

## Airway inflammation and functional changes after exposure to different concentrations of isocyanates

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**Background:** Isocyanates are a common cause of occupational asthma (OA).

**Objectives:** We sought (1) to examine whether asthmatic reactions to isocyanates could be induced at concentrations as low as 1 ppb in subjects with OA caused by isocyanates previously diagnosed in our center and (2) to compare the inflammatory and functional changes after exposure to 1 and 15 ppb of isocyanates with similar total doses (concentration of isocyanates  $\times$  duration of exposure).

**Methods:** Specific inhalation challenges were performed in 12 asthmatic subjects with previously confirmed OA caused by isocyanates. Eight subjects were exposed to 1 ppb at 10% of the dose of isocyanates that induced an asthmatic reaction at the time of the diagnosis. Seven subjects were exposed to the same total dose of isocyanates by using concentrations of 1 and 15 ppb 1 month apart.

**Results:** Exposure to 1 ppb at 10% of the dose that had induced functional changes at the time of diagnosis induced an asthmatic reaction in 3 of 8 subjects. There was a significant correlation between the percentage of maximum decrease in FEV<sub>1</sub> after exposure to 1 ppb and the increase in sputum neutrophils ( $\rho = 0.8$ ). By keeping the total dose (concentration of isocyanates  $\times$  duration of exposure) of isocyanates similar, 4 of 7 subjects experienced an asthmatic reaction after exposure to 1 ppb, whereas only one subject experienced an 18.5% decrease in FEV<sub>1</sub> after exposure to 15 ppb.

**Conclusion:** Isocyanates can induce functional and inflammatory changes (mainly neutrophilic) at concentrations as low as 1 ppb. For the same total dose of exposure, low concentrations of isocyanates are as harmful as or even more harmful than higher concentrations for subjects with OA to isocyanates. (*J Allergy Clin Immunol* 2002;110:641-6.)

**Key words:** Occupational asthma, specific inhalation challenges, airway inflammation, neutrophils

### Abbreviations used

GMD: Gas-monitoring device  
OA: Occupational asthma  
SIC: Specific inhalation challenge

Diisocyanates are widely used and represent the leading cause of occupational asthma (OA). Lately, industries have tried to reduce the concentrations of isocyanates in the workplace to prevent sensitization and occurrence of OA. In Canada the threshold value short-term exposure limit for isocyanates has been reduced from 20 to 5 ppb. We have previously shown that inflammatory changes preceded the onset of functional changes after exposure to occupational agents in subjects with OA.<sup>1</sup> However, we do not know whether such changes in airway inflammation can be induced by low doses of isocyanates (1 ppb) in subjects who are already sensitized to isocyanates and have OA caused by isocyanates.

The airway inflammation induced by exposure to isocyanates is variable. Some authors have shown that exposure to isocyanates can induce sputum eosinophilia,<sup>2</sup> whereas others have more recently shown sputum neutrophilia.<sup>3</sup> The determinants favoring airway neutrophilia or eosinophilia after exposure to isocyanates or other occupational agents are unknown.

In this study we sought to investigate whether exposure to low concentrations of isocyanates (1 ppb) was able to induce asthmatic reactions in subjects with OA caused by isocyanates. We also wanted to assess the type of airway inflammation induced by these exposures and whether a low concentration of exposure (1 ppb) was equivalent to a high concentration (15 ppb) of isocyanates in inducing airway inflammation and asthmatic reactions for the same total dose of exposure (concentration of isocyanates  $\times$  duration of exposure) in subjects with OA caused by isocyanates who were already sensitized to this agent.

## METHODS

### Subjects

We recruited subjects with OA caused by isocyanates previously diagnosed by means of specific inhalation challenges (SICs) in our center, in whom the dose of occupational agent necessary to induce an asthmatic reaction had been determined at the time of the SIC. These subjects (1) had not experienced a severe asthmatic

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reaction ( $\geq 50\%$  decrease in  $FEV_1$ ) after the earlier exposure, (2) had an  $FEV_1$  value of greater than 2 L with well-controlled asthma, (3) did not have significant comorbidity, and (4) were willing to participate in the study. The subjects were no longer exposed to isocyanates and had not been exposed to these agents since they underwent the specific challenge to isocyanates at the time of the diagnosis. The subjects who were treated with inhaled corticosteroids took their medication at bedtime only. Long-acting  $\beta_2$ -agonists were stopped 72 hours before starting the SIC, whereas short-acting  $\beta_2$ -agonists were stopped 12 hours before the SIC.

