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Pulmonary effects of short-term exposure to low levels of toluene diisocyanate in asymptomatic subjects

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ABSTRACT: Isocyanates may be involved in the development of chronic obstructive airway disease among exposed workers. A short-term exposure to toluene diisocyanate (TDI) at concentrations near the permissible levels was investigated to examine whether there was an association with changes in pulmonary function tests and in potential markers of airway injury and inflammation in bronchial lavage (BL) and bronchoalveolar lavage (BAL).

Seventeen subjects without respiratory symptoms (eight smokers and nine nonsmokers) were exposed once to ambient air and once to TDI (5 parts per billion (ppb) for 6 h followed by 20 ppb for 20 min) in a randomized, single-blind sequence. Pulmonary function tests were repeatedly assessed during exposure and BAL was performed 1 h after each exposure. Biochemical studies on lavage fluids included albumin, immunoglobulins, antiproteases (α_2-macroglobulin and α_1-proteinase inhibitor), potential indicators of epithelial cell function (secretory component and Clara cell protein), and cytokines (tumour necrosis factor-α, interleukin (IL)-4, IL-5, IL-6, and IL-8).

Exposure to TDI caused a modest decrease in specific airway conductance (sG_{aw}) (p=0.053) and in maximal expiratory flow at 25% of forced vital capacity ($MEF_{25\%}$) (p=0.015) when compared with ambient air. Exposure to TDI resulted in a slight increase in BAL albumin level (TDI: 26.4±12.5 *versus* air: 21.8±8.6 $\mu g \cdot mL^{-1}$, p=0.044) and in BL α_2-macroglobulin concentration (TDI: 0.07±0.061 *versus* air: 0.05±0.04 $\mu g \cdot mL^{-1}$, p=0.021).

This study suggests that exposure to low toluene disocyanate concentrations is associated with minimal but detectable changes in airway calibre and in epithelial barrier permeability. The pulmonary effects of long-term exposure to low levels of isocyanates require further investigation.

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Isocyanates are characterized by the presence of highly reactive N=C=O groups. These chemicals are used in the production of polyurethane polymers, which have a wide variety of applications in the manufacture of flexible and rigid foams, elastomers, adhesives, and surface coatings [1]. The most commonly used diisocyanate monomers include toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI) and hexamethylene diisocyanate (HDI).

Isocyanates can cause occupational asthma and hypersensitivity-pneumonitis-like reactions in exposed workers, presumably through immunologically-mediated mechanisms [2]. However, there is epidemiological evidence that workplace exposure to isocyanates could lead to the development of obstructive airway disease through nonimmunological mechanisms [1]. A number of investigators have documented across-shift changes in airway calibre among workers exposed to isocyanates [1]. Several longitudinal studies have demonstrated an accelerated decline in forced expiratory volume in one second (FEV_1) in TDI-exposed workers [3–5]. Several studies [5–7] failed, however, to detect adverse respiratory effects in workers exposed to TDI levels below the recommended threshold limit values (TLV) of 5 parts per billion (ppb) as an 8-h time-weighted average (TWA) and 20 ppb as a 10-min short-term exposure limit (STEL) [1, 8].

The aim of this controlled exposure study was to investigate the short-term effects of TDI levels near the permissible exposure limits on pulmonary function tests. The study also examined whether exposure to low levels of TDI was associated with airway injury and inflammation by examining the cellular and biochemical changes in the airways, as assessed by bronchoalveolar lavage (BAL).

Subjects and methods

Subjects

Seventeen volunteers (eight males and nine females) without occupational exposure to isocyanates were recruited for this study. Inclusion in the study protocol was based on the absence of respiratory symptoms suggestive

of asthma and chronic bronchitis, and a normal physical examination. The participants included nine lifetime nonsmokers and eight current smokers with a mean smoking history of 9 pack-yrs (range 2–25 pack-yrs). The mean age of the subjects was 35 yrs (range 19–51 yrs). Nonspecific bronchial responsiveness was assessed by inhaling doubling concentrations of histamine (0.03–16 $mg \cdot mL^{-1}$) at tidal breathing for 2-min periods according to the method described by COCKCROFT *et al.* [9]. The level of bronchial responsiveness to histamine was expressed as the concentration of histamine causing a 20% fall in forced expiratory volume in one second (FEV_1) (PC_{20}). Histamine PC_{20} values were not taken into account in the selection of the subjects and remained blinded to the investigators until the analysis of results. The participants were informed of the purpose and procedures of the study and signed a statement of informed consent. The study protocol and consent form were approved by the Ethics Committee of the institute.

