associated with BHR and lung function parameters indicative of obstruction. These associations were weaker or did not exist for COPD-like symptoms, indicating that these symptoms may be due to other respiratory conditions.

The low prevalence of specific IgE antibodies in this population of workers who were actively working at the time of the study complicates the assessment of its association with exposure as well as with health effects. Nevertheless, an association between specific IgE to N100–HSA and work-related chest tightness as well as exposure to isocyanates was indicated. The results suggest that, at most, specific IgE plays a role in a minority of

individuals with symptoms. Thus, other mechanisms, like cell-mediated allergic reactions or pulmonary irritation (4, 43), are likely to be involved. The association between IgE to each of the isocyanate conjugates and work-related rhinitis in the absence of a statistically significant association with exposure is remarkable and needs to be further explored.

IgG antibodies are usually considered an effect of exposure. The observed relationship between IgG and exposure can therefore be regarded as an external validation of the exposure assessment in this study. In addition, it shows that, despite the low prevalence of specific IgE, the conjugates used are suitable reagents for the detection of isocyanate-specific immune responses. The significantly stronger association between specific IgG and exposure in atopic subjects, despite their lower exposure levels, suggests that they are immunologically more responsive to isocyanates than nonatopic individuals. A remarkable high prevalence of IgG to HDI_L–HSA was found in office workers. A recent study demonstrated specific IgG to HDI–HSA in 13% of 139 individuals without known exposure to isocyanates (44). Whether specific IgG antibodies to HDI_L–HSA in office workers represents actual exposure needs to be further explored.

Taken together, despite a possible healthy worker effect, exposure–response relationships were demonstrated for respiratory symptoms and sensitization in this population of spray painters exposed mainly to oligomers of HDI. Specific IgG antibodies seem to be primarily a marker of exposure. The association between specific IgE to N100–HSA and symptoms on one hand and exposure on the other hand is suggestive of an IgE-mediated mechanism in only a small proportion of the symptomatic individuals. A more detailed evaluation of immunologic and physiological endpoints is needed to gain insight in the nature of symptoms induced by isocyanates and the role of specific antibodies in this population.

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