The study was approved by a research ethics committee of Sacre-Coeur Hospital, and all subjects provided written informed consent.

## Study design

The study comprised 2 parts. The first part was designed to investigate whether low concentrations of isocyanates (1 ppb) could induce asthmatic reactions, airway inflammation, or both. On the first day (control day), the subjects were given a questionnaire regarding their respiratory symptoms, smoking habits, and current treatment for asthma. Their  $FEV_1$  values were monitored for 7 hours to ensure the stability of their asthma. Methacholine challenge followed by sputum induction were performed at the end of the day. On day 2, the next day, subjects were exposed to 1 ppb at 10% of the total dose of isocyanates that induced an asthmatic reaction at the time of the diagnosis. At the time of the diagnosis, all subjects were exposed to isocyanates by using a closed-circuit apparatus, which allowed us to deliver and monitor a predetermined concentration of isocyanates. We were therefore able to calculate the total dose of isocyanates to which they were exposed by calculating the product of the concentration of isocyanates delivered by the duration of exposure. If no reaction occurred on day 2, the subjects were exposed to 15 ppb on day 3, either until a reaction occurred or for a maximum duration of 2 hours. Methacholine inhalation challenge followed by sputum induction was performed 7 hours after the end of exposure.

The second part of the study was set to verify whether low concentrations of isocyanates (1 ppb) could induce asthmatic reactions and airway inflammation in the same proportion of subjects as exposure to a higher isocyanate concentration (15 ppb) for the same total dose of isocyanates delivered. Two SICs were performed 1 month apart in subjects with OA to isocyanates who previously received a diagnosis in our center. The first SIC was performed by using a concentration of 1 ppb until the occurrence of a 20% decrease in  $FEV_1$  or for a maximum of 120 minutes. This challenge was preceded by a control day, as described above. During the second SIC 1 month later, subjects were exposed to the same total dose (concentration  $\times$  duration of exposure) of isocyanates by using a concentration of 15 ppb. The exposure to isocyanates was also preceded by a control day. Seven hours after the end of exposure, methacholine challenge followed by sputum induction was performed.

## Procedures

Spirometry was performed according to American Thoracic Society standards.<sup>4</sup> Methacholine challenge tests were performed as previously described.<sup>5</sup> Sputum was induced by using inhalations of increasing concentrations (3%, 4%, and 5%) of hypertonic saline<sup>6</sup> and processed as previously described.<sup>7</sup>

## Specific inhalation challenges

SICs were performed as previously described.<sup>8</sup> On the control day, spirometry was performed with a water-filled spirometer (Collins W.E. Collins Inc).  $FEV_1$  was measured every 10 minutes for the first hour, every 30 minutes for the second hour, and then hourly for a total of 7 hours to ensure that there was no significant fluctua-

tion. On subsequent days, isocyanate exposure was carried out by using a closed-circuit apparatus described previously and online monitoring of isocyanate concentrations (see below).<sup>9</sup> No modification of the apparatus was required to generate isocyanates as an aerosol at concentrations of close to 1 ppb. However, to generate stable low concentrations of isocyanates as vapor, the generator of isocyanates was slightly modified. First, the outlet of the stainless-steel inlet tube of the generator was covered with a diffusor (20  $\mu$ m, Dionex Corp). Then an insert of foam (2 cm  $\times$  2 cm  $\times$  2 cm) was forced to the bottom of the 17  $\times$  60-mm vial that is usually fitted in the generator. The latter piece of foam was then covered with the isocyanates. The role of the foam insert is to prevent the surface oscillation of the liquid poured into the vial. Airflow on the order of 0.2 to 0.4 L/min was then circulated through the generator containing the isocyanates and mixed with the 50 L/min nominal airflow of the exposure chamber. Isocyanate concentration in the exposure chamber was assessed with a gas-monitoring device (GMD) systems instrument (model: Remote Intelligent Sensor). All GMD readings were calibrated by comparison with HPLC readings used as the standard when the generation of isocyanate was carried out for 3 to 4 hours. In general, GMD readings were twice the actual concentrations of isocyanates, namely by means of HPLC assessments:  $1.2 \pm 0.1$  (SD) ppb ( $n = 12$ ) in the case of toluene diisocyanate and  $1.2 \pm 0.2$  (SD) ppb ( $n = 8$ ) in the case of hexamethylene diisocyanate (Julie Paradis, Jacques Lesage, and Yves Cloutier, personal communication). The correspondence between GMD readings and isocyanate assessments by means of HPLC is linear over the range of concentrations used in our protocol for all types of isocyanates.