Exposure control

The subjects were randomly exposed to ambient air and TDI (5 ppb for 6 h followed by 20 ppb for 20 min) in a single-blind crossover design. At least 4 weeks separated the two exposure events. Exposure was postponed if the subject presented with an acute respiratory infection within the previous 4 weeks. The exposure protocol started at 09:00 h (±15 min). Exposures were performed in a 5 m^3 chamber designed to carry out inhalation challenges for diagnostic purposes in subjects with possible isocyanate-induced occupational asthma [2]. TDI was generated by passing air over the surface of pure liquid TDI (80% 2,4-TDI and 20% 2,6-TDI isomers) (Merck, Hohenbrunn, Germany) contained in a glass flask. TDI concentrations were measured continuously using a MDA Model 7100 tape monitor (MDA Scientific Inc, Glenview, IL, USA), which displays a concentration value every 2 min. The tip of the sampling tube was located at a distance of ~50 cm from the subject′s mouth. Optical calibration of the MDA 7100 tape monitor was performed before each exposure. The concentration of TDI in the exposure chamber was maintained at the target level by regulating the exhaust ventilation and/or the flow of air passing over the pure liquid TDI. The MDA readings were used to calculate the mean actual concentration during each exposure period. The mean (SD) of the actual values of TDI recorded in the chamber was 4.2 (0.4) ppb when the target concentration was 5 ppb and 19.6 (3.7) ppb when it was 20 ppb. Exposure to ambient air was obtained using the same equipment and protocol, with the exception that liquid TDI was replaced by water.

Pulmonary function measurements

Forced vital capacity (FVC), FEV_1, FEV_1/FVC ratio, and maximal expiratory flow at 50% ($MEF_{50\%}$) and 25% of FVC ($MEF_{25\%}$) were assessed with a pneumotachograph (Medisoft, Dinant, Belgium) according to the standards of the American Thoracic Society [10]. Specific airway conductance (sG_{aw}), functional residual capacity (FRC) and total lung capacity (TLC) were measured in a constant-volume body plethysmograph (Medisoft) [11]. The mean value of sG_{aw} was derived from three successive measurements and was used for the analysis. Predicted values for pulmonary function parameters were derived from the European Community for Coal and Steel standard tables [12].

At the beginning of each study day, the subjects underwent a complete series of pulmonary function tests, including measurement (in sequence) of sG_{aw}, FRC, TLC, FVC, FEV_1, $MEF_{50\%}$ and $MEF_{25\%}$. Pulmonary function tests were reassessed every hour during the 6-h exposure to 5 ppb TDI or ambient air and repeated after completion of the 20-min exposure period to 20 ppb TDI or air. Venous blood was collected before and 1 h after the end of each exposure for cellular and biochemical studies.

Bronchoscopy and lavage procedure

Bronchoscopy and BAL were performed 1 h after the end of exposure as previously described [13]. The subjects were premedicated with 0.5 mg atropine. After topical anaesthesia of the upper respiratory tract with 4% lidocaine, the fibreoptic bronchoscope was wedged in a subsegment of the right middle lobe or lingula. Four 50-mL aliquots of sterile 0.9% saline solution at 37°C were instilled through the bronchoscope channel. After each instillation, the fluid was gently aspirated with a syringe, collected in siliconized glass flasks and kept on ice throughout the procedure. The first recovered aliquot was considered a bronchial lavage (BL) sample and was analysed separately from a pool of the following three aliquots. Immediately after the end of BAL procedure, the recovered fluid was filtered through a layer of gauze to remove gross mucus and an aliquot was saved for total cell count with a Coulter FN cell counter (Courter, Miami, FL, USA). Lavage fluid was centrifuged at $500 \times g$ for 10 min and the cell pellet was used in lymphocyte subpopulation studies, while the supernatant was kept at -80°C for biochemical analyses.

