
Evaluation of a 4-Steps-1-Day Whole Body Challenge Protocol for the Diagnosis of Occupational Asthma due to Diisocyanates

41

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Abstract

Inhalative challenges are important in the diagnosis of occupational asthma due to diisocyanates. As existing protocols are time-consuming and costly, it was the aim of this study to develop a short duration whole body exposure protocol. Ninety three subjects with suspected occupational diisocyanate-induced asthma and verified current or previous occupational exposure to diisocyanates and ten control subjects without diisocyanate exposure but with bronchial hyperresponsiveness were investigated. After baseline examination on the first day, subjects underwent a standardized whole body multiple-steps-1-day challenge with exposures of up to four times 30 min to concentrations of 5, 10, 20, and 30 ppb of the dominant diisocyanate used at work on the second day. Common spirometric and body plethysmographic parameters were used as positivity criteria. Overall, 14 subjects demonstrated a positive diisocyanate challenge, 19 were considered doubtful, and 60 were negative. All controls had negative challenges. Positive reactions occurred during the challenge ($n = 10$) or during follow-up ($n = 4$). Eight subjects showed $>40\%$ fall of FEV_1 . These severe reactions occurred after 5 ppb ($n = 2$) or 10 ppb ($n = 3$), while isolated late reactions after 2 h of follow-up were not observed. Multivariate analysis showed an association between a positive challenge and both the degree of previous occupational exposure and the presence of baseline bronchial hyperresponsiveness. In summary, the proposed 4-steps-1-day diisocyanate challenge protocol induced pronounced bronchial reactions in a small number of subjects. As these reactions were more likely to occur after low concentrations, it is recommended to shift the initial concentration/dose step to lower exposures.

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Keywords

Asthma • Bronchial hyperresponsiveness • Challenge • Diagnosis • Diisocyanates • Occupational

1 Introduction

Inhalation challenges are considered an important tool to confirm the diagnosis of occupational asthma (OA) (Vandenplas et al. 2006). While in most forms of OA the need for inhalative challenges may be questioned due to the relatively high prediction of sensitization in subjects with work-related symptoms (van Kampen et al. 2008), the demonstration of sensitization in diisocyanate asthma is rare. The sensitivity of *in vitro* assays for the detection of diisocyanate-specific IgE is too low to serve as a reliable diagnostic marker (Wisnewski 2007). Thus, exposure protocols of diisocyanate challenges are needed that yield a high diagnostic efficiency, without posing a danger to the subjects' health.

Diisocyanate challenges have been performed as whole body exposures and by a closed-circuit apparatus. To our knowledge, the closed-circuit method was used exclusively in Canada (Malo et al. 1999; Vandenplas et al. 1992a), a comparison between both methods showed minor differences with respect to challenge results. Whole body exposures are performed with different protocols in many countries; most studies used stepwise challenges with 2–4 dose steps (Vandenplas et al. 1992a; Vogelmeier et al. 1991; Smith et al. 1980). Whereas exposures were performed with the most common diisocyanates (isophorone diisocyanate (IPDI), toluene diisocyanate (TDI), diphenylmethane-4,4'-diisocyanate (MDI), naphthylene-1,5-diisocyanate (NDI), and 1,6-hexamethylene diisocyanate (HDI)), protocols differ with respect to exposure and test duration, and minimal and maximal diisocyanate concentrations. Exposure monitoring is almost exclusively performed using tape monitor systems; with minimal concentrations of mostly 5 ppb and maximal concentrations of mostly 20 ppb showing little

differences between studies (Karol et al. 1994; Vandenplas et al. 1993; Banks et al. 1989; Mapp et al. 1986a, b; Malo et al. 1983; O'Brien et al. 1979). Only few researchers used higher concentrations, such as 30 ppb (Smith et al. 1980), 40 ppb (Paggiaro et al. 1987), or 250 ppb (Paggiaro et al. 1986). While the total exposure duration of stepwise tests showed little variation with a range of approximately 30–120 min (Malo et al. 1999; Vandenplas et al. 1996; Moscato et al. 1993; Tossin et al. 1989; Vogelmeier et al. 1991; Paggiaro et al. 1987; Mapp et al. 1986a, b; O'Brien et al. 1979), the most important differences between study protocols were the total test duration, i.e., most researchers included long periods without exposure to yield total test duration of 2 days (Vandenplas et al. 1992a, 1996; Malo et al. 1983; Smith et al. 1980) to 3 days (Malo et al. 1999; Kopferschmitt-Kubler et al. 1998).