An SIC result was considered positive if there was a decrease in  $FEV_1$  of 20% or greater. Methacholine challenge and sputum induction were performed at the end of each day. Significant changes in  $PC_{20}$  were set at a 3.2-fold difference.<sup>10</sup>

## Skin testing

Skin tests were performed with commercial extracts from common inhalants (trees, weeds, ragweed, house dust mites, cockroach, *Aspergillus* species, *Alternaria* species, *Cladosporium* species, cat, and dog; Omega). A positive reaction was set at a wheal diameter of 3 mm or greater in the absence of reaction to a control diluent and with a positive reaction to histamine phosphate (1 mg/mL). A subject was considered atopic if at least one skin prick test response to common inhalants was positive.

## Analysis of results

Results were expressed as means and SD, except for data with nonnormal distribution, which were expressed as medians and interquartile ranges. Paired  $t$  tests and the Wilcoxon signed-rank test were used to compare  $PC_{20}$  and cell counts, respectively, before and after exposure to isocyanates. Correlations between differences in sputum neutrophils before and after exposure and maximum decreases in  $FEV_1$  after exposure were examined with the Spearman rank order test. We considered an increase in sputum eosinophils and neutrophils after exposure to isocyanates as being clinically significant if there was an increase in eosinophils of at least  $0.26 \times 10^6$  and an increase in sputum neutrophils of at least  $0.64 \times 10^6$  neutrophils.<sup>11</sup> Significance was accepted at the level of 95%. The analysis was performed by using the SPSS 10.0 statistical package.

## RESULTS Subjects

Eight subjects were enrolled in the first part of the study, and 7 subjects took part in the second part of the study (3 subjects who were enrolled in the first part also

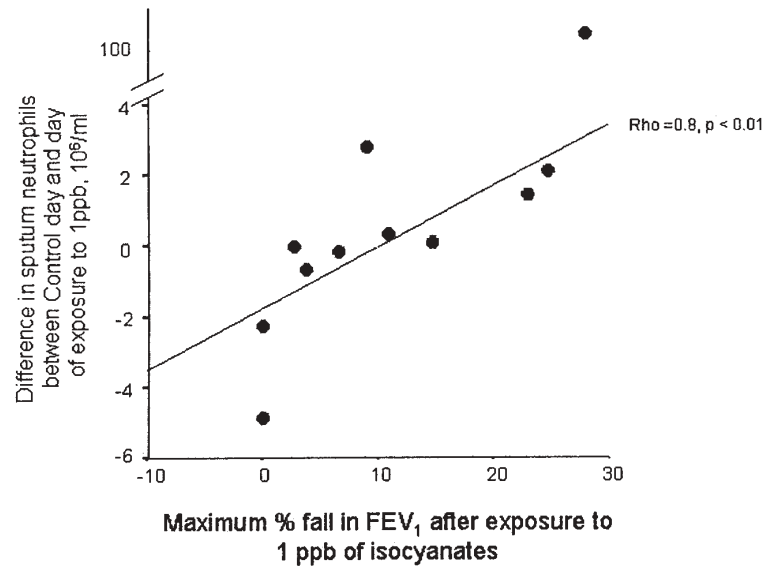


**FIG 1.** Correlation between changes in sputum neutrophil counts and maximum percentage decrease in FEV<sub>1</sub> after exposure to 1 ppb of isocyanates.