Cellular studies

Cell differentials were performed on cytospin preparations using the same centrifuge (Cytospin Shandon Elliot, Runcorn, UK). Smears for differential counts were stained with May–Grünwald-Giemsa stain (Merck, Darmstadt, Germany) and at least 400 cells were counted on stained slides. The lymphocyte subpopulations in blood and lavage samples were determined using flow cytometry (Facscalibur, Becton-Dickinson, Mountain View, CA, USA) in a two-colour direct immunofluorescence assay. Lymphocyte subsets in BL samples could be determined in only eight subjects after both exposures, since the number of lymphocytes was too small to allow subpopulation analysis on all lavages samples. Monoclonal antibodies used in phenotyping studies included the following: anti-CD2 (pan-human T-cells), anti-CD3 (pan-T-cells), anti-CD4 (T-helper cells), anti-CD8 (T-cytotoxic/suppressor cells), anti-CD19 (B-cells), and anti-CD56 (natural killer (NK) cells and cytotoxic T-cells) (Becton-Dickinson). The reagents were used according to the manufacturer′s instructions.

Measurement of biochemical constituents in lavage fluids

Biochemical constituents were assessed on unconcentrated BAL and BL fluids. The levels of albumin, immunoglobulin (Ig)G, IgA, IgM, secretory component of IgA, α_2-macroglobulin, and α_1-proteinase inhibitor were measured by an immunoradiometric assay (IRMA). This assay provides a sensitivity in the ng·mL^{-1} range [14]. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of tumour necrosis factor (TNF)-α (Cytoscreen US, BioSource International Inc, Camarillo, CA, USA), interleukin (IL)-4 (Cytoscreen US), IL-5 (Cytoscreen US), IL-6 (Cytoscreen US) and IL-8 (EASIA, Medgenix Diagnostics, Fleurus, Belgium). The lower detection limit of these assays was 0.115 pg·mL^{-1}, 2 pg·mL^{-1}, <4 pg·mL^{-1}, 0.104 pg·mL^{-1}, and 0.7 pg·mL^{-1}, respectively. The concentration of Clara cell protein (CC16) was measured by an immunoassay technique based on the agglutination of latex particles [15, 16]. The concentrations of IL-5, IL-6, IL-8 and CC16 were also determined on blood samples collected before and after each exposure.

Analysis of results

Pre-exposure pulmonary function tests were compared using paired Student's t-tests. Changes in pulmonary function tests measured throughout the exposure periods were assessed by regression analysis for repeated measures using generalized estimating equations (GEE) that included as covariates exposure (TDI or air), time of measurement, and the pre-exposure value [17, 18]. The role of potential determinants of the response to TDI was further assessed using the difference between TDI and air in pulmonary function tests as the response variable. The covariates of this analysis included the mean actual concentration of TDI (target concentration of 5 ppb *versus* 20 ppb), the cumulative dose of TDI at each functional assessment, smoking status (smoker *versus* nonsmoker), and the level of nonspecific bronchial responsiveness to histamine (log PC20 value). The changes from before-to-after exposure in blood parameters were analysed using two-way analysis of variance (ANOVA). Cell counts and protein concentrations in BL and BAL after exposure to ambient air and TDI were compared using the Wilcoxon signed rank test. A p-value ≤ 0.05 was considered significant.

Results

Pulmonary function tests

The characteristics of the subjects are summarized in table 1. The results of pulmonary function tests conducted before and at the end of exposure to ambient air and TDI are presented in table 2. No significant differences in pre-exposure values was observed between the two exposures. None of the subjects experienced significant respiratory symptoms in response to the exposures. Regression analysis of repeated measures using GEE demonstrated that exposure to TDI resulted in a slight decrease in sG_{aw} (β= -0.574, SE=0.297, p=0.053) and MEF25% (β= -0.152, SE=0.063, p=0.015). The mean±SEM values of sG_{aw} and MEF25% recorded during exposure to air and TDI are illustrated in figures 1 and 2. Multivariate regression analysis of the time-point differences in sG_{aw} showed that the mean concentration of TDI was a significant determinant of the response (β= -0.027, SE=0.013, p=0.044), while the level of nonspecific responsiveness to histamine had a significant effect on changes in MEF25% induced by TDI exposure (β= -0.066, SE=0.029, p=0.022).