To our knowledge, there is only one single multiple-steps-1-day protocol reported in the literature, with concentrations between 5 and 20 ppb, but the authors did not describe the concentrations of the steps in detail (Vogelmeier et al. 1991).

2 Methods

2.1 Subjects

The study was approved by the Ethics Committee of the Ruhr University and all subjects gave informed consent. Between 2001 and 2008, a total of 122 subjects with current or previous occupational exposure to diisocyanates were investigated in our institute for suspected occupational asthma (OA) or hypersensitivity pneumonitis (HP) due to diisocyanates. Fourteen subjects were not challenged because of baseline

airways obstruction, one subject did not allow the challenge, one asymptomatic subject had transient acute symptoms after accidental high exposure, and in two subjects the diagnosis of HP due to diisocyanates was made by a typical history and increased specific IgG antibodies to diisocyanates. Eleven subjects did not complete the challenge protocol due to burning eyes ($n = 1$) or hyperventilation ($n = 10$). In total, 93 subjects were included in the final analysis. In addition, ten control subjects with mild to very mild asthma and bronchial hyperresponsiveness without exposure to diisocyanates were challenged with TDI and MDI (Table 41.1). The challenges in exposed subjects were performed for a medical opinion.

2.2 Clinical Investigations

A complete examination was performed on three consecutive days. During the first day, baseline parameters were recorded, including methacholine testing and repeated lung function measurements to document a stable respiratory status. On the second day, the diisocyanate challenge was performed, and follow-up measurements were terminated on the third day. Medication was stopped since the first day. Anamnestic data were recorded with a self-administered questionnaire that was corrected during a physician's interview. Detailed information about job titles and exposure conditions were obtained from both the patients and the files of the technical services of the accident insurance, which were available for each patient. Both the quality of the dominant diisocyanate exposure and a quantitative expert assessment of inhalative exposure to diisocyanate vapors or aerosols (ratings 1-low, 2-intermediate, and 3-high) were recorded by last author (RM). Environmental monitoring at workplaces was available in 20 cases, the results were considered in the expert judgement. Personal data (age, gender, and smoking habits), current or previous work-related symptoms (shortness of breath, wheezing, recurrent cough, runny or itching nose, sneezing, burning or itching eyes, and skin complaints of face, forearms or hands) and current

asthma medication (bronchodilators and inhalative steroids) and the following information about the course of symptoms were gathered: overall duration of occupational exposure to diisocyanates, period from the beginning of exposure to the onset of symptoms (latency), period of symptomatic exposure, and period from the end of exposure to diagnosis. After a physical examination, standard laboratory tests, electrocardiograms and chest radiographs were performed. Total IgE and specific IgE antibodies to HDI, TDI and MDI were measured in the patients' sera by ImmunoCAP (Phadia, Uppsala, Sweden) according to the manufacturer's recommendations. Skin prick tests (SPT) were performed with a panel of 20 environmental allergens (various manufacturers). Atopy was defined by a wheal diameter of at least 3 mm greater than the saline control (in the presence of a positive histamine control). Body plethysmography and spirometry were performed with MasterScreen (CareFusion, Würzburg, Germany), and specific airway resistance (sRt) as well as forced expiratory volume in 1 s (FEV_1) in percent of predicted (American Thoracic Society 1995) were recorded. Methacholine testing was done with a reservoir method (American Thoracic Society 2000). A positive reaction was assumed if FEV_1 decreased by at least 20 % with the highest dose. As this test is comparable to the ATS dosimeter test, this definition includes subjects that would be considered borderline according to ATS (Baur et al. 1998).

