Prepolymers of hexamethylene diisocyanate as a cause of occupational asthma

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Background: *Occupational asthma (OA) caused by products that contain hexamethylene diisocyanate (HDI) has been ascribed to the highly volatile monomer of HDI. Most two-component paints are now made up primarily of nonvolatile prepolymers of HDI (30% to 60%) with only trace amounts (<0.1%) of the monomer. The respective role of the two chemical forms of HDI in causing OA has never been investigated.*

Methods: *Twenty workers who were consecutively referred for possible OA that resulted from exposure to spray paints underwent inhalation challenges on separate days with pure HDI monomer and the commercial formulation of HDI prepolymers to which they had been exposed at work.*

Results: *Specific inhalation challenges elicited a positive asthmatic reaction in 10 of the 20 subjects. Among these subjects, four had positive bronchial reactions (two early, one late, and one dual) to both the monomer and the prepolymers. Four other subjects had asthmatic reactions (two early, one late, and one dual) after exposure to the prepolymers but not after exposure to the monomer. The discordance in bronchial response elicited by the monomer and the prepolymers could not be due to differences in the level of baseline nonspecific bronchial reactivity or in HDI concentrations during the tests. One subject showed an atypical progressive reaction after exposure to the monomer but not after exposure to the prepolymer. In this case, the discordant response could be explained by differences in HDI concentration.*

Conclusion: *These observations show that, although they are nonvolatile, the prepolymers of HDI can induce OA and that asthmatic reactions as a result of exposure to prepolymers but not the monomer is not a rare occurrence. (J ALLERGY CLIN IMMUNOL 1993;91:850-61.)*

Key words: *Asthma, occupational diseases, bronchial provocation tests, isocyanates*

Isocyanates, mainly the diisocyanates such as toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), hexamethylene diisocyanate (HDI), and isophorone diisocyanate (IPDI), are used extensively in the production of polyurethane compounds, which have a wide variety of industrial applications.[1] These low molecular weight chemicals can cause occupational asthma (OA) in 5% to 15% of exposed workers,[2, 3] and are currently the principal cause of OA in industrialized countries, accounting for approximately 30% of identified cases.[4, 5]

The monomers of TDI and HDI are highly volatile at ambient temperatures.[1] To reduce respiratory hazards that are due to inhalation of monomer vapors, new types of isocyanates, referred to as prepolymers, have been progressively introduced. They result either from the reaction of a polyhydroxyl compound or water with an excess of diisocyanate molecules or from the self-combination of diisocyanate monomers.[1] The prepolymers have higher molecular weights and are consequently less volatile than their parent monomer. They still contain reactive isocyanate groups, which may be inhaled when generated in an aerosol form during spraying processes.[6, 7] Although they are generally considered to be of a lower toxicity

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Abbreviations used

ELISA: Enzyme-linked immunosorbent assay
FEV_1: Forced expiratory volume in 1 second
HDI: Hexamethylene diisocyanate
HSA: Human serum albumin
Ig: Immunoglobulin
IPDI: Isophorone diisocyanate
MDI: Diphenylmethane diisocyanate
OD: Optical density
PBS: Phosphate-buffered saline
PC20: Concentration of methacholine that induces a 20% decrease in forced expiratory volume in 1 second
ppb: Parts per billion
TDI: Toluene diisocyanate

than monomers, their respiratory effects remain largely unknown. Recent data suggest that long-term exposure to prepolymers could result in decreased lung function in smokers.[8] We recently described two subjects who had asthmatic reactions after challenge exposure to a prepolymer of TDI but not after exposure to the monomer of TDI.[9]

HDI is an aliphatic diisocyanate that is used almost exclusively in the manufacture of paints and surface coatings because the presence of the aliphatic radical provides excellent light and weather stability to the polyurethane end product.[1] Because the HDI monomer is highly volatile, most paints are made predominantly of HDI prepolymers with only a small residual amount of monomer (<1%). The prepolymers include primarily the biuret and the isocyanurate ring (trimer) structures of HDI (Fig. 1). It has been shown that workers can be exposed to high concentrations of airborne prepolymers during spray painting operations.[8, 10] Occupational asthma in workers who are exposed to HDI-based paints has been well documented.[11-17] In a few reports[14-17] prepolymers of HDI were suspected but not definitively demonstrated as being the causal agent because inhalation challenges were not performed[14, 17] or were performed with a paint that contained both the monomer and the prepolymer.[15, 16] Specific antibodies against HDI prepolymers have been found in some workers who were exposed to spray paints, but these antibodies were not closely related to the presence of respiratory symptoms.[17-19] To the best of our knowledge, the distinction between the two chemical forms of HDI in causing OA has never been made, and OA that is due to one but not the other type of HDI has never been described.

The aim of this study was to assess the respective importance of the monomer and the prepolymers of HDI in the development of HDI-induced asthma. Twenty subjects, who were referred consecutively for possible OA caused by spray paints, underwent inhalation challenges with the monomer and prepolymers of HDI on separate days. The subjects' sera were also assessed for the presence of specific antibodies (IgE and IgG) against HDI monomer and HDI prepolymers.