**TABLE I.** Characteristics of the subjects

| No. | Sex | Age (y)         | Smoking | Atopy | Agent | Duration of exposure (y) | Duration since last exposure (y) | Treatment          | FEV <sub>1</sub> (% predicted) |
|-----|-----|-----------------|---------|-------|-------|--------------------------|----------------------------------|--------------------|--------------------------------|
| 1   | M   | 58              | ExS     | NA    | MDI   | 0.6                      | 4.0                              | ICS, $\beta_2$ -A  | 95.0                           |
| 2   | M   | 61              | ExS     | A     | HDI   | 28.0                     | 1.0                              | ICS, $\beta_2$ -A  | 81.7                           |
| 3   | F   | 41              | No      | NA    | HDI   | 1.6                      | 1.0                              | ICS, $\beta_2$ -A  | 64.1                           |
| 4   | M   | 33              | S       | A     | HDI   | 2.1                      | 1.0                              | $\beta_2$ -A       | 71.5                           |
| 5   | M   | 49              | ExS     | A     | TDI   | 17.0                     | 1.0                              | $\beta_2$ -A       | 78.8                           |
| 6   | M   | 39              | S       | A     | HDI   | 0.6                      | 8.0                              | $\beta_2$ -A       | 85.3                           |
| 7   | M   | 57              | S       | NA    | TDI   | 10.0                     | 8.0                              | $\beta_2$ -A       | 72.1                           |
| 8   | M   | 74              | S       | A     | TDI   | 20.0                     | 10.0                             | ICS, $\beta_2$ -A  | 104.1                          |
| 9   | M   | 68              | S       | NA    | HDI   | 35.0                     | 4.0                              | $\beta_2$ -A       | 84.8                           |
| 10  | M   | 45              | S       | NA    | TDI   | 1.3                      | 0.08                             | $\beta_2$ -A       | 74.6                           |
| 11  | M   | 50              | ExS     | NA    | HDI   | 30.0                     | 0.2                              | None               | 112.3                          |
| 12  | M   | 39              | ExS     | A     | MDI   | 0.5                      | 3.0                              | ICS, $\beta_2$ -LA | 91.9                           |
|     |     | 50.3 $\pm$ 12.8 |         |       |       | 8.1 $\pm$ 11.4           | 4.0 $\pm$ 3.5                    |                    | 84.7 $\pm$ 14.1                |

ExS, Exsmoker; NA, nonatopic; MDI, methylene diphenyldiisocyanate; ICS, inhaled corticosteroids;  $\beta_2$ -A, short-acting  $\beta_2$ -agonists; A, atopic; HDI, hexamethylene diisocyanate; No, never smoked; S, current smoker; TDI, toluene diisocyanate;  $\beta_2$ -LA, long-acting  $\beta_2$ -agonists.

participated in the second part). Six subjects had OA caused by hexamethylene diisocyanate, 4 had OA caused by toluene diisocyanate, and 2 had OA caused by methylene diphenyldiisocyanate. They had not been exposed to isocyanates for an average of  $4.0 \pm 3.5$  years. The characteristics of the subjects are presented in Table I.

### First part of the study

Exposure to a concentration of 1 ppb and to a dose as low as  $10.7\%$  ( $47 \pm 37.3$  ppb  $\cdot$  min) of the total dose of isocyanates ( $371.3 \pm 361.1$  ppb  $\cdot$  min) that had induced an asthmatic reaction at the time of diagnosis caused an asthmatic reaction in 3 subjects (2 late reactions and 1 dual reaction in subjects 1 and 5 and 6, respectively, in Table II). These subjects were not exposed to 15 ppb during this part of the study. They had an increase in their

sputum neutrophil count, and 2 (subjects 1 and 5) of them had an increase in both sputum eosinophil and neutrophil counts compared with that on the control day.