Table 1. – Characteristics of the subjects

Subject No.	Sex	Age yrs	Height cm	FVC L	FVC % pred	FEV1 L	FEV1 % pred	FEV1/FVC (%)	Histamine PC20 mg·mL^{-1}
Nonsmokers									
2	F	38	162	3.87	118	2.98	105	77	>16
3	F	35	169	4.39	125	3.26	102	74	1.3
4	F	45	168	4.53	134	3.72	128	82	2.3
5	F	51	165	3.65	114	2.65	100	73	3.3
6	F	34	162	4.24	121	3.23	109	76	9.4
7	F	46	165	3.55	111	2.70	98	76	6.3
8	M	33	178	5.82	110	4.59	109	79	>16
10	M	46	182	5.92	120	4.55	114	77	8.8
12	F	38	158	3.04	104	2.71	101	89	>16
Smokers									
1	M	33	170	4.53	101	3.41	88	75	6.0
9	M	19	182	4.62	79	4.00	84	87	3.5
11	M	21	185	5.82	104	4.66	96	80	6.1
13	F	47	178	4.18	109	3.01	92	72	5.2
14	M	28	180	5.47	100	4.63	104	85	4.0
15	F	28	172	4.41	105	3.23	92	73	2.4
16	M	32	175	4.41	94	3.76	92	85	2.9
17	M	25	183	6.29	110	4.92	106	78	3.6

FVC: forced vital capacity; FEV1: forced expiratory volume in one second; PC20: concentration of histamine causing a 20% fall in FEV1; M: male; F: female.

Table 2. – Pre-exposure and post-exposure values of pulmonary function test

	Ambient Air		TDI	
	Pre	Post	Pre	Post
FVC L	4.63±0.90	4.62±0.92	4.65±0.88	4.58±0.90
FEV_1 L	3.63±0.80	3.63±0.79	3.63±0.78	3.59±0.75
FEV_1/FVC %	78±6	79±6	78±5	78±5
$MEF_{50\%}$ $L \cdot s^{-1}$	4.43±1.36	4.38±1.34	4.32±1.36	4.43±1.15
$MEF_{25\%}$ $L \cdot s^{-1}$	1.75±0.83	1.82±0.84	1.72±0.95	1.74±0.72
sG_{aw} $kPa^{-1} \cdot s^{-1}$	3.75±1.87	4.45±2.48	3.79±2.29	3.38±1.91
FRC L	3.53±0.99	3.41±0.98	3.41±0.83	3.42±0.89
TLC L	6.47±1.28	6.37±1.29	6.32±1.20	6.42±1.11

TDI: toluene diisocyanate; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; $MEF_{50\%}$: maximal expiratory flow at 50% of FVC; $MEF_{25\%}$: MEF at 25% of FVC; sG_{aw}: specific airway conductance; FRC: functional residual capacity; TLC: total lung capacity.

Cell populations in BL and BAL

There were no significant differences in the volume of fluid recovered and total and differential cell counts in BL and BAL after exposure to TDI and ambient air (table 3). However, analysis of lymphocyte subpopulations showed that exposure to TDI was associated with a decrease in the proportion of CD19 cells both in BL (TDI: 0.8±0.7 *versus* air: 1.6±0.7%; p=0.063) and in BAL (TDI: 0.7±0.7% *versus* air: 1.4±1.4%; p=0.036), although absolute numbers of CD19 cells were not significantly modified.

Biochemical constituents in lavage fluids

TNF-α and IL-4 were not detected in unconcentrated lavage fluids. The results of the other biochemical constituents in BL and BAL are presented in table 4. Exposure to TDI was associated with a slight but significant increase in BAL albumin levels (26.4±12.5 $\mu g \cdot mL^{-1}$) as compared with exposure to ambient air (21.8±8.6 $\mu g \cdot mL^{-1}$, p=0.044). The concentration of α_2-macroglobulin in BL was higher after exposure to TDI (0.07±0.06 $\mu g \cdot mL^{-1}$) than after exposure to ambient air (0.05±0.04 $\mu g \cdot mL^{-1}$, p=0.021). When the results of biochemical constituents were normalized to albumin concentration in lavage fluids, the α_2-macroglobulin/albumin ratio in BL remained higher after TDI exposure (0.0019±0.0016) than after air exposure (0.0012±0.0010, p=0.036). No changes in white blood cells or in blood protein levels were observed in response to TDI exposure.