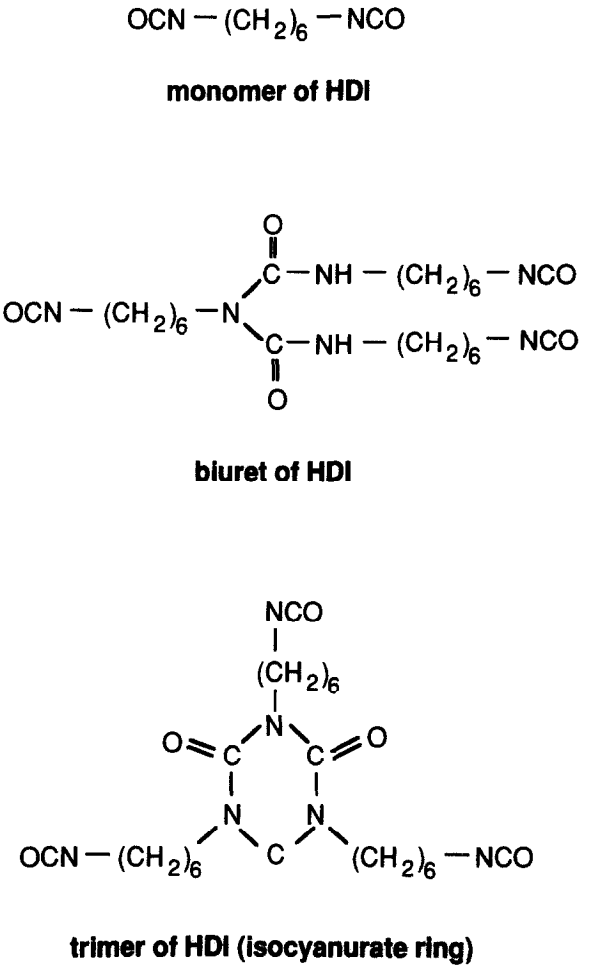

FIG. 1. The chemical structure of the monomer and the principal prepolymers of HDI (i.e., the biuret and the trimer structures). Note that the prepolymers of HDI have three functional isocyanate NCO groups.

METHODS

Subjects

Twenty subjects who underwent inhalation challenges as part of their diagnostic evaluation for possible OA caused by spray paints that contain HDI were included in this study. The data from 11 of the subjects were reported in a previous study in which we validated a recently developed closed-circuit method for inhalation challenges with vapors of isocyanate monomers.[20] The subjects first completed a detailed medical and occupational questionnaire, which was administered by trained physicians, and identified symptoms, which were consistent with asthma and were temporally related to work exposure. Their atopic status was assessed by skin prick testing with 15 common inhalant

TABLE I. Baseline clinical and functional features of the subjects with negative inhalation challenges

No.	Sex	Age	Atopy*	Smoking habits	Duration of exposure (yr)	Duration of symptoms (yr)	Interval of time since last work exposure (mo)
1	M	29	−	Smoker	12.0	0.2	6.0
3	M	54	+	Ex-smoker	20.0	1.0	0.5
4	M	42	−	Smoker	6.0	5.0	1.0
7	M	35	−	Smoker	10.0	8.0	0.2
10	M	29	−	Nonsmoker	15.0	4.0	3.0
11	M	30	+	Nonsmoker	12.0	4.0	3.0
12	F	40	−	Smoker	1.0	0.5	6.0
15	M	58	−	Smoker	15.0	2.0	0.1
17	M	32	−	Smoker	5.0	4.0	0.2
19	M	48	−	Nonsmoker	14.0	3.0	0.1
Mean		39			11.0	3.1	2.0
SD		10			5.6	2.3	2.3

FVC, Forced vial capacity; *SD*, standard deviation.
*Atopy is defined as the presence of one or more positive skin reactions to common inhalent allergens.
†See text for source of predicted values.

TABLE II. Baseline clinical and functional features of the subjects with positive inhalation challenges

No.	Sex	Age	Atopy*	Smoking habits	Duration of exposure (yr)	Duration of symptoms (yr)	Interval of time since last work exposure (mo)
Subjects with positive reactions to the monomer and the prepolymer of HDI (group 2A)							
5	M	48	−	Nonsmoker	36	8	1
6	M	53	+	Ex-smoker	30	5	8
8	M	50	−	Nonsmoker	37	2	2
9	M	29	+	Nonsmoker	11	5	2
20	M	46	+	Ex-smoker	25	3	0.1
Mean		45			27	4.6	2.7
SD		9			10	2.3	3.5
Subjects with positive reactions to the prepolymer of HDI only (group 2B)							
13	M	49	−	Ex-smoker	30	6	5
14	M	36	−	Nonsmoker	18	4	1
16	M	48	−	Ex-smoker	14	7	3
18	M	50	+	Non-smoker	30	6	3
Mean		45			23	5.7	3.0
SD		6			8	1.2	1.6
Subject with positive reaction to the monomer of HDI only (group 2C)							
2	M	36	−	Smoker	0.5	0.2	

FVC, Forced vital capacity.
†See text for source of predicted values.
‡Geometric mean and standard deviation.

allergens. Atopy was defined as the presence of a positive skin reaction to one or more of the allergen extracts.