2.3 Diisocyanate Challenges

Diisocyanate challenges were performed with the predominant diisocyanate used at work. Challenges were performed as a whole body exposure as described recently (Marczynski et al. 2003). Briefly, subjects were exposed to MDI (technical grade; $n = 51$), TDI ($n = 6$), HDI ($n = 30$), NDI ($n = 4$) or IPDI ($n = 2$). NDI was obtained from Bayer, Leverkusen (Germany), the other diisocyanates from Merck, Darmstadt (Germany). During the challenges subjects were continuously exposed to diisocyanates at four different concentrations

Table 41.1 Personal data, symptoms, and clinical data according to challenge results

	Exposed subjects					P*
	Challenge result					
	Positive (n = 14)	Doubtful (n = 19)	Negative (n = 60)	Total (n = 93)	Controls (n = 10)	
Age; year (median, range)	43 (20–60)	48 (29–63)	43 (20–72)	44 (20–72)	39 (27–52)	0.9
Male gender; n (%)	10 (71)	17 (90)	55 (92)	82 (88)	2 (20)	0.06
Current smoker; n (%)	6 (43)	2 (11)	20 (33)	28 (30)	3 (30)	0.6
Current asthma medication; n (%)	12 (86)	12 (63)	33 (55)	57 (61)	1 (10)	0.03
Job titles; n (%)						0.4
Spray painter	1 (7)	3 (17)	19 (32)	23 (25)	0 (0)	0.06
Foam/casting	3 (21)	3 (16)	6 (10)	12 (13)	0 (0)	0.2
Foundry	1 (7)	2 (11)	1 (2)	4 (4)	0 (0)	0.3
Isocyanate production	2 (14)	4 (21)	7 (12)	13 (14)	0 (0)	0.8
Miner	0 (0)	1 (5)	1 (2)	2 (2)	0 (0)	0.6
Others	7 (50)	6 (32)	26 (43)	39 (42)	10 (100)	0.7
Duration of exposure; mo (median, range)	43 (7–348)	202 (6–459)	120 (0–492)	136 (0–492)	–	0.3
Beginning of exposure to onset of symptoms; months (median, range)	13 (1–255)	168 (1–276)	69 (1–396)	73 (1–396)	–	0.3
Symptomatic exposure; months (median, range)	27 (3–180)	72 (1–291)	34 (1–360)	34 (1–360)	–	0.8
End of exposure to diagnosis; months (median, range)	9 (0–22)	17 (1–220)	8 (0–156)	9 (0–220)	–	0.9
Work-related symptoms						
Airways; n (%)	14 (100)	16 (84)	56 (93)	86 (93)	–	0.3
Nose or eyes; n (%)	5 (36)	5 (26)	24 (41)	34 (37)	–	0.7
Skin; n (%)	3 (23)	2 (11)	9 (15)	14 (15)	–	0.5
FEV ₁ ; %pred (median, range)	91.1 (61.8–136.6)	92.1 (53.7–127.2)	94.4 (59.9–130.0)	92.7 (53.7–136.6)	101.5 (72–126)	0.5
sRt; (median. range)	1.2 (0.6–2.7)	0.9 (0.5–2.9)	1.0 (0.5–3.0)	1.0 (0.5–3.0)	1.3 (0.6–2.1)	0.03
Hyperresponsiveness; n (%)	12 (86)	6 (32)	32 (53)	50 (54)	10 (100)	0.03
Atopy; n (%) ^a	2 (15)	3 (16)	23 (39)	28 (31)	–	0.11
Total IgE; kU/L (median. range)	61 (4–833)	19 (4–284)	53 (1–949)	41 (1–949)	30 (11–355)	0.6

Percentages refer to the number of tests performed (^a2 missings)

*Challenge positives were compared with challenge negatives

for 30 min each: 5, 10, 20, and 30 ppb within 1 day. After the 20 and 30 ppb exposures, a pause of 90 min was made to detect initial late reactions. Thus, the total exposure period was 2 h, the total challenge duration 5 h. Follow-up measurements were performed immediately after the last exposure step, and 30 min, 2 h, 4 h, and about 20 h (next day) afterwards. Peak expiratory flow (PEF) measurements were performed by the

subjects between 4 and 20 h follow-up measurements at intervals of 1 h, with the exception of sleeping periods. Body plethysmography and spirometry were performed before and after each step. The diisocyanate atmospheres were generated in an air-tight glass chamber. The concentrations of diisocyanate vapor in the chamber were measured continuously with a calibrated tape monitor system (Toxic Gas