Discussion

Epidemiological surveys have documented asymptomatic decreases in FEV_1 and expiratory flow rates at low pulmonary volumes during the course of a work-shift among workers exposed to TDI [3, 19]. The functional changes were dose-related, although determination of exposure levels was based mainly on job categories and area samplings for short periods [3, 19]. In an uncontrolled exposure protocol, VOGELMEIER *et al.* [20] found a >50% decrease in sG_{aw} in one of 10 nonasthmatic subjects and in three of 19 subjects with asthma unrelated to isocyanates who were exposed for 2 h to 10–20 ppb of TDI. The present controlled exposure study confirmed that TDI levels near the permissible exposure limits can cause a reduction in airway calibre, as evidence by modest, but significant, decreases in sG_{aw} and expiratory flow rate at low pulmonary volumes ($MEF_{25\%}$). It remains uncertain why the detected effect of TDI was limited to these two indices, which are assumed to reflect changes

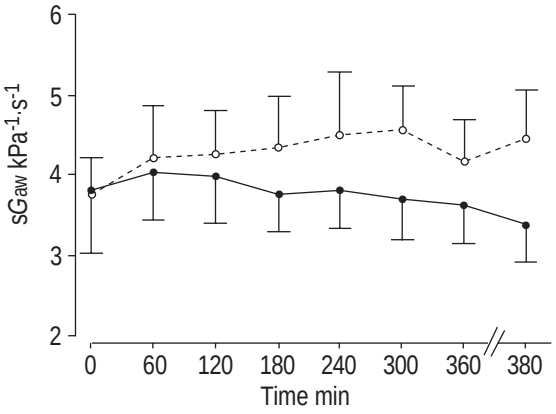

Fig. 1. – Mean±SEM values of specific conductance (sG_{aw}) during exposure to ambient air (- -○- -) and toluene diisocyanate (—●—) for 370 min (5 parts per billion (ppb) for 360 min and 20 ppb for 10 min).

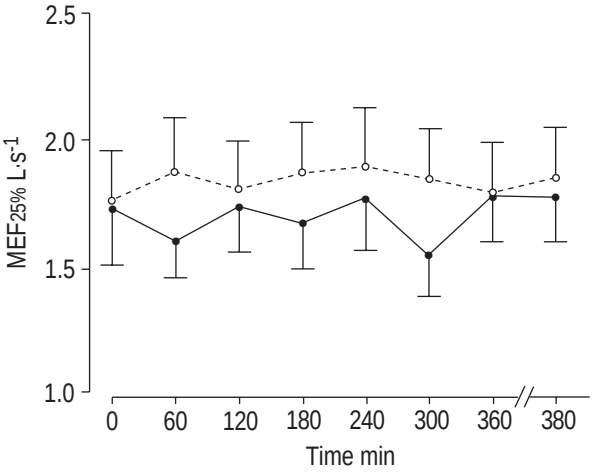

Fig. 2. – Mean±SEM values of maximal expiratory flow at 25% of forced vital capacity ($MEF_{25\%}$) during exposure to ambient air (- -○- -) and toluene diisocyanate (—●—) for 370 min (5 parts per billion (ppb) for 360 min and 20 ppb for 10 min).

Table 3. – Cell populations in bronchial and bronchoalveolar lavage

	Bronchial lavage		Bronchoalveolar lavage	
	Ambient air	TDI	Ambient air	TDI
Volume mL	17.8±5.8	17.1±7.5	123±17	121±15
Total cells $10^3 \cdot mL^{-1}$	530±330	502±285	322±241	331±216
Macrophages % TC	95.8±3.4	94.9±3.2	96.2±2.3	95.9±2.7
Lymphocytes % TC	2.7±2.6	3.1±2.2	2.9±2.3	3.3±1.9
Neutrophils % TC	1.4±1.5	1.7±1.6	0.8±0.5	0.7±0.8
Eosinophils % TC	0.1±0.3	0.2±0.3	0.2±0.3	0.1±0.3
CD2 % lymp[#]	90.5±4.3	91.2±3.0	92.0±3.4	92.8±3.0
CD19 % lymp[#]	1.6±0.7	0.8±0.7[+]	1.4±1.4	0.7±0.7[‡]
CD4 % lymp[#]	53.8±12.1	53.0±13.5	50.8±14.1	52.4±15.0
CD8 % lymp[#]	33.4±13.7	34.1±14.1	35.5±15.2	35.6±17.3
CD3 % lymp[#]	86.6±5.0	86.5±4.2	87.6±3.6	82.0±20.0
CD56 % lymp[#]	11.1±3.9	9.9±5.2	9.2±5.4	11.1±5.4

TDI: toluene diisocyanate; TC: total cells; lymp: lymphocytes. [#]: n=8. [+]: p=0.06; [‡]: p=0.036, between groups.

in different portions of the airways. The finding that s*G*aw and MEF25% were affected by TDI exposure suggests that TDI could exert an effect on both small and large airways. However, further investigations including assessment of partial flow-volume curves are required to determine the portion of the airway that is most sensitive to TDI. The tape-monitor method used in the present study is less precise than chromatographic techniques for measuring TDI concentrations [1]. However, continuous monitoring was necessary to regulate the concentration of TDI generated in the exposure chamber. In addition, the tape monitor method has been used extensively in field studies [5, 7].