Ten of the 20 subjects had at least one positive asthmatic reaction during inhalation challenges. The main baseline clinical and functional features are shown in Table I for the subjects with negative challenges (group 1, $n = 10$) and in Table II for subjects with positive challenges (group 2, $n = 10$). There was no significant difference between subjects with positive challenges and those with negative challenges in terms of age, atopic status, duration of work exposure to spray paint, duration of symptoms, and interval of time between last work exposure and inhalation challenges. The proportion of current smokers was significantly lower among subjects with positive challenges (1 of 10) as

FEV_1 (L)	FEV_1 (% predicted value)†	FEV_1/FVC (%)	Baseline methacholine PC20 (mg/ml)
3.7	96	83	>128
2.3	65	72	0.5
3.7	104	87	64.0
3.9	80	83	>128
3.1	77	69	50.0
3.0	68	62	0.2
2.5	73	81	40.0
3.0	86	65	2.3
4.1	89	75	10.5
3.9	110	80	7.8
3.3	84.8	75.7	
0.6	15.0	8.4	

FEV_1 (L)	FEV_1 (% predicted value)†	FEV_1/FVC (%)	Baseline methacholine PC20 (mg/ml)
3.11	86	77	0.25
3.05	89	70	0.85
2.85	99	82	2.25
3.15	80	67	0.22
4.36	108	78	0.25
3.3	92.4	74.8	0.48‡
0.6	11.1	6.1	2.7
3.14	87	67	1.80
3.44	97	73	0.08
3.45	105	74	2.00
2.39	88	69	0.08
3.1	94.2	70.7	0.38‡
0.5	8.4	3.3	6.22
4.34	101	73	11.0

compared with those with negative challenges (6 of 10). The mean of baseline forced expiratory volume in 1 second and the FEV_1/forced vital capacity ratio were within normal limits both in subjects with positive (92.4% ± 9.2% predicted value for FEV_1 and 73.0% ± 4.9% for FEV_1/forced vital capacity) and negative (84.8% ± 15.0% predicted value and 75.7% ± 8.4%) challenges. Surprisingly, a baseline FEV_1 ≤80% predicted was more frequently noted among subjects with negative challenges (5 of 10) than among those with positive challenges (1 of 10) (Fisher's exact test, $p = 0.07$). In contrast, all subjects with positive challenges had significant bronchial hyperresponsiveness to methacholine (concentration of methacholine that induces a 20% decrease in FEV_1 [PC20] ≤16 mg/ml as opposed to only 5 of 19 subjects with negative challenges (Fisher's exact test, $p = 0.01$). The mean interval between the two series of tests was 2.3 ± 2.5 days in subjects with negative reactions and 4.3 ± 3.8 days in subjects with positive reactions.

Functional investigation

Spirometry was performed according to recommended standards[21] on a Vitalograph apparatus (Vitalograph Ltd., Buckingham, England) for the specific inhalation tests and on a Collins spirometer (W.E. Collins Ltd., Braintree, Mass.) for methacholine tests.

Nonspecific bronchial responsiveness to methacholine was assessed with a Wright nebulizer (Aerosol Medical Ltd., Colchester, U.K.) (output = 0.14 L/min) during tidal breathing for 2 minutes according to the procedure outlined by Cockcroft et al.[22] Individual dose-response curves to methacholine were drawn on a semi-logarithmic noncumulative scale.

Specific inhalation challenges

Inhalation challenges were carried out according to a well-standardized protocol.[20, 23-25] Spirometry was assessed before each challenge exposure and reassessed every 10 minutes for the first hour, every 30 minutes for the second hour, and hourly for a total of at least 8 hours after the end of exposure. Prechallenge FEV_1 had to be within ± 10% of the control day value for the subject to continue with the tests.

The following sequence of tests was performed on each subject. On the first day, subjects were exposed to a control product (i.e., the diluent usually mixed with the paint that the subject used at work). The diluents contained various hydrocarbons (such as xylene and toluene) and polyols. The subjects were asked to remain on an 8 m^3 challenge room where the diluent was nebulized for 30 minutes. Fluctuations in FEV_1 during the control day had to be less than 10% of the baseline value. At the end of the day, baseline methacholine PC20 was determined, blood was taken, and the serum was stored at −20° C for subsequent immunologic studies.

On the following days, subjects underwent two series of inhalation tests with HDI. One series of tests included exposure to pure HDI monomer and the other included exposure to prepolymers of HDI (i.e., the paint hardener to which the subject had been exposed at work because pure polymers do not exist). These commercial hardeners are made of a mixture of prepolymers (30% to 60%) and the monomer (<1%). The sequence of challenges was randomized for the first 11 subjects,[20] whereas for the nine remaining subjects, exposure to the monomer was performed first.

TABLE III. Challenge tests: Concentrations of HDI and reactions

	Challenges with the monomer of HDI						
		HDI concentration*					
No.	Duration of exposure (min)	Mean	SD	Range	Prechallenge methacholine PC20 (mg/ml)	Maximum fall in FEV_1†	Pattern of reaction
Subjects with positive reactions to the monomer and the prepolymers of HDI (group 2A)							
5	30	20.7	—	—	0.25	39.9	Dual
6	1	14.0	—	—	0.50	22.6	Immediate
8	5	9.0	—	ND	27.4	Immediate	
9	240	9.3	3.6	4-18	0.75	22.5	Late
20	150	14.4	3.7	6-22	0.25	24.1	Dual
Subjects with positive reactions to the prepolymers of HDI (group 2B)							
13	120	12.9	3.6	9-25	1.8	6.3	—
13							
14	120	14.0	1.6	10-17	0.08	2.9	—
16	120	15.2	3.2	10-26	2.0	8.0	—
18	120	16.7	2.7	14-22	0.08	5.1	—
Subjects with positive reaction to the monomer of HDI (group 2C)							
2	240	17.4	3.1	12-24	11.0	22.5	Progressive

*Isocyanate concentrations measured every 2 minutes during the tests are expressed in parts per billion (ppb); standard deviations are not provided when there are less than 20 concentration values, and range is not expressed when there are less than 3 values.