Monitor Series 7100; Zellweger Analytics, Inc., Lincolnshire, USA). The test was considered positive (end-of-test-criterion) if the following three criteria were fulfilled: (1) decrease of FEV₁ of at least 20 %, (2) increase of sRt of at least 100 %, and (3) increase of sRt to at least 2 kPa · s. A test was considered doubtful if the first criterion, but not the second and third, was fulfilled, or if any combination of two of the three criteria was measured. A challenge was defined negative if all three criteria were not fulfilled. Early reactions were defined as positive reactions during the challenge (including the measurement after the last exposure step of 30 ppb); late reactions fulfilled the positivity criterion afterwards. For safety reasons, the subjects spent the night after the challenge in an adjacent hospital, irrespective of the challenge result.

2.4 Statistical Analysis

Characteristics and clinical data of the subjects with positive and negative diisocyanate challenges were compared by a Chi² test for categorical variables and a Mann–Whitney *U* test or *t*-Test as appropriate. An extended Mantel-Haenszel Chi² test for linear trend was used for the comparison of challenge positive and negative subjects with respect to the three exposure categories. Multivariate analysis was performed with logistic regression analysis. Goodness of fit was assessed by a Hosmer and Lemeshow test. For multivariate analysis only positive and negative challenges were included. $P < 0.05$ was considered statistically significant. Data were analyzed with SPSS version 19, IBM, Ehningen, Germany.

3 Results

The patients were predominantly males (88 %), the median age was 44 (range 20–72) years (Table 41.1). Overall, they had near-to-normal lung function with a mean FEV₁ of 93 % predicted, FEV₁ variability during the first day was below ± 10 %. About one third of subjects

were current smokers and atopics. Sixty one percent of the subjects were prescribed asthma medication. Job titles were diverse with some preponderance of spray painting, foam/casting and diisocyanate production. Twenty-one subjects (23 %) were still exposed at their workplace during the last month. Work-related airways symptoms were more often reported than symptoms of nose, eyes, or skin. A higher number of control subjects than patients were considered hyperresponsive to methacholine (100 vs. 54 %, respectively), but fewer controls were on current asthma medication (10 vs. 61 %, respectively; Table 41.1).

Overall, 14 subjects (15 %) demonstrated a positive challenge, 19 (20 %) were considered doubtful, and 60 (65 %) were negative. Challenge positive subjects were more likely to take asthma medication than challenge negatives (86 vs. 55 %, respectively) and to experience bronchial hyperresponsiveness (86 vs. 53 %, respectively). They also had somewhat shorter exposure duration and latency (beginning of exposure to onset of symptoms), although this was not statistically significant. There were no further relevant differences between the groups with respect to personal characteristics, exposure data, symptoms, spirometry, or total IgE (Table 41.1). None of the subjects showed sensitization to any diisocyanate.

Of the subjects with positive challenges, ten reactions were positive with MDI (20 % of MDI challenges), three with NDI (75 %) and one with HDI (3 %). None of the ten control subjects showed a positive reaction (data not shown). Five subjects preferred to terminate the positive reaction by a bronchodilator (additional two subjects with negative challenges took medication). By definition, challenge negatives did not show an effect on lung function, but in doubtful cases there was some variation of FEV₁ (Fig. 41.1), but not in sRt (Fig. 41.2). The subjects showed positive reactions (both FEV₁ and sRt-criterion fulfilled) after all challenge steps: 5 ppb ($n = 3$), 10 ppb ($n = 3$), 20 ppb ($n = 2$), and 30 ppb ($n = 2$), or follow-up measurements after 30 min ($n = 1$), 2 h ($n = 2$), or 4 h ($n = 1$). PEF recordings did not show any positive reactions in subjects with doubtful or negative challenges (data