The relevance of TDI-induced acute functional changes to the development of chronic airway obstruction remains uncertain. Longitudinal studies of workers exposed to TDI have produced conflicting results. Early studies found a correlation between one-day and long-term decrements in pulmonary function tests [3, 4]. Subsequent studies, however, failed to demonstrate an accelerated decline in pulmonary function tests among workers exposed to average concentrations of isocyanates <5 ppb [5–7].

Multivariate regression analysis of the factors that could affect the airway response to TDI indicated that the changes in s*G*aw were mainly influenced by the concentration of TDI. By contrast, the changes in MEF25% did not appear to be dose-dependent, although higher levels of bronchial hyperresponsivenness to histamine were associated with a more pronounced decrease in MEF25%. In the present study, an unusually high proportion of asymptomatic subjects showed a mild to moderate level of nonspecific bronchial hyperresponsiveness (histamine PC20 value <8 $mg \cdot mL^{-1}$). This finding could be, at least in part, related to the smoking status of the subjects, since all smokers showed a histamine PC20 value <8 $mg \cdot mL^{-1}$ as opposed to four out of six nonsmokers. In addition, six subjects reported a history of allergic rhinitis, which is frequently associated with asymptomatic bronchial hyperresponsiveness. Although multivariate regression analysis was used to control for histamine PC20 value, it cannot formally rule out that there was an effect of bronchial hyperresponsiveness on parameters other than MEF25%. Smoking was not a significant determinant of the functional response to TDI. This is in agreement with epidemiological surveys, which did not detect any additive effect of smoking and isocyanate exposure [3, 5–7, 19].

The observed increase in BAL albumin content after TDI exposure is likely to represent indirect evidence of changes in permeability of the epithelial barrier and slight leakage of blood plasma components into the alveolar compartment. Such increases in albumin levels have already been documented after exposure of humans to ozone [21, 22]. Discordance between increased albumin concentrations in distal airways and unchanged levels in proximal airways has also been observed after exposure to ozone [22]. The increase in BL α_2-macroglobulin level, even when expressed as a ratio to albumin, could reflect a selective increase in epithelial permeability

Table 4. – Biochemical constituents in bronchial and bronchoalveolar lavage fluids

	Bronchial lavage		Bronchoalveolar lavage	
	Ambient air	TDI	Ambient air	TDI
Albumin $\mu g \cdot mL^{-1}$	41.0±19.9	40.3±17.7	21.8±8.6	26.4±12.5[‡]
α_1-protease inhibitor $\mu g \cdot mL^{-1}$	0.80±0.86	0.92±0.83	0.44±0.51	0.62±0.63
α_2-macroglobulin $\mu g \cdot mL^{-1}$	0.05±0.04	0.07±0.06[+]	0.04±0.03	0.06±0.05
IgA $\mu g \cdot mL^{-1}$	2.16±1.28	2.64±1.51	0.98±0.60	1.36±0.83
Secretory component $\mu g \cdot mL^{-1}$	18.6±7.6	18.1±5.7	8.0±5.7	9.0±4.8
IgG $\mu g \cdot mL^{-1}$	7.7±5.9	8.5±7.2	3.8±2.5	4.6±2.5
IL-5 $pg \cdot mL^{-1}$	1.2±2.3	3.3±6.2	2.2±3.9	1.8±3.4
IL-6 $pg \cdot mL^{-1}$	4.9±4.0	4.8±3.6	1.9±1.5	1.9±1.3
IL-8 $pg \cdot mL^{-1}$	29.4±19.0	32.9±31.8	17.4±23.9	20.2±25.1
CC16 $\mu g \cdot L^{-1}$	1498±954	1782±1103	575±608	467±223

TDI: toluene diisocyanate; Ig: immunoglobulin; IL: interleukin; CC16: Clara cell protein. [+]: p=0.021; [‡]: p=0.044.