†The maximum fall in FEV_1 is the lowest value of FEV_1 observed anytime after isocyanate exposure and is expressed in percent of baseline value.

‡The subject was exposed for 5 minutes to a paint hardener that contained HDI and IPDI both in the form of monomers and prepolymers and 5 months later to the pure HDI prepolymer (which was obtained from the manufacturer) for 60 minutes.

Inhalation challenges with the monomer of HDI were performed with the closed-circuit method that we recently designed to obtain stable concentrations of isocyanates.[20] Briefly, vapors of HDI monomer were generated by passing a controlled airflow onto the surface of pure liquid monomer of HDI (Eastman Kodak Company, Rochester, N.Y.) which was contained in a glass flask deposited in a silicon bath at constant temperature. The air that originated from the flask was mixed with a second larger air stream for which flow rate, temperature, and humidity were kept constant (32 L/min, 24° C, and 50%, respectively) with the use of an MNR monitor (model HCS-301, Miller Nelson Research Inc., Monterey, Calif.). The resulting air was sent to a 0.25 m^3 plexiglass cylinder coated with Teflon. The subject inhaled HDI monomer vapors during tidal breathing through an orofacial mask that was connected to the central part of the reservoir. The orofacial mask was supplied with a unidirectional valve so that expired air was evacuated outside of the system. The pressure inside the reservoir was maintained at a constant level even though the subject was breathing through a system of solenoid valves, which regulate an exhaust pump.

Since prepolymers are not volatile at ambient temperatures, exposure to the prepolymers of HDI was performed in an 8 m^3 challenge room, which was adapted from the one used by Pepys and Hutchcroft.[26] The paint hardener, which had been mixed with the diluent to reduce its viscosity, was nebulized with an Intertech 7760-E nebulizer (Trudel Medical, Montreal, Canada). Adequate mixing of the air in the room was ensured by a small fan. The room was supplied with an exhaust ventilation system near the ceiling.

The duration of exposure was progressively increased on separate days, from a total of 1 minute on the first day to 5, 30, 60, and 120 minutes on the following days. Furthermore, each daily exposure was subdivided into shorter periods after which FEV_1 was assessed. After the first series of tests, positive or negative, the methacholine PC20 was reassessed in all subjects with the exception of subjects nos. 8 and 15. When challenges did not induce a decrease in $FEV_1 > 20\%$ of the prechallenge value but a significant decrease in methacholine PC20 was observed, an additional exposure of 240 minutes was included. The second series of challenges was performed only when the FEV_1 and the methacholine PC20 were back to the control day value. During the second series of tests, the subjects were also exposed for progressively longer periods of time, except those who had no asthmatic reaction during the first set of tests. In that case, exposure periods were increased over 1 to 3 days to a total of 120 or 240 minutes. After the second set of tests, methacholine PC20 was reassessed.

HDI concentrations in the cylinder of the closed-circuit apparatus and in the challenge room were assessed every 2 minutes with an MDA 7100 tape monitor (MDA Scientific Inc., Glenview, Ill.). The technicians in charge of the tests were instructed to keep HDI concentrations below the recommended threshold limit value ceiling of 20 parts per billion (ppb).[27, 28] For the closed-circuit method, the tip of the

Challenges with the prepolymers of HDI						
Duration of exposure (min)	HDI concentration*			Prechallenge methacholine PC20 (mg/ml)	Maximum fall in FEV_1†	Pattern of reaction
	Mean	SD	Range			
1	15.0	—	—	0.50	32.3	Dual
0.1	10.0	—	—	0.85	37.3	Immediate
1	12.3	—	—	2.25	38.8	Immediate
240	13.1	7.2	4-36	0.50	28.7	Late
30	16.7	5.9	7-26	0.43	26.6	Progressive
5	11.3	—	8-16	3.4	22.2	Dual‡
60	14.2	4.9	8-22	2.7	32.2	Dual‡
7	15.0	—	12-19	0.5	28.0	Immediate
50	16.0	8.3	5-22	1.0	25.1	Immediate
120	14.9	3.8	9-21	0.125	42.5	Progressive
240	13.0	3.5	7-29	7.20	7.6	