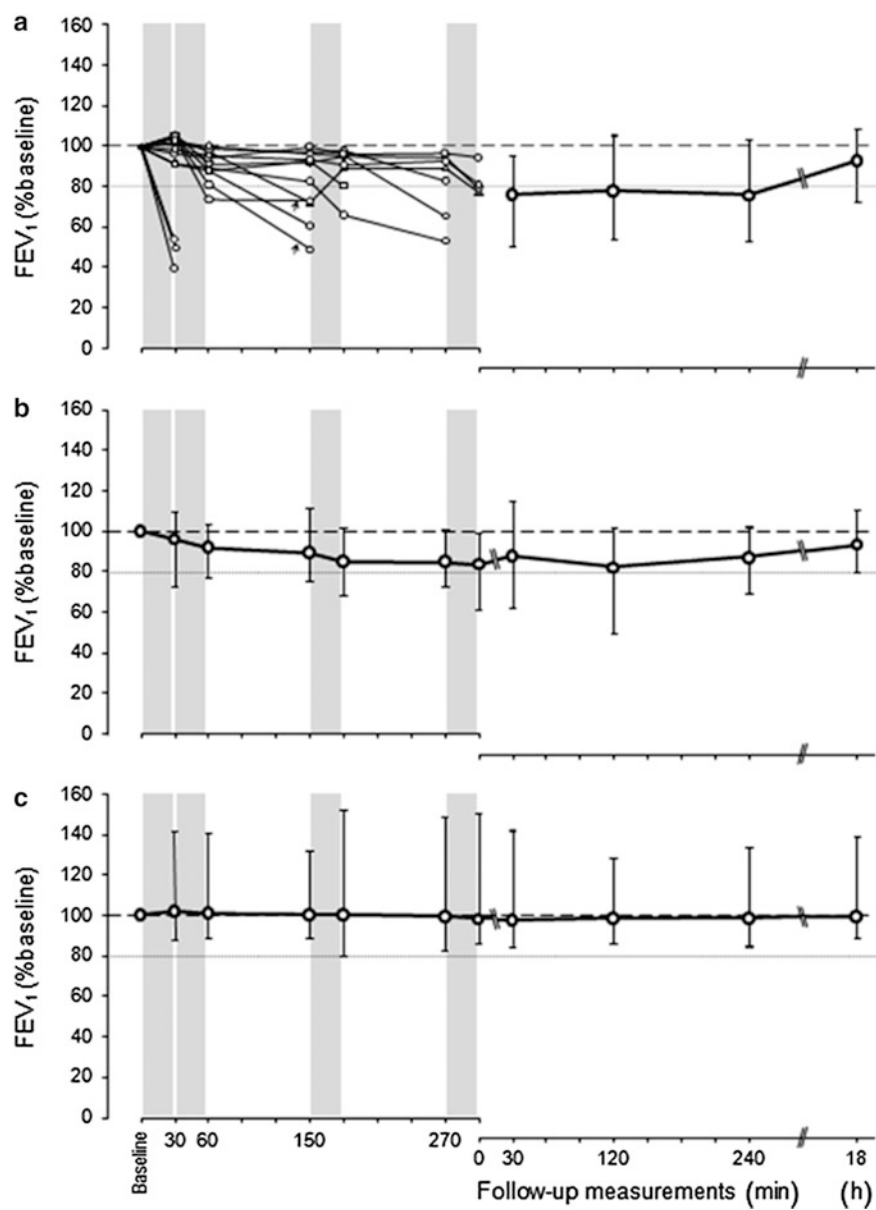


Fig. 41.1 Time response curves of FEV₁ during challenge and follow-up periods. For challenge positives (a), the individual curves are shown for the challenge period, whereas medians and ranges are given for the follow-up period. For subjects with doubtful (b) or negative (c) challenges, medians and ranges are given for both challenge

and follow-up periods. Exposures (4 × 30 min) are represented by grey columns. The two arrow heads in *Panel a* indicate two subjects who showed a significant decrease in FEV₁, but no increase in sRt after the exposure step. In these subjects the test was terminated after a significant reaction was obtained after a pause of 90 min

not shown). However, there were discrepancies between FEV₁ and sRt with respect to the time of significant reactions (data not shown in detail). Of the four subjects with reactions in the follow-up

period, three subjects were near-positive during the challenge period. The subject without any reaction during the challenge period became positive at 2 h of follow-up.