Five subjects were subsequently exposed to 15 ppb, for a total dosage of  $579 \pm 535.3$  ppb  $\cdot$  min. All of them experienced at least a 20% decrease in FEV<sub>1</sub> after exposure to 15 ppb. One (subject 7) of them had neutrophilic inflammation, whereas another (subjects 3) had both sputum neutrophilia and eosinophilia.

There was a significant correlation between the percentage of maximum decrease in FEV<sub>1</sub> after exposure to 1 ppb and the increase in sputum neutrophil count ( $\rho = 0.8$ ,  $P < .001$ , Figure 1). This analysis was performed by considering the 11 subjects who were exposed to 1 ppb. We did not find such a correlation between the percentage decrease in FEV<sub>1</sub> and sputum eosinophil count.

**TABLE II.** Changes in sputum cell counts and airway responsiveness after exposure to 1 and 15 ppb of isocyanates at different doses of exposure

| Subject no. | Control                     |                                      |                                      | 1 ppb                       |                               |                                      |                                      |
|-------------|-----------------------------|--------------------------------------|--------------------------------------|-----------------------------|-------------------------------|--------------------------------------|--------------------------------------|
|             | PC <sub>20</sub><br>(mg/mL) | Eosinophils<br>(10 <sup>6</sup> /mL) | Neutrophils<br>(10 <sup>6</sup> /mL) | PC <sub>20</sub><br>(mg/mL) | Duration of<br>exposure (min) | Eosinophils<br>(10 <sup>6</sup> /mL) | Neutrophils<br>(10 <sup>6</sup> /mL) |
| 1           | 1.4                         | 0.03                                 | 2.7                                  | ND                          | 30.0                          | 0.6                                  | 111.8                                |
| 2           | 0.7                         | 0.5                                  | 18.0                                 | 0.7                         | 18.0                          | 0.5                                  | 15.8                                 |
| 3           | 0.03                        | 0.03                                 | 0.9                                  | 0.06                        | 60.0                          | 0.03                                 | 0.3                                  |
| 4           | S                           | 0.4                                  | 0.4                                  | 0.04                        | 120.0                         | 0.04                                 | 0.8                                  |
| 5           | 1.2                         | 0.03                                 | 1.4                                  | ND                          | 45.0                          | 0.7                                  | 2.8                                  |
| 6           | 4.4                         | 0.002                                | 0.2                                  | ND                          | 30.0                          | 0.0                                  | 3.3                                  |
| 7           | 0.3                         | 0.01                                 | 0.5                                  | 0.2                         | 6.0                           | 0.002                                | 0.3                                  |
| 8           | 1.0                         | 0.0                                  | 0.5                                  | 1.5                         | 6.0                           | 0.0                                  | 0.5                                  |
|             | 0.2 (0.2)                   | 0.03 (0.3)                           | 0.7 (1.4)                            | 20.2 (0.2)                  | 39.4 ± 37.4                   | 0.04 (0.6)                           | 1.6 (12.1)                           |

ND, Not done; L, late reaction; N, no reaction; I, immediate reaction; S, 20% decrease in FEV<sub>1</sub> after administration of normal saline during methacholine challenge; D, dual reaction.

**TABLE III.** Changes in sputum cell counts and airway responsiveness to methacholine after exposure to one and 15 ppb of isocyanates with the same total dose of exposure