sampling tube of the MDA monitor was connected to the central part of the cylinder. The concentrations of HDI could be modified by regulating the flow of air as it passed through the isocyanate-containing flask. For the tests in the challenge room, the monitor was located in the adjacent room to avoid contaminating the chemical tape. The tip of the sampling tube of the MDA monitor crossed the window of the challenge room and was put at a distance equivalent to that which separated the source of isocyanates and the subject's mouth (approximately 70 cm). The concentration of HDI could be stabilized by regulating the room ventilation and the output of the nebulizer. Assessment of the concentrations of HDI prepolymers would have required the use of chromatographic methods[7] because the MDA 7100 tape monitor has not been validated for measuring concentrations of isocyanate prepolymers. The isocyanate groups on aerosolized HDI prepolymers may react, although to an unknown extent, with the chemical substrate of the tape used in the MDA 7100 monitor. However, chromatographic techniques do not allow for direct visualization of the "instantaneous" concentrations to which the subject is being exposed during the tests. We compared the concentrations of HDI that were recorded by the MDA 7100 tape monitor and by high-performance liquid chromatography during nebulization of a commercial mixture of the monomer and prepolymers of HDI (Du Pont, hardener 793-S, Du Pont Company, Wilmington, Del.).[29] Four samples for 15 minutes each at four different concentrations were realized. We found a satisfactory correlation between the MDA 7100 reading and the high-performance liquid chromatography results ($r = 0.99$). However, the MDA reading underestimated the real values, that is, readings of 5 and 18 ppb, respectively, which corresponded to values of 7.5 and 27 ppb as measured by high-performance liquid chromatography.

Immunologic tests

Preparation of isocyanate protein conjugates. The monomer and prepolymers of HDI were conjugated to human serum albumin (HSA) with a previously described procedure.[19, 24, 30] The HSA was purchased as a 25% solution (American Red Cross Blood Services, Washington, D.C.). The HDI prepolymer was obtained from a commercial mixture that contained HDI monomer (0.7%) and HDI prepolymers (30% to 60%) (Du Pont, hardener 793-S). The mixture was dried under vacuum to further reduce the HDI monomer content. The isocyanates (1 mg/ml HSA) were mixed with the HSA in 7% sodium bicarbonate, stirred at room temperature for 1 hour, dialyzed extensively against phosphate-buffered saline (PBS), and filtered in sterile fashion. As a control, all of the above steps were applied to HSA without the addition of an isocyanate. To confirm that conjugation had occurred, the number of free amino groups present in the conjugates was determined according to the method of Synder and Sobocinski's.[31] Isocyanates react with other chemical groups such as sulfhydryl groups. The degree of ligand binding is an important determinant of antigenicity. Immunoelectrophoresis was also performed on all of the conjugates with Immuno-tec II immunoelectrophoresis plates (Calbiochem-Behring, La Jolla, Calif.) to determine whether conjugation had been achieved, which could be demonstrated by altered mobility of the isocyanate-HSA conjugates.

ELISA. The ELISA procedure was performed with modifications of previously described methods.[32, 33] Briefly, wells of polystyrene Immulon micro-ELISA plates (Greiner & Sons, Nurtingen, Germany) were coated with either HDI monomer (Aldrich Chemical Co., Milwaukee, Wis.) HSA, HDI prepolymer HSA, or HSA alone in carbonate coating buffer, pH 9.6. The plate was then incubated overnight at

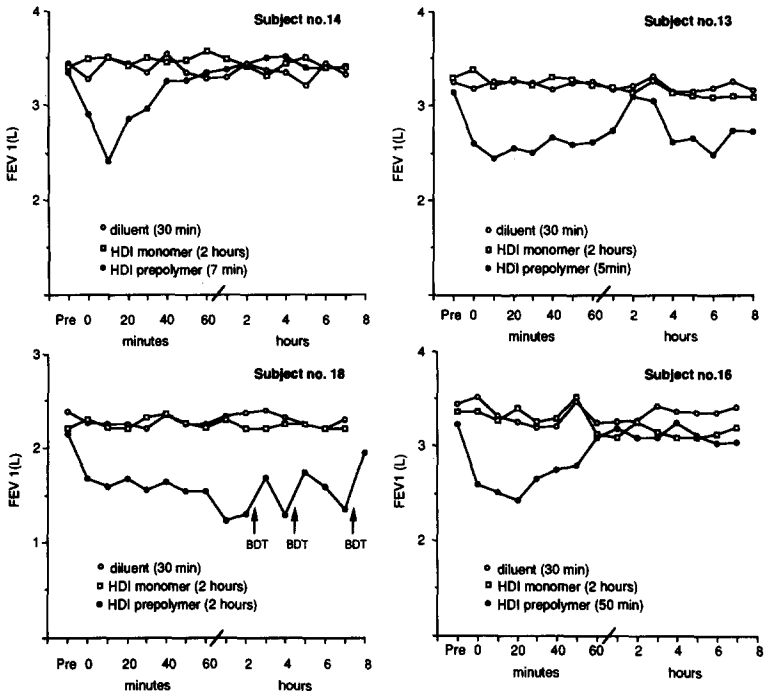

FIG. 2. Changes in FEV_1 among subjects in group 2B after exposure to the control substance (a nebulized paint diluent) *(open circles),* to the vapors of HDI monomer *(open squares),* and to nebulized HDI prepolymers *(closed squares).* In these subjects, exposure to the prepolymers of HDI elicited an asthmatic reaction (two immediate, one dual, and one atypical progressive).