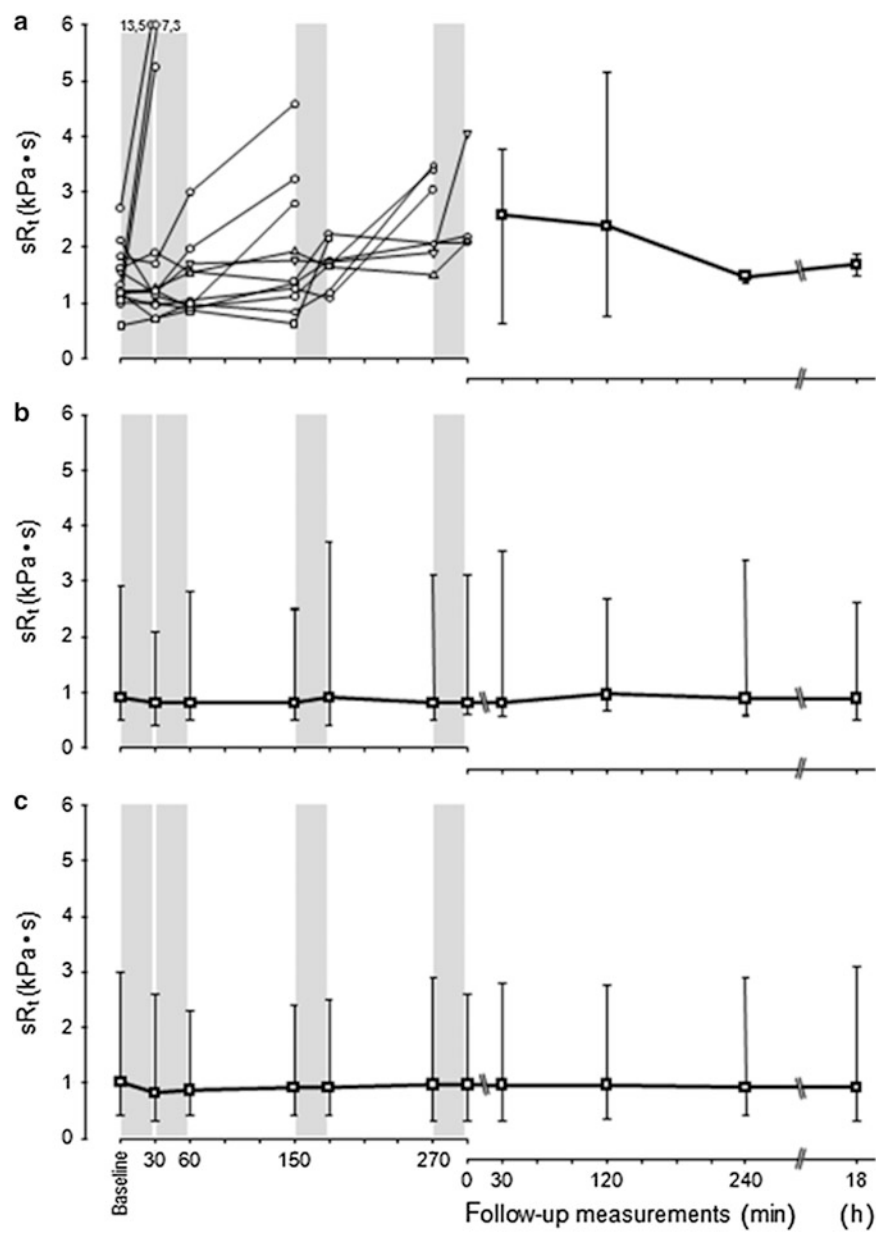


Fig. 41.2 Time response curves of specific airway resistance (sRt) during challenge and follow-up periods. For challenge positives (a), the individual curves are shown,

whereas for subjects with doubtful (b) or negative (c) challenges, medians and ranges are given. The exposure periods (4×30 min) are represented by grey columns

Severity of reactions during the challenge period and afterward was comparable. The maximal fall of FEV_1 was 60.7 % from baseline during the challenge period, while it was 65.1 % during the follow-up (Fig. 41.3). Eight subjects showed a

more than 40 % fall of FEV_1 at any time during the study, five of these inhaled a bronchodilator after the positive reaction. These reactions occurred after 5 ppb ($n = 2$) or 10 ppb ($n = 3$). Three subjects needed a second and one subject a third

bronchodilation. In the morning after the challenge day, two subjects showed a FEV₁ decrease >20 % (24 and 28 %) from baseline (both subjects inhaled bronchodilators earlier). These subjects were again treated with a short acting beta-agonist and airway obstruction was fully reversible. They were given topical steroids afterward for a few days and told to contact their general practitioners if symptoms should persist.