| Subject no. | Control                     |                                      |                                      |                             | 1 ppb                         |                                      |                                      |                                               |
|-------------|-----------------------------|--------------------------------------|--------------------------------------|-----------------------------|-------------------------------|--------------------------------------|--------------------------------------|-----------------------------------------------|
|             | PC <sub>20</sub><br>(mg/mL) | Eosinophils<br>(10 <sup>6</sup> /mL) | Neutrophils<br>(10 <sup>6</sup> /mL) | PC <sub>20</sub><br>(mg/mL) | Duration of<br>exposure (min) | Eosinophils<br>(10 <sup>6</sup> /mL) | Neutrophils<br>(10 <sup>6</sup> /mL) | Reaction (%<br>decrease in FEV <sub>1</sub> ) |
| 1           | 1.4                         | 0.03                                 | 2.7                                  | ND                          | 30.0                          | 0.6                                  | 111.8                                | L (28.5)                                      |
| 5           | 1.2                         | 0.03                                 | 1.4                                  | ND                          | 45.0                          | 0.7                                  | 2.8                                  | L (23.0)                                      |
| 6           | 4.4                         | 0.002                                | 0.2                                  | ND                          | 30.0                          | 0.009                                | 2.4                                  | D (24.7)                                      |
| 9           | 0.3                         | 0.01                                 | 0.6                                  | 0.2                         | 120.0                         | 0.0                                  | 3.3                                  | N (9.0)                                       |
| 10          | 12.5                        | 0.01                                 | 5.2                                  | 5.1                         | 120.0                         | 0.04                                 | 0.14                                 | N (0.0)                                       |
| 11          | 17.5                        | 0.04                                 | 0.5                                  | 6.2                         | 120.0                         | 0.05                                 | 0.6                                  | N (14.7)                                      |
| 12          | 1.0                         | 0.0                                  | 2.4                                  | ND                          | 30.0                          | ND                                   | ND                                   | D (46.4)                                      |
|             | 2.5 (1.9)                   | 0.02 (0.02)                          | 1.0 (2.9)                            | 1.8 (1.5)                   | 60 ± 41.5                     | 0.03 (0.6)                           | 2.6 (29.9)                           | 20.9 ± 14.9                                   |

ND, Not done; L, late reaction; N, no reaction; I, immediate reaction; S, 20% decrease in FEV<sub>1</sub> after administration of normal saline during methacholine challenge; D, dual reaction.

## Second part of the study

Seven subjects took part in the second phase of the study. One subject (subject 12) did not produce enough sputum for a reliable analysis. Three subjects who were enrolled in the first part of the study also participated in the second part. For these subjects, the control day and the exposure at 1 ppb were not repeated in the second part of the study, and the data from the first part of the study were kept. The second exposure to 15 ppb, as well as the second control day, were performed 1 month after the first SIC.

The total dose of exposure was estimated by calculating the product of the concentration of isocyanates delivered and the duration of exposure. The respiratory rate and the tidal volume were monitored but recorded in only 4 subjects (subjects 1, 5, 6, and 7). The data obtained in these subjects were similar for exposure at both 1 and 15 ppb.

Four subjects experienced an asthmatic reaction (late reactions in 2 subjects [subjects 1 and 5] and dual reactions in 2 subjects [subjects 6 and 12] after exposure to 1 ppb at a total dose of isocyanates of  $60.7 \pm 40.9$  ppb/min), whereas only one subject experienced an immediate 18.5% decrease in FEV<sub>1</sub> after exposure to 15 ppb at the total dose of  $66.4 \pm 42.3$  ppb · min (subject 9 in Table III). This subject only showed a 9% decrease in

FEV<sub>1</sub> after exposure to 1 ppb. Two subjects (subjects 10 and 11) did not experience a 20% decrease in FEV<sub>1</sub> either at 1 or 15 ppb. However, one of them (subject 11) had a 15% decrease in FEV<sub>1</sub> at both 1 and 15 ppb. Overall, there was a greater decrease in FEV<sub>1</sub> after exposure to 1 ppb ( $20.9 \pm 14.9\%$ ) than after exposure to 15 ppb ( $8.6 \pm 7.6\%$ ,  $P < .05$ ).

Significant changes in neutrophil counts occurred in 4 subjects without significant changes in functional parameters (FEV<sub>1</sub> and PC<sub>20</sub>, subject 9 at 1 ppb and subjects 1, 5, and 10 at 15 ppb); 2 subjects also had an increase in their sputum eosinophil counts (subjects 5 and 10 at 15 ppb). Increases in neutrophil counts occurred in all subjects who experienced an asthmatic reaction; 2 subjects (subjects 1 and 5 at one ppb) also had an increase in sputum eosinophil counts compared with those on the control day.