4° C. Appropriate concentrations of reagents and serum dilutions had previously been determined in a checkerboard fashion. All volumes used were 200 μl. After incubation with antigen and between all subsequent layers, the plates were washed three times with PBS that contained 0.05% Tween (Sigma Chemical Company, St. Louis, Mo.). Dilutions of sera in PBS-Tween were added and incubated at 37° C for 1 hour. Rabbit anti-human IgG (Accurate Chemical, Westbury, N.Y.) or goat anti-human IgE (Sigma) diluted 1:1000 in PBS-Tween was added and incubated for 45 minutes at 37° C. Next, goal anti-rabbit IgG or rabbit anti-goat IgG (Sigma) conjugated with alkaline phosphatase was added and incubated for 30 minutes. The last step was the addition of the substrate, p-nitrophenyl phosphate (Sigma phosphatase 104) at a concentration of 1 mg/ml in 10% diethanolamine buffer, pH 9.8. Development was allowed to proceed until the positive control sample reached a predetermined optical density (OD). The OD at 405 nm was read with a Bio-Tek model EL-312 automated ELISA reader (Bio-Tek Instruments, Inc., Winooski, Vt.). The individuals who performed the serologic assays ha no knowledge of the clinical information or challenge status of the subjects.

Analysis of results

Reference values for spirometry were taken from Knudson et al.[34] The concentration of methacholine that caused a 20% decrease in FEV_1 (PC20) was interpolated on the individual dose-response curve. Significant bronchial hyperresponsiveness was defined as a PC20 value <16 mg/ml.[35] Changes in methacholine PC20 of more than 3.2 times from one assessment to the next were considered significant according to the reproducibility of the test in our laboratory.[36] Isocyanate inhalation tests were considered positive when the subject had a sustained decrease in FEV_1 >20% over the prechallenge value, provided that FEV_1 fluctuations were <10% on the control day. The temporal pattern of asthmatic reactions was characterized as immediate, late, dual, or atypical according to previously described criteria.[25, 26] Specific IgE and IgG antibody levels were expressed as an ELISA index calculated by a modification of a previously published formula[30]: OD worker's serum for isocyanate HSA/Mean OD normal control sera for HSA. A serum sample with an ELISA index >2 was considered positive for the presence of specific anti-HDI monomer or anti-HDI prepolymer antibodies. Student's *t* test for paired and unpaired samples, chi square test, and Fisher's exact test were used as appropriate for comparison of variables. Probability *(p)* values <0.05 were considered to be statistically significant.

RESULTS

Inhalation challenges

The results of inhalation challenges in subjects with positive reactions (group 2, $n = 10$) are detailed in Table III. Among the 10 subjects, five had positive bronchial reactions to both HDI monomer and prepolymers (group 2A). The pattern of asthmatic reactions included two early, one late,

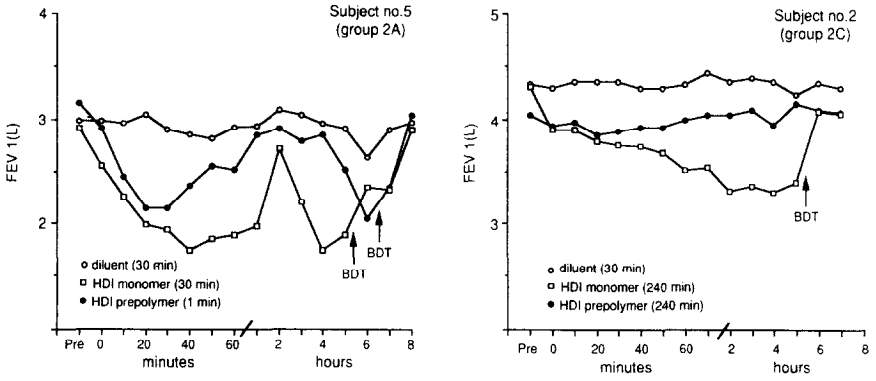

FIG. 3. The pattern of bronchial responses to the different chemical forms of HDI observed in subjects in group 2A ($n = 5$) who reacted to both the monomer and the prepolymers of HDI *(left panel)* and a subject in group 2C (subject no. 2) who had an asthmatic reaction after exposure to the monomer but not after exposure to the prepolymer *(lower panel)*. Subjects in group 2B ($n = 4$) exhibited an asthmatic reaction after exposure to the prepolymers but not after exposure to the monomers (see Fig. 2).

and two dual reactions. Four subjects had asthmatic reactions after exposure to HDI prepolymers but not after exposure to the HDI monomer (group 2B). These results are shown in Fig. 2. Subject no. 13 had a dual reaction after exposure to a paint hardener that contained HDI and IPDI in the form of both monomers and prepolymers. The subject was subsequently challenged with the different types of isocyanates separately. Exposure to the prepolymer of HDI obtained from the manufacturer induced a dual reaction similar to that observed after exposure to the paint hardener, whereas exposure to the vapors of monomeric IPDI and to the nebulized prepolymer of IPDI did not induce any significant bronchial response. Subject no. 4 (group 2C) had an atypical progressive reaction when challenged with HDI monomer but not with HDI prepolymers. The pattern of bronchial response to the different forms of HDI is illustrated for one subject in each group (subject no. 5 for group 2A, subject no 13 for group 2B, and subject no. 2 for group 2C) in Fig. 3.

Although the number of subjects in group 2A ($n = 5$) and 2B ($n = 4$) was too limited for adequate statistical comparison, there was no obvious difference between these two groups regarding their clinical and functional features (Table II). All subjects in groups 2A and 2B had an FEV_1 > 80% of predicted values. The baseline methacholine PC20 was similar in both groups (geometric mean ± geometric standard deviation was 0.48 ± 2.7 mg/ml in group 2A and 0.38 ± 6.22 mg/ml in group 2B).