The degree of exposure to diisocyanates during employment, as assessed by expert rating, was associated with positive challenges, although a prediction was not possible in each case. Low exposure assignment did not exclude a positive challenge (Table 41.2). This finding was corroborated by multivariate analysis, which in

addition showed an association between a positive challenge and bronchial hyperresponsiveness (Table 41.3).

4 Discussion

We describe a highly selected group of subjects with previous or current exposure to diisocyanates and work-related respiratory symptoms. Interestingly, specific IgE antibodies to diisocyanates in serum were not detected in any subject, although we used a common commercially available test. We cannot explain this unexpected finding, but it showed convincingly that positive challenges with diisocyanates may occur in subjects without obvious sensitization. Total IgE was not different between challenge positive and negative subjects, indicating no IgE-dependent mechanism.

To our knowledge there are no studies in the literature that compare different protocols of challenge tests for the diagnosis of occupational asthma with respect to test duration or minimal and maximal doses during challenge steps. Evaluations of existing exposure protocols are descriptive as direct comparisons cannot be performed due to ethical reasons, thus the conclusions that can be drawn from such studies remain limited. This is also true for the present study which intended to shorten the test duration without loss of safety for the patients. However, there are several important findings that may help to establish optimized challenge protocols.

As diisocyanate challenges have the distinctive feature of frequent late reactions, immediate reactions, e.g., after the final dose step cannot be distinguished from late reactions to previous

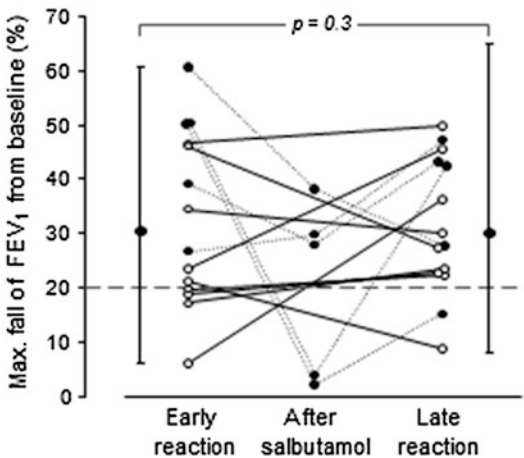


Fig. 41.3 Maximal fall of FEV₁ during the challenge (early reaction) and during follow-up (late reaction) in 14 challenge positives. In addition, medians and ranges are presented. Five subjects received a short acting beta-agonist during the challenge (black dots and dashed lines)

Table 41.2 Association of challenge results with exposure intensity according to expert ratings

Exposure group	Challenge results			Total (n = 93)	OR (95 % CI) ^a pos. vs. neg.
	Positive (n = 14)	Doubtful (n = 18)	Negative (n = 61)		
1 = low	6	13	45	65	1
2 = intermediate	4	4	14	21	2.1 (0.5–8.7)
3 = high	4	2	1	7	30 (2.9–315)

^aExtended Mantel-Haenszel chi square test for linear trend

Table 41.3 Odds ratios for a positive challenge from multivariate analysis

	n	OR	95 % CI	p
Exposure degree				
Small (Ref.)	51	1.00		
Medium	18	1.52	0.29–7.93	0.6198
High	5	23.00	1.05–503.03	0.0464*
Latency (month)		0.98	0.92–1.04	0.5025
Symptomatic exposure duration (month)		1.00	0.99–1.01	0.9820
Hyperresponsiveness				
No (Ref.)	30	1.00		
Yes	44	10.19	1.04–99.85	0.0462*
Atopy				
No (Ref.)	47	1.00		
Yes	25	0.27	0.04–1.74	0.1690
Smoking				
Never (Ref.)	31	1.00		
Current	26	0.71	0.12–4.11	0.6994
Ex	17	0.16	0.01–1.94	0.1481
Age (year)		0.96	0.89–1.03	0.2699