associated, to some unknown extent, with local production. An isolated increase in BAL α_2-macroglobulin content has also been found after a 3-h exposure to 0.6 ppb of nitrogen dioxide [23]. The concentrations of potential indicators of epithelial cell dysfunction (secretory component and CC16) and pro-inflammatory cytokines (TNF-α, IL-4, IL-5, IL-6, and IL-8) in BL and BAL were not significantly altered by TDI exposure. Nor did cellular studies provide evidence of an influx of inflammatory cells into the airway compartment in response to TDI. An inflammatory response of the airways to TDI exposure could have been missed, since the BAL procedure was performed at a short interval after the end of the exposure. However, the present study suggests that the observed changes in pulmonary function tests were not directly related to airway inflammation or injury. A decrease was found in the percentage of CD19+ B-cells in both BL and BAL. There is some suggestion that CD19+ B-lymphocytes could be involved in chronic obstructive lung disease [24]. In particular, BOSKEN *et al.* [24] found a significantly higher number of CD19+ B-lymphocytes in the adventitia of the small airways of smokers with airway obstruction. This study is difficult to compare with the present data considering that there are documented differences in inflammatory and immune cells between bronchial biopsies and BAL. More recently, a study by SANDSTRÖM *et al.* [25] demonstrated a marked reduction in both percentage and absolute number of CD19+ B-cells after repeated exposure to high levels of nitrogen dioxide (4 ppm) in humans. Although the relevance of these findings remains to be explored, this study together with the present results, supports the concept of subtle imm-unological changes in the airways after exposure to irritant chemicals.

The mechanisms leading to the nonimmunological respiratory health hazards caused by isocyanates remain largely speculative. Isocyanates are highly reactive chemicals capable of interacting with airway proteins. *In vitro* experiments have suggested that isocyanates could exert a direct pharmacological effect by inhibiting acetylcholinesterase activity [26] and β-adrenergic adenylate cyclase activity [27], although these effects have not been substantiated *in vivo* [27]. Studies in guinea-pigs have shown that *in vivo* exposure to TDI induces an increase in airway responsiveness to acetylcholine. These experiments provided evidence that the increased smooth muscle contractility is mediated by the release of tachykinins [28] and occurs independently of airway inflammation [29]. TDI can also cause an increase in airway responsiveness to substance P through inactivation of neutral endopeptidase [30]. *In vitro* experiments have demonstrated that TDI causes contraction of airway smooth muscle in guinea-pigs through the release of tachykinins by capsaicin-sensitive sensory nerves [31]. The effects detected in the present study on pulmonary function tests and airway proteins are consistent with the known physiological effects of tachykinins, although the role of neuropeptides was not specifically assessed.

It was concluded that short-term exposure to toluene diisocyanate levels near the permissible limits for workplaces can cause detectable, although minimal, changes in airway calibre and epithelial permeability. These findings provide rationale for conducting additional epidemiological studies to explore the respiratory effects of long-term exposure to low levels of isocyanates.

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References

1. Musk AW, Peters JM, Wegman DH. Isocyanates and respiratory disease: current status. *Am J Ind Med* 1988; 13: 331–349.
2. Vandenplas O, Malo JL, Saetta M, Mapp CE, Fabbri LM. Occupational asthma and extrinsic alveolitis due to isocyanates: current status and perspectives. *Br J Ind Med* 1993; 50: 213–228.
3. Peters JM, Murphy RLH, Pagnotto LD, Whittenberger JL. Respiratory impairment in workers exposed to "safe" levels of toluene diisocyanate (TDI). *Arch Environ Health* 1970; 20: 364–367.
4. Wegman DH, Peters JM, Pagnotto LD, Fine LJ. Chronic pulmonary function loss from exposure to toluene diisocyanate. *Br J Ind Med* 1977; 34: 196–200.
5. Diem JE, Jones RN, Hendrick DJ, *et al*. Five-year longitudinal study of workers employed in a new toluene diisocyanate manufacturing plant. *Am Rev Respir Dis* 1982; 126: 420–428.
6. Musk AW, Peters JM, Bernstein L. Absence of respiratory effects on subjects exposed to low concentrations of TDI and MDI–a reevaluation. *J Occup Med* 1985; 27: 917–920.
7. Jones RN, Rando RJ, Glindmeyer HW, *et al*. Abnormal lung function in polyurethane foam producers. Weak relationship to toluene diisocyanate exposures. *Am Rev Respir Dis* 1992; 146: 871–877.
8. American Conference of Governmental Industrial Hygienists. 1995–1996 Threshold limit values (TLVs) for chemical substances and physical agents, and biological exposure indices (BEIs). ACGIH, Cincinnati, Ohio. ((ISBN): 1-882417-11-9). 1995–1996.
9. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clinical Allergy* 1977; 7: 235–243.
10. American Thoracic Society. Standardization of spirometry. 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–1136.
11. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests European Community for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993; 6: Suppl. 16, 5–40.
12. European Community for Coal and Steel. Standardization of lung function tests. *Bull Eur Physiopath Respir* 1983; 19: Suppl. 5, 7–10.
13. Vandenplas O, Depelchin S, Delaunois L, Delwiche JP, Sibille Y. Bronchoalveolar lavage immunoglobulin A and G and antiproteases correlate with changes in diffusion indices during the natural course of pulmonary sarcoidosis. *Eur Respir J* 1994; 7: 1856–1864.
14. Delacroix DL, Marchandise FX, Francis C, Sibille Y. Alpha-2-macroglobulin, monomeric and polymeric immunoglobulin A, and immunoglobulin M in bronchoalveolar lavage. *Am Rev Respir Dis* 1985; 132: 829–835.
15. Bernard A, Lauwerys R, Noel A, Vandeleene B, Lambert A. Determination by latex immunoassay of protein 1 in

