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Inhalation challenges with occupational agents: Threshold duration of exposure

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Summary

Objectives: The aim of this study was to characterize the threshold duration of exposure needed to elicit an asthmatic reaction during specific inhalation challenges (SIC) with various occupational agents and to determine the duration of exposure that should be completed before the test can be considered negative.

Methods: This retrospective study analysed the cumulative duration of challenge exposure that was required to elicit a $\geq 20\%$ fall in forced expiratory volume in one second in 335 consecutive subjects with a positive SIC.

Results: The threshold duration of challenge exposure required to induce an asthmatic reaction was ≤ 60 min in 179 (53%) subjects, between 61 and 120 min in 74 (22%) subjects, and longer than 120 min in 82 (25%) subjects. The multivariate linear regression analysis showed that a longer duration of exposure was associated with exposure to low-molecular-weight agents ($p < 0.001$), a higher level of baseline non-specific bronchial hyperresponsiveness to histamine ($p = 0.015$), increasing age ($p = 0.011$), and a shorter duration of asthma symptoms at work ($p = 0.060$).

Conclusions: This study demonstrates that the sensitivity of SICs for diagnosing OA is highly dependent upon the duration of the challenge exposure. These data may provide useful guidance for improving the reliability of SICs performed with realistic methods of exposure.

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Abbreviations: FEV₁, forced expiratory volume in one second; HMW, high molecular weight; LMW, low molecular weight; OA, occupational asthma; PC₂₀, provocative concentration of histamine causing a 20% fall in FEV₁; SIC, specific inhalation challenge; TD_{20%FEV₁}, threshold duration of exposure inducing a $\geq 20\%$ fall in FEV₁.

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Introduction

A specific inhalation challenge (SIC) with occupational agents is still regarded as the "reference" method to establish the diagnosis and aetiology of immunologically-mediated occupational asthma (OA).^{1–3} A major limitation of SICs is their potential for inducing false-negative results when specific bronchial reactivity to the sensitizing agent has declined after cessation of workplace exposure.^{4–9} Using a closed-circuit device that allows for generating quantified levels of various agents, Lemi re and co-workers elegantly demonstrated that the specific bronchial reactivity to sensitizing occupational agents almost never completely disappear after cessation of exposure,^{8,9} although the dose of the agent required for inducing a positive SIC response significantly increases.⁸

Since standardized preparations are not available for most occupational agents, SICs are still usually performed by exposing the subjects to the suspected occupational agent using the "realistic" approach originally described by Pepys and Hutchcroft in the early 1970s.¹⁰ This methodology aims at reproducing as close as possible the conditions of exposure at the workplace in terms of the chemical and physical characteristics of the agent, for example, dust, aerosol, vapour, or fume.^{10–13} The concentrations of the agents generated during these SICs are usually not measured due to the wide diversity of the tested agents and the high technical demands for controlling exposures. In these realistic SICs, the duration of the challenge exposure is used as a surrogate of the dose of the agent delivered to the subjects. However, the cumulative duration of exposure to the suspected agent, which should be completed before the test can be considered negative, has never been thoroughly evaluated.

The aim of this study was to characterize the threshold duration of exposure that was required to elicit a $\geq 20\%$ fall in FEV₁ (TD_{20%FEV1}) in a large series of positive SICs induced by various occupational agents as well as to identify the factors that can affect this parameter.

Methods

Study design and population

In this retrospective study, the records of all subjects investigated for possible OA who showed a positive SIC, defined by a $\geq 20\%$ fall in FEV₁, from January 1992 to December 2011 at our institution were reviewed. These tests are routinely performed in our centre in the investigation of work-related asthma. The protocol of the study was approved by the *Comit   d'  thique m  dicale du Centre Hospitalier Universitaire de Mont-Godinne* (approval number 84-2012). A statement of informed consent was not required because the data were analysed retrospectively in an anonymous way.

Specific inhalation challenges

SICs were completed according to a previously described protocol^{12,14,15} that remained unchanged over the reviewed

period. Briefly, occupational agents were generated in five-cubic-meter cubicles using a realistic approach.^{10,12} Asthma medications were withdrawn according to their duration of action,¹¹ while inhaled corticosteroids were halted 72 h prior to the tests. The level of exposure during SICs was continuously monitored only for isocyanates using an MDA 7100 monitor (MDA Scientific Inc., Glenview, IL) and was kept below the ceiling value of 20 ppb.