Hosmer and Lemeshow Goodness of fit test: $p = 0.5001$. * significant association with responder status

dose steps in 1-day protocols. Thus, maximal doses with optimal sensitivity and specificity cannot be derived precisely from our study. In a limited number of controls we had no false positives, thus specificity seems high up to 30-min exposures of 30 ppb. Concerning sensitivity, an important number of subjects, i.e., 6 of the 14 responders (43 %) demonstrated a positive reaction after the final concentration of 30 ppb. Although some of them might have become positive also with a concentration of 20 ppb at follow-up, a higher concentration than 20 ppb for 30 min may be warranted. Vandenplas et al. (1993) observed that neither the concentration nor the duration of exposure *per se* is the main determinant of the occurrence of an asthmatic reaction to diisocyanates, but the dose that is the product of both factors. As the generation of higher exposure concentrations by heating might be linked with the degradation of immunogenic epitopes, it may be wise to keep the maximal concentration at 20 ppb and to expand the exposure duration, possibly to 1 h. For the initial concentration/dose the present study suggests that lower doses should be used in order to prevent severe reactions in highly susceptible individuals.

Most authors used multiple-steps-challenge protocols that take several days (Malo et al. 1999; Kopferschmitt-Kubler et al. 1998; Vandenplas et al. 1992a; Banks et al. 1989). The test duration in these studies amounts up to 4 days, while the time of exposure varied from 20 to 240 min. Whether 1-day protocols are of sufficient safety needs further study. The comparable lung function impairment of early and late reactions in this study argues for 2-days protocols. Although all reactions were well controlled by bronchodilators, the severity of reactions in this study was considered not acceptable.

It is one of the most important findings of this study that bronchial reactions did not occur later than 2 h after the end of the challenge. Pauses of 90 min between steps were chosen in the present study after 10 and 20 ppb steps. In view of the higher risk of low concentration steps, a pause should be included after the 5 ppb step as well, the duration of the pauses was probably adequate. With pauses of 60–90 min 1-day protocols may be designed with both low initial doses and maintaining dose augmentation by doubling doses. Such protocols have the advantage of lower costs and also a lower chance to produce difficult-to-explain non-linear dose response

relationships of multiple-days protocols (Malo et al. 1999).

The overall low sensitivity of challenge testing in this study of 15 % is probably mainly due to selection, but also false negatives may have occurred, as described earlier (Vandenplas et al. 1992b). That is especially true for doubtful test results in a considerable number of subjects in this study ($n = 19$; 20 %). As FEV₁, but not specific airway resistance which is less dependent on cooperation of the patient, varied widely in this group, it is obvious that most subjects were in fact negatives. However, as some uncertainty remains, this group was excluded from further analysis. We preferred to include body plethysmography as an effect parameter because spirometry was considered less reliable in this group of subjects who were examined for compensation purposes. Overall, a combination of spirometry and body plethysmography as effect parameters was considered useful, but probably the inclusion of further effect parameters, like serial methacholine testing or the measurement of exhaled nitric monoxide may increase the sensitivity of diisocyanate challenges.

There was no preponderance of a specific profession among responders, but there was an association between the previous degree of exposure to diisocyanates as assessed by expert rating with the responder status. This is highly plausible and points to an overall reliable test system. There was also an association between responder status and bronchial hyperresponsiveness: 86 % of the responders demonstrated bronchial hyperresponsiveness whereas this percentage was much lower in doubtful or negative reactors (32 and 53 %). This was also reflected in a higher number of subjects on current asthma medication in the responders. The most probable explanation is selection of the subjects, most of them had only negligible exposures to diisocyanates in the past, and work-related symptoms were not suggestive of OA due to diisocyanates.

Overall, this study indicates that lower initial doses should be used and that the need for follow-up monitoring longer than 2 h after negative challenges may be abandoned. Whether the

compromise between time and effort, on the one hand, and safety, on the other hand, may be obtained with 1-day protocols needs further evaluation.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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