normal and pathological urine. *Clin Chim Acta* 1991; 201: 231–246.
16. Bernard A, Marchandise FX, Depelchin S, Lauwerys R, Sibille Y. Clara cell protein in serum and bronchoalveolar lavage. *Eur Respir J* 1992; 5: 1231–1238.
17. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear model. *Biometrika* 1986; 73: 13–22.
18. Davies CS. A computer program for regression analysis of repeated measures using generalized estimating equations. *Comput Meth Prog Biomed* 1993; 40: 15–31.
19. Wegman DH, Pagnotto LD, Fine LJ, Peters JM. A dose-response relationship in TDI workers. *J Occup Med* 1974; 16: 258–260.
20. Vogelmeier C, Baur X, Fruhmann G. Isocyanate-induced asthma: results of inhalation tests with TDI, MDI and methacholine. *Int Arch Occup Environ Health* 1991; 63: 9–13.
21. Devlin RE, McDonnell WF, Mann R, *et al*. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* 1991; 4: 72–81.
22. Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993; 148: 1363–1372.
23. Frampton MW, Finkelstein JN, Roberts NJ, Smeglin AM, Morrow PE, Utell MJ. Effects of nitrogen dioxide exposure on bronchoalveolar lavage proteins in humans. *Am J Respir Cell Mol Biol* 1989; 1: 499–505.
24. Bosken CH, Hards J, Gatter K, Hogg JC. Characterization of the inflammatory reaction in the peripheral airways of cigarette smokers using immunocytochemistry. *Am Rev Respir Dis* 1992; 145: 911–917.
25. Sandström T, Helleday R, Bjermer L, Stjernberg N. Effects of repeated exposure to 4 ppm nitrogen dioxide on bronchoalveolar lymphocyte subsets and macrophage in healthy men. *Eur Respir J* 1992; 5: 1092–1096.
26. Brown WE, Green AH, Karol MH, Alarie YC. Inhibition of cholinesterase activity by isocyanates. *Toxicol Appl Pharmacol* 1982; 63: 45–52.
27. McKay RT, Brooks SM. Effect of toluene diisocyanate on beta adrenergic receptor function. Biochemical and physiologic studies. *Am Rev Respir Dis* 1983; 128: 50–53.
28. Sheppard D, Scypinski L. A tachykinin receptor antagonist inhibits and an inhibitor of tachykinin metabolism potentiates toluene diisocyanate-induced airway hyperresponsiveness in guinea pigs. *Am Rev Respir Dis* 1988; 138: 547–551.
29. Cibulas W, Brooks SM, Murlas CG, Miller ML, McKay RT. Toluene diisocyanate-induced airway hyperreactivity in guinea pigs depleted of granulocytes. *J Appl Physiol* 1988; 64: 1773–1778.
30. Sheppard D, Thompson JE, Scypinski L, Dusser D, Nadel JA, Borson DB. Toluene diisocyanate increases airway responsiveness to substance P and decreases airway neutral endopeptidase. *J Clin Invest* 1988; 81: 1111–1115.
31. Mapp CE, Boniotti A, Papi A, *et al*. The effect of phosphoramidon and epithelium removal on toluene diisocyanate-induced contractions in guinea-pig bronchi. *Eur Respir J* 1992; 5: 331–333.