## DISCUSSION

This study shows that (1) exposure to isocyanates at 1 ppb can induce significant functional and inflammatory changes, (2) the inflammatory changes can precede the changes in airway caliber, and (3) isocyanates can induce both eosinophilic and neutrophilic inflammation, but neutrophilic inflammation seems to be more common.

| 1 ppb                                      |                          | 15 ppb                     |                                   |                                   |                                            |
|--------------------------------------------|--------------------------|----------------------------|-----------------------------------|-----------------------------------|--------------------------------------------|
| Reaction (% decrease in FEV <sub>1</sub> ) | PC <sub>20</sub> (mg/mL) | Duration of exposure (min) | Eosinophils (10 <sup>6</sup> /mL) | Neutrophils (10 <sup>6</sup> /mL) | Reaction (% decrease in FEV <sub>1</sub> ) |
| L (28.5)                                   |                          |                            |                                   |                                   |                                            |
| N (0.0)                                    | 0.8                      | 12.0                       | 0.8                               | 11.4                              | I (20.0)                                   |
| N (6.3)                                    | ND                       | 90.0                       | 5.5                               | 8.2                               | L (35.0)                                   |
| N (10.9)                                   | 0.1                      | 5.0                        | 0.06                              | 0.7                               | I (21.9)                                   |
| L (23.0)                                   |                          |                            |                                   |                                   |                                            |
| D (20.0)                                   |                          |                            |                                   |                                   |                                            |
| N (6.6)                                    | ND                       | 60.0                       | 0.03                              | 0.7                               | L (33.8)                                   |
| N (2.7)                                    | 0.07                     | 26.0                       | 0.004                             | 0.9                               | I (23.3)                                   |
|                                            | 0.2 (0.1)                | 25.0 ± 32.8                | 0.06 (3.1)                        | 0.9 (9.1)                         |                                            |

| Control                  |                                   |                                   | 15 ppb                   |                            |                                   |                                   |                                            |
|--------------------------|-----------------------------------|-----------------------------------|--------------------------|----------------------------|-----------------------------------|-----------------------------------|--------------------------------------------|
| PC <sub>20</sub> (mg/mL) | Eosinophils (10 <sup>6</sup> /mL) | Neutrophils (10 <sup>6</sup> /mL) | PC <sub>20</sub> (mg/mL) | Duration of exposure (min) | Eosinophils (10 <sup>6</sup> /mL) | Neutrophils (10 <sup>6</sup> /mL) | Reaction (% decrease in FEV <sub>1</sub> ) |
| 52.0                     | 0.01                              | 1.1                               | 5.8                      | 2.0                        | 0.0                               | 31.5                              | N (0.0)                                    |
| 1.4                      | 0.01                              | 5.4                               | 1.2                      | 3.0                        | 0.2                               | 8.8                               | N (1.6)                                    |
| 23.0                     | 0.0                               | 0.2                               | 4.2                      | 2.0                        | 0.04                              | 0.4                               | N (14.8)                                   |
| 0.14                     | 0.002                             | 0.3                               | 0.06                     | 6.0                        | 0.07                              | 7.0                               | N (18.5)                                   |
| 18.0                     | 0.2                               | 3.5                               | 12.5                     | 8.0                        | 0.6                               | 8.6                               | N (3.7)                                    |
| 5.8*                     | 0.07                              | 1.5                               | 5.6                      | 8.0                        | 0.01                              | 1.4                               | N (15.6)                                   |
| 0.7                      | 0.0                               | 1.3                               | S                        | 2.0                        | ND                                | ND                                | N (2.8)                                    |
| 4.6 (3.9)                | 0.04 (0.1)                        | 1.3 (3.4)                         | 0.5*(0.5)                | 4.4 ± 2.8                  | 0.04 (0.3)                        | 7.8* (13.3)                       | 8.6 ± 7.6*                                 |

Threshold limit values for short-term exposure to isocyanates were set at a concentration of 20 ppb and are currently at 5 ppb.<sup>12</sup> However, these concentrations represent the threshold at which irritant effects on the mucosa are unlikely to occur. They do not represent concentrations at which the risk for development of sensitization to isocyanates is unlikely to occur, nor do they represent concentrations at which already sensitized individuals are unlikely to react. There are indeed cases of subjects who describe asthmatic symptoms on exposure to apparently low concentrations of isocyanates, causing the investigator to suspect that they can react to low concentrations on laboratory exposure. In these individuals it is therefore justified to consider exposure to even lower concentrations than the 5- or 15-ppb threshold previously suggested for such testing.<sup>9,13</sup>