Spirometry^{16,17} was obtained at baseline, immediately after each exposure, then every 15 min for the first hour, every 30 min for the second hour, and hourly thereafter for a total of at least six hours after the end of exposure. The baseline level of non-specific bronchial hyperresponsiveness to histamine¹⁴ and, since 2006, sputum eosinophil counts¹⁵ were assessed at the end of the control day and re-assessed 6 and 24 h after the active challenges. The degree of non-specific bronchial hyperresponsiveness was expressed as the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀).¹⁸ A SIC was considered positive when a sustained $\geq 20\%$ fall in FEV₁ was recorded on two consecutive assessments. The pattern of bronchial responses was characterized as immediate, late, dual, or atypical according to previously described criteria.^{10,19}

On the first day, the subjects were exposed to a 'control' agent for 30 min to ensure that FEV₁ fluctuations were $\leq 12\%$ of the baseline value. The 'control' substance was selected according to the nature of the occupational agent suspected of causing OA. For instance, lactose powder was used as the 'control' substance for SIC with agents in powder form (e.g. flour, drugs, persulphates), pine dust for SIC with wood dusts, vinyl gloves for SIC with latex gloves, and diluents for polyurethane products and other resins. On the following day, the subjects were challenged with the suspected occupational agent (s). The duration of exposure to both high-molecular-weight (HMW) and low-molecular-weight (LMW) agents was gradually increased (i.e. 1 min, 4 min, 10 min, 15 min, 30 min, and 60 min) until a $\geq 20\%$ fall in FEV₁ occurred or a cumulative exposure of two hours was completed. Those subjects who did not demonstrate a $\geq 20\%$ fall in FEV₁ during the first active test day underwent a repeated challenge in the same way for a maximum of 2–3 h on the next day. Accordingly, all subjects were challenged for at least 240 min before considering the SIC as being negative. In addition, further challenges were proposed when there was a significant (>3 -fold) decrease in the post-challenge PC₂₀ value¹⁴ or an increase in sputum eosinophils $>3\%$,¹⁵ as compared to the control day values.

Data analysis

Quantitative data are presented as median with 25th and 75th percentiles. Comparison between subgroups of subjects was made using the Chi-square test for categorical variables and the Wilcoxon rank-sum test for numerical variables. A multivariate linear regression analysis was conducted to identify the factors that affected the TD_{20%FEV1}. The independent variables incorporated into the regression model included the relevant demographic and clinical characteristics of the subjects (i.e. age, gender, and smoking status, atopy, duration of asthma symptoms at

work, and time elapsed between the last work exposure and SIC), indices reflecting asthma severity (dose of inhaled corticosteroids needed to control asthma, FEV₁ % predicted, FEV₁/forced vital capacity ratio, and baseline histamine PC₂₀ value), and the category of the tested agent (i.e. HMW vs. LMW agent). The regression analysis was performed using a backward selection by Wald test. All statistical tests are two-tailed. A *p*-value <0.05 was considered significant. Statistical analysis was performed using the IBM SPSS Statistics 19.0 software (SPSS Inc, Chicago, Ill).

Results

During the period from 1992 to 2011, a total of 335 subjects showed a positive SIC in terms of the changes in FEV₁. The main demographic and clinical characteristics of the subjects are summarized in Table 1. The agents having caused a positive SIC were HMW agents in 206 (61%) subjects and

LMW agents in 123 (37%) instances, while the causal agent remained indeterminate in six cases. Among the HMW substances, latex was the main causative agent (*n* = 91, 27%), followed by flour (*n* = 89, 27%), and enzymes (*n* = 14, 4%). The principal LMW causal agents were metals (*n* = 24, 7%), isocyanates (*n* = 23, 7%), wood dusts (*n* = 21, 6%), quaternary ammonium compounds (*n* = 10, 3%), and persulfate salts (*n* = 8, 2%).

The distribution of TD_{20%FEV1} values is presented in Table 2. Noticeably, 82 of the 335 (25%) subjects required a challenge exposure of more than two hours (Table 1). These subjects were slightly older (40 [25th–75th percentile: 32–49] yr vs. 36 [25th–75th percentile: 31–44] yr, *p* = 0.038), were more often current or ex-smokers (49% vs. 35%, *p* = 0.028), showed less often an atopic status (50% vs. 64%, *p* = 0.020), and had a higher histamine PC₂₀ value (3.20 [0.94–4.84] mg/ml vs. 1.36 [0.35–4.16] mg/ml, *p* = 0.001) as compared to the other subjects. The SICs with LMW agents required more frequently (39 of 123

Table 1 Demographic and clinical characteristics of the subjects.