The mean concentration of HDI during challenges with HDI monomer was slightly higher for subjects with negative reactions (group 1; 14.7 ± 3.15 ppb) as compared with those with positive reactions (group 2B; 11.7 ± 5.7 ppb; $t = 6.23$; $p < 0.001$). Among subjects in group 2, there was no difference in the mean ± standard deviation concentration of HDI during challenges with the HDI monomer (14.7 ± 3.1 ppb) as compared with challenges with HDI prepolymers (15.1 ± 5.3 ppb) ($p > 0.05$). This indicated that the lack of bronchial response to the HDI monomer (group 2B) was not due to lower concentrations of HDI during the tests with the monomer. In subjects in group 2B 17.5% of all 2-minute assessments were above the recommended limit of 20 ppb during challenges with the HDI monomer, a figure that was not significantly different from 15.8% during challenges with HDI prepolymers. This suggests that the bronchial responses to HDI prepolymers, but not to HDI monomers, in subjects in group 2 were not related to exposure to irritant concentrations of HDI. In contrast, for the subject in group 2C, the mean concentration of HDI was significantly higher during the positive test to HDI monomer (17.4 ± 3.1 ppb) than during the negative test to HDI prepolymer (13.0 ± 3.5 ppb; $t = 5.73$; $p < 0.001$). The discordance in bronchial response to the monomer and the prepolymers in this subject could therefore be due to differences in concentration of HDI.

Further inhalation challenges were performed to investigate the factors that could have accounted for the discordance in bronchial response to the HDI monomer and HDI prepolymers. Two of the five subjects in group 2A were first tested with the HDI monomer, whereas the four subjects in group 2B were first challenged with the HDI monomer and subsequently with HDI prepolymers. To determine whether the sequence of the tests could have influenced the bronchial response, two subjects in group 2B (subjects nos. 16 and 18) were rechallenged with the HDI monomer for 240 minutes one and 4 days, respectively, after the positive reaction was elicited by the prepolymers. Their methacholine PC20 was similar to the value noted before exposure to HDI prepolymers. Neither of the subjects had a significant decrease in FEV_1 after these exposures. The HDI monomer was generated in gaseous form with the closed-circuit method.[20] In contrast, the prepolymers of HDI had to be nebulized in the challenge room because they are not volatile. To assess the potential role of the physical state of HDI, subjects nos. 14 and 18 were exposed for 120 minutes to HDI monomer generated in an aerosol form. During these tests, pure HDI monomer was nebulized in

TABLE IV. Immunologic test results

No.	Antibodies to HDI-HSA		Antibodies to HDI resin-HSA	
	IgG index 1:100 dilution	IgE index 1:10 dilution	IgG index 1:100 dilution	IgE index 1:10 dilution
Subjects with negative challenges				
1	Neg	Neg	Neg	Neg
3	2.4	Neg	2.2	Neg
4	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg
17	ND	ND	ND	ND
19	8.2	Neg	5.9	2.3
Subjects with positive reactions to the monomer and the prepolymer of HDI (group 2A)				
5	10.4	2.1	6.6	2.6
6	Neg	Neg	Neg	Neg
8	12.6	2.6	7.4	2.3
9	Neg	Neg	Neg	Neg
20	7.1	Neg	3.3	Neg
Subjects with positive reactions to the prepolymer of HDI only (group 2B)				
13	Neg	Neg	Neg	Neg
14	2.8	Neg	2.1	Neg
16	2.6	2.1	Neg	Neg
18	Neg	Neg	Neg	Neg
Subject with positive reaction to the monomer of HDI only (group 2C)				
2	Neg	Neg	Neg	Neg

An index <2 is considered negative.
ND, Not done.

the challenge room with the same procedure as that used for HDI prepolymers. The tests were performed 4 and 15 days, respectively, after the positive reaction was elicited by the prepolymers at a time when the subject's methacholine PC20 was similar to the value observed before the positive challenge with HDI prepolymers. These exposures to the nebulized HDI monomer did not induce significant changes in FEV_1.

Immunologic tests

Serum samples of 19 of the 20 subjects were available. The results are listed in Table IV. Of the 10 subjects with negative challenges, one had a positive IgG antibody and one had both positive IgG and IgE antibody. Of the five subjects with positive challenges to both monomer and prepolymer, one had positive IgG and two had both positive IgG and IgE. Of the five subjects who had positive challenges to only prepolymer or monomer, one had a positive IgG and one had both positive IgG and IgE.

Of the six subjects who had positive challenges with an immediate component to their response (nos. 5, 6, 8, 13, 14, and 16), three had both IgG and IgE, and two had IgG. Of the four subjects without an immediate response (nos. 2, 9, 18, and 20), only one had IgG, and none had IgE. There was a very good correlation between the results of specific IgG and IgE to HDI resin and to HDI.