Ten of the 12 subjects enrolled in the studies showed a significant reaction to isocyanate challenges (subjects 9 and 11 demonstrated a borderline reaction), which confirms a satisfactory reproducibility of testing, even if these tests were carried out an average of 4 years after the diagnosis was confirmed, with similar testing being used.<sup>14</sup> Some participants (subjects 1, 2, and 3 in Table II) reacted to far lower doses of isocyanates when concentrations of 1 ppb were used than when they were exposed to high-

er concentrations, both in comparison with the time of diagnosis 2 or more years before and within the time scheme of our protocol. We would indeed have expected to have had to administer a similar or even higher total dose of isocyanate to induce a similar reaction. We previously showed that 9 (60%) of 15 subjects showed an improvement in specific bronchial responsiveness to an occupational agent when they were exposed 2 or more years after initial testing, therefore requiring an increase in the dose to elicit an asthmatic reaction documented by changes in FEV<sub>1</sub> and or PC<sub>20</sub>.<sup>14</sup> Among the 9 subjects described in this former publication, there were 4 cases of OA caused by isocyanates. Moreover, in the second part of our protocol, 4 subjects showed a reaction when they were exposed to 1 ppb but not when they were exposed to 15 ppb. The reasons for this enhanced specific reactivity at low concentrations are hypothetical. We do not think that it can be explained by technical factors, such as variability of concentrations obtained. As shown in the "Methods" section, the SDs around the average isocyanate concentrations obtained were narrow. We know that to mediate their effects, inhaled isocyanates are coupled to human proteins, forming isocyanate-protein adducts.<sup>15,16</sup> It is possible that these adducts are either more readily produced, more biologically active, or both

in terms of inducing the inflammatory asthmatic cascade if the exposure to isocyanates occurs at lower concentrations for a longer period of time.

Inflammatory changes documented in induced sputum were detected by exposing individuals at concentrations of 15 ppb and, interestingly, 1 ppb. We<sup>17</sup> and others<sup>18</sup> previously suggested the use of induced sputum in the investigation of OA. We more recently showed that eosinophils can increase in induced sputum on the day before functional changes significantly occur.<sup>1</sup> This was shown in subjects exposed to high- and low-molecular-weight occupational agents, including 3 exposed to isocyanates. Fabbri et al<sup>19</sup> originally described an influx of neutrophils in the bronchoalveolar lavage fluid of asthmatic subjects who experienced a late reaction to isocyanates. In the current study we predominantly observed an increase in sputum neutrophils, with a combined increase in sputum eosinophils in a few cases. These changes occurred in some subjects before they experienced functional changes or, almost invariably, on the day functional changes occurred. Neutrophilia can therefore precede the occurrence of functional changes after exposure to isocyanates. Various types of cells, including neutrophils, are involved in the pathogenesis of asthma.<sup>20</sup> Therefore as in asthma, neutrophils seem to be involved in the pathophysiology of OA and more specifically in the pathophysiology of OA caused by isocyanates. The underlying mechanisms of the inflammatory changes after exposure to isocyanates need to be further explored.

It has been suggested that subjects with OA caused by latex can be maintained in an environment with low concentrations of latex without significant worsening of their asthma.<sup>21</sup> Although our study has not been performed in the workplace, the results show that exposure to low concentrations of isocyanates is as harmful as or even more harmful than exposure to higher concentrations for subjects with documented OA to isocyanates. Therefore contrary to the situation for latex, lowering the concentrations of isocyanates at the workplace is unlikely to allow subjects with OA to isocyanates to remain at work without a substantial risk of worsening their asthma.

In conclusion, OA caused by isocyanates seems to be enhanced when isocyanates are generated at low concentrations for a long period of time compared with when they are generated at a higher concentration for a shorter duration of exposure. The mechanism for this remains to be explored, as does the detailed mechanism of neutrophil participation in this disease.

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