	All patients with positive SIC (<i>n</i> = 335)	Duration of SIC exposure ≤120 min (<i>n</i> = 253)	Duration of SIC exposure >120 min (<i>n</i> = 82)	<i>p</i> -Value
Gender (male)	204 (61)	150 (59)	54 (66)	0.290
Age, yr ^a	37 (31–46)	36 (31–44)	40 (32–49)	0.038
Current and ex-smokers	129 (39)	89 (35)	40 (49)	0.028
Atopy ^b	204 (61)	163 (64)	41 (50)	0.020
Duration of asthma before SIC, mo ^a	30 (13–65)	32 (14–67)	28 (13–54)	0.210
Delay since last work exposure, mo ^a	3 (0.1–14)	3 (0.1–15)	1.8 (0.1–14)	0.619
Inhaled corticosteroid ^c				
Low dose	31 (9)	24 (9)	7 (9)	0.707
Medium dose	73 (22)	58 (23)	15 (18)	
High dose	48 (14)	33 (13)	15 (18)	
Baseline FEV ₁ , % pred ^a	92 (81–101)	92 (80–100)	92 (82–102)	0.370
Baseline FEV ₁ <80% pred	72 (21)	58 (23)	14 (17)	0.262
Baseline FEV ₁ /FVC, % ^a	75 (69–80)	75 (69–80)	76 (69–81)	0.255
Baseline FEV ₁ /FVC <70%	87 (26)	66 (26)	21 (26)	0.932
Baseline histamine PC ₂₀ , mg/mL ^a	1.75 (0.43–5.50)	1.36 (0.35–4.16)	3.20 (0.94–4.84)	0.001
>16 mg/ml	39 (12)	25 (10)	14 (17)	0.005
>2–16 mg/ml	108 (32)	75 (30)	33 (40)	
>0.25–2 mg/ml	123 (37)	99 (39)	24 (29)	
≤0.25 mg/ml	65 (19)	54 (21)	11 (13)	
Type of occupational agents				
Low molecular weight	123 (37)	84 (33)	39 (48)	0.015
High molecular weight	206 (61)	166 (66)	40 (49)	
Indeterminate	6 (2)	3 (1)	3 (4)	
Pattern of asthmatic reaction				
Immediate	187 (56)	134 (53)	53 (65)	0.005
Dual	60 (18)	54 (21)	6 (7)	
Isolated late	19 (6)	10 (4)	9 (11)	
Atypical prolonged immediate	61 (18)	48 (19)	13 (16)	
Atypical progressive	8 (2)	7 (3)	1 (1)	

Data are presented as *n* (% of available data) unless otherwise specified. FEV₁: forced expiratory volume in one-second; FVC: forced vital capacity; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁; SABA: (inhaled) short-acting beta2-agonist; SIC: specific inhalation challenge.

^a Median value with 25th–75th percentiles in parentheses.

^b Atopy defined by the presence of a positive skin-prick test to at least one common inhalant allergen.

^c Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent; low dose: equal or less than 500 µg per day; medium dose: more than 500 µg but equal or less than 1000 µg per day; and high dose: more than 1000 µg per day.

Table 2 Duration of challenge exposure required to induce an asthmatic reaction.

Cumulative duration of exposure to occupational agents inducing a $\geq 20\%$ fall in FEV ₁	Number (%) of SICs		
	All agents (n = 335)	HMW agents (n = 204)	LMW agents (n = 126)
≤ 5 min	61 (18)	49 (24)	12 (10)
6–15 min	39 (12)	33 (16)	5 (4)
16–30 min	38 (11)	24 (12)	14 (11)
31–60 min	41 (12)	24 (12)	17 (13)
61–120 min	74 (22)	34 (17)	38 (30)
121–240 min	62 (19)	31 (15)	29 (23)
> 240 min	20 (6)	9 (4)	11 (9)

HMW: High-molecular weight; LMW: Low-molecular-weight; SIC: Specific inhalation challenges.

[31.7%] SICs) an exposure longer than two hours than those performed with HMW agents (40 of 206 [19.4%] SICs, $p = 0.015$). $TD_{20\%FEV_1}$ was more frequently ≤ 120 min in dual reactions and > 120 min in isolated late reactions (Table 1).

The multivariate linear regression analysis showed that a longer threshold duration of exposure was associated with LMW agents (beta coefficient [SE]: 44.09 [10.06], $p < 0.001$), a higher baseline histamine PC_{20} value (1.18 [0.53], $p = 0.028$), increasing age (1.44 [0.56], $p = 0.010$), and a shorter duration of asthma symptoms at work (-0.15 [0.79], $p = 0.062$).

Discussion

This study showed that a substantial proportion of subjects who completed an SIC procedure using a “realistic” methodology required a prolonged exposure to the causal occupational agent for developing an asthmatic reaction. Namely, the $TD_{20\%FEV_1}$ was longer than one hour in 47% of the subjects and longer than two hours in 25%. These data demonstrate that the sensitivity of SICs for diagnosing OA may be highly dependent upon the duration of challenge exposure.

It has been shown that the total dose of the agent – rather than either the concentration or duration of exposure – determines the bronchial response to LMW and HMW occupational agents in an individual subject with OA.^{20,21} In this study, the duration of exposure to the occupational agents was used as a rough estimate of the dose that was delivered to the subjects. The data reflect a “real-life” setting, wherein the concentrations of agents generated through the realistic methods are highly variable and cannot be easily controlled.^{22,23} Indeed, determining the concentrations of various LMW chemicals requires appropriate equipment that is not easily available and that most often provide an a posteriori estimate of the mean concentration to which the subject has been exposed during the SIC. In our centre, instantaneous concentrations were assessed only for isocyanates, which can induce severe asthmatic reactions at very low levels. On the other hand, water-soluble HMW agents^{24,25} and some LMW agents (e.g. platinum salts,⁷ reactive dyes,²⁶ and persulfate salts²⁷) can be administered through the nebulization of increasing concentrations of the agent diluted in saline. However,

standardized and validated extracts of occupational agents are lacking for most occupational agents.²⁸ Closed-circuit devices that make it possible to deliver known and stable concentrations of occupational agents in various forms have been developed,^{23,29,30} but these apparatuses are expensive and require a pre-calibration process for each tested agent.³¹ Even with such sophisticated devices, measuring the levels of airborne particles during the SIC does not accurately reflect the actual dose of allergens inhaled by the subjects.

Our retrospective data were used to investigate the host factors that could help in identifying the subjects who need a long duration of exposure in order to induce an asthmatic reaction. The multivariate linear regression analysis revealed that a low level of baseline non-specific bronchial hyperresponsiveness (i.e. a high histamine PC_{20} value) appeared as the principal determinant of a longer $TD_{20\%FEV_1}$. The dose of non-occupational inhalant allergens required to induce an asthmatic reaction is dependent upon both the level of non-specific bronchial hyperresponsiveness and the degree of immunological reactivity to the allergen, as assessed either by the skin end-point or the level of specific IgE antibodies.^{32,33} There are only scarce data pertaining to occupational agents. With regards to HMW and LMW occupational agents, the level of specific bronchial reactivity has been inconsistently related to the degree of non-specific bronchial hyperresponsiveness.^{9,30,34,35} For HMW agents, the main determinant of the specific bronchial reactivity seemed to be the level of specific IgE sensitization,^{9,35} which was not taken into account in our analysis because of the diversity of the tested HMW agents. The multivariate analysis also suggested that the duration of exposure required for eliciting a positive FEV₁ response increased with increasing age and decreasing duration of work-related asthma symptoms. The latter finding may suggest that subjects with a shorter duration of OA require a higher dose of the sensitizing agent to induce an asthmatic response.

A high proportion of the subjects had been removed from workplace exposure for a long time before referral for an SIC procedure. However, the median delay since last work exposure was three months, which is similar to the figure reported by Malo and co-workers (i.e. 76 days) in a large series of subjects investigated for possible OA.³⁶ Disconcertingly, the interval of time elapsed since the last work exposure did not affect directly the $TD_{20\%FEV_1}$. Several

studies that involved re-challenging subjects with OA at variable intervals after the initial SIC documented that the specific bronchial reactivity to occupational agents may decrease after cessation of the sensitizing agent,^{4–6,8} with the exception of a study involving platinum salts [Merget, 1994 #477]. Using the same concentrations of the agents as those generated during the initial SIC, Lemi re and co-workers found that the duration of exposure, and consequently the total dose, required for inducing a positive SIC response significantly increased (~3-fold on average) in subjects with OA due to various agents who were re-challenged two years after removal from exposure.⁸ However, in a similar study conducted in subjects with OA caused by HMW agents who had been removed from exposure for a longer interval (on average 5.7 yr) and who had normalized their non-specific hyperresponsiveness to methacholine, the dose needed to elicit a bronchial response was not modified at the follow-up assessment.⁹

The multivariate linear regression analysis also revealed that LMW agents more frequently required a longer TD_{20%} FEV₁ than HMW agents. This retrospective study does not allow for determining whether this finding was related to differences in the mode of exposure or in the intrinsic 'asthmagenic' properties between these two categories of agents, which act through presumably different immunologic mechanisms.³⁷ Nevertheless, differences in the levels of exposure did not appear to account for the observed difference, since the proportion of subjects with a TD_{20%} FEV₁ of more than two hours still remain significantly higher for LMW agents (45%) as compared to HMW agents (29%, $p = 0.025$) after exclusion of SICs with isocyanates, whose concentrations were continuously measured and kept below the permissible levels (data not detailed). It has been shown that LMW agents induce more frequently isolated late asthmatic reactions than HMW agents.^{19,38,39} According to our protocol, challenge exposures are discontinued as soon as an early reaction occurs, while late reactions develop only after the end of the 2-h challenge exposure, which may contribute to the longer duration of exposure observed in SIC with LMW agents.

Altogether, the findings of this retrospective study provide some evidence that the duration of exposure may be regarded as a useful, though very crude, parameter for guiding the exposure during realistic SICs. However, since the concentrations of the tested occupational agents were not known with the exception of isocyanates, our study aimed only at providing practical guidance for performing SIC with "realistic" methods of exposure, and our findings cannot be directly applied to other challenge procedures. An accurate identification of the respective role of the host and exposure factors that determine the specific bronchial reactivity to occupational agents would require further investigations with a precise quantification of the level of exposure during SIC.^{23,29,30}

Despite its inherent limitations, this study has practical implications in the investigation of OA through SIC using realistic methods of exposure to occupational agents. The data indicate that an SIC involving a short period of exposure to the suspected occupational agent (usually 30–60 min) may not be sensitive enough for accurately excluding the diagnosis of OA. This finding further emphasize the need for using markers of the bronchial response to

occupational agents that are more sensitive than the assessment of airway calibre, such as the changes in the post-challenge level of non-specific bronchial responsiveness to histamine/methacoline^{14,40} and in sputum eosinophil counts^{15,41}. Alternatively, the sensitivity of SICs for diagnosing OA can be markedly improved by increasing the duration of exposure.

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Conflict of interest

There is no conflict to declare in relation to this article.

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References

1. Nicholson PJ, Cullinan P, Taylor AJ, Burge PS, Boyle C. Evidence based guidelines for the prevention, identification, and management of occupational asthma. *Occup Environ Med* 2005;**62**: 290–9.
2. Beach J, Russell K, Blitz S, Hooton N, Spooner C, Lemi re C, et al. A systematic review of the diagnosis of occupational asthma. *Chest* 2007;**131**:569–78.
3. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and management of work-related asthma: American college of chest physicians consensus statement. *Chest* 2008;**134**:15–41S.
4. Mapp CE, Corona PC, De Marzo N, Fabbri L. Persistent asthma due to isocyanates. A follow-up study of subjects with occupational asthma due to toluene diisocyanate (TDI). *Am Rev Respir Dis* 1988;**137**:1326–9.
5. Paggiaro PL, Vagaggini B, Dente FL, Bacci E, Bancalari L, Carrara M, et al. Bronchial hyperresponsiveness and toluene diisocyanate. Long-term change in sensitized asthmatic subjects. *Chest* 1993;**103**:1123–8.
6. Pisati G, Baruffini A, Bernabeo F, Cerri S, Mangili A. Rechallenge subjects with occupational asthma due to toluene diisocyanate (TDI), after long-term removal from exposure. *Int Arch Occup Environ Health* 2007;**80**:298–305.
7. Merget R, Reineke M, Rueckmann A, Bergmann EM, Schultze-Werninghaus G. Nonspecific and specific bronchial responsiveness in occupational asthma caused by platinum salts after allergen avoidance. *Am J Respir Crit Care Med* 1994;**150**: 1146–9.
8. Lemi re C, Cartier A, Dolovich J, Chan-Yeung M, Grammer L, Ghezzo H, et al. Outcome of specific bronchial responsiveness to occupational agents after removal from exposure. *Am J Respir Crit Care Med* 1996;**154**:329–33.
9. Lemi re C, Cartier A, Malo JL, Lehrer SB. Persistent specific bronchial reactivity to occupational agents in workers with normal nonspecific bronchial reactivity. *Am J Respir Crit Care Med* 2000;**162**:976–80.

10. Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975;112: 829–59.
11. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. *Eur Respir J Suppl* 1993;16:53–83.
12. Vandenplas O, Malo JL. Inhalation challenges with agents causing occupational asthma. *Eur Respir J* 1