DISCUSSION

Although the prepolymers of HDI are now extensively used in the manufacture of polyurethane compounds, particularly paints and surface coatings, their role in the development of OA caused by products that contain HDI has never been specifically documented. We previously described two cases of OA that were due to the prepolymer but not to the monomer of TDI.[9] However, OA that is caused by prepolymers of HDI has not been described to our knowledge, and the relevance of this finding stems from the fact that HDI is currently more commonly used that TDI. The consequences of exposure to the prepolymers are difficult to assess because prepolymers do not exist in a pure form. The prepolymers of HDI always contain a small amount of residual monomer, usually less than 1%. Furthermore, the prepolymers are in fact a mixture of several different chemical

structures (Fig. 1), the proportions of which may vary considerably from one commercial product to the next. In this study we compared bronchial responses after separate exposures to pure monomer of HDI and to the commercial formulation of HDI prepolymers to which the subjects had been exposed at work. Four subjects had an asthmatic reaction after exposure to the prepolymers but not after exposure to the monomer. In theory, several factors could have accounted for the observed differences in bronchial response to the monomer and the prepolymers. First, the subjects could have been exposed to higher concentrations of HDI during the challenges with the prepolymers than during those with the monomer. Assessment of HDI levels with the MDA 7100 tape monitor during the tests showed no difference in the mean concentration or in the proportion of concentrations above the recommended threshold limit value ceiling of 20 ppb. Furthermore, we found that the concentrations of HDI measured by the tape monitor were similar to those obtained by a chromatographic method. Second, the negative challenges with the monomer could have induced a progressive increase in specific or nonspecific bronchial responsiveness, which would lead to positive reactions during the subsequent challenges with the prepolymers. There was no significant difference in the value of the methacholine PC20 assessed before the two series of inhalation challenges. Furthermore, in two subjects rechallenge with the monomer 1 and 10 days after a positive reaction to the prepolymer did not elicit a significant bronchial response. Third, the physical state of HDI, that is, vapor for the monomer and aerosol for the prepolymers, could have influenced the bronchial responses. Two subjects were challenged with the nebulized monomer and did not have any asthmatic reaction. It is, however, our impression that the physical state of isocyanates (gas or vapor as opposed to aerosol) is not a determinant of the bronchial response. In a recent study we showed that five of six subjects demonstrated similar asthmatic reactions after exposure to the monomer of isocyanate generated as a gas through a closed-circuit device and after exposure to the isocyanate generated as an aerosol.[20] So, although one could not exclude entirely the possibility that subjects with negative challenge to HDI but positive challenge with prepolymers might have responsed to HDI delivered through the aerosol or adsorbed in the prepolymer particles, there are several lines of evidence that indicate that the discordance in the reactions induced by the monomer and the prepolymers of HDI was actually due to a difference in bronchial reactivity to the two types of HDI.

The pathogenesis of isocyanate-induced asthma remains controversial.[2, 3, 37] It seems that at least two reactive NCO isocyanate groups are required to cause asthma,[38] a feature shared by both the monomer and prepolymers of HDI. The existence of an antibody response is supported by the presence, at least in some subgroups of affected workers, of specific antibodies (IgE or IgG) against isocyanates.[24, 39, 40] Immunologic[15, 30, 41] and asthmatic[13, 15, 42] cross-reactivity between different diisocyanate monomers has been demonstrated. This may be related to the fact that isocyanate antibodies are directed not only against the isocyanate hapten but also against new antigenic determinants, which are altered portions of the carrier molecule that result from the interaction of highly reactive isocyanates with homologous proteins. Though the number of subjects is small, there is some evidence from our studies that the observed differences in bronchial response are due to differences in antibody-mediated sensitization. Of those 10 subjects with negative challenges, only one (10%) had specific IgG antibody and one (10%) had specific IgE antibody. Of those six workers who had positive challenges with an immediate component, five (83%) had antibody: three (50%) with both IgE and IgG and two (33%) with only IgG. In contrast, of the four subjects with no immediate component, only one (25%) had IgG and none had IgE. These results suggest the hypothesis that immediate or dual bronchial responses are associated with antibody response, whereas negative or delayed responses are not. The testing of this hypothesis will require more intense study with a larger number of subjects.

It is also interesting that a recent work published in abstract form documented that products of the reaction between TDI and water (including toluene diammine, biuret, and possibly polymers) maintain the same ability of the monomer to contract guinea pig airway smooth muscle in vitro.[43] These findings could support data presented in this work, which show that TDI-related products can cause occupational asthma.

Our observations are relevant to the diagnostic investigation of isocyanate-induced asthma. If inhalation tests are required to establish the diagnosis, the subjects should be challenged with the type of isocyanate to which they were exposed at work. We recently developed a closed-circuit method for inhalation tests with isocyanates, which makes it possible to have more accurate control over the level of exposure and therefore the magnitude of the induced asthmatic reaction.[20] To achieve standardization of the tests the method was designed to produce vapors of isocyanates generated from the pure monomers. The results of this study demonstrate that the closed-circuit

method should be further developed to make it possible to generate controlled levels of isocyanate prepolymers. Our findings also raise questions about workplace hygiene. The fact that prepolymers of isocyanates are capable of causing asthma indicates that exposure limits should be established not only for isocyanate monomers but for all isocyanates regardless of their chemical form, as has already been done in some countries.[7, 8]

In conclusion, we demonstrated that some workers may have asthmatic reactions after exposure to prepolymers of HDI but not after exposure to the corresponding monomer. Furthermore, our study suggests that such elective reactions are not a rare occurrence because four of 10 subjects with HDI-induced asthma reacted to the prepolymer form of HDI only. The prepolymers of isocyanates should definitely be considered as a potential cause of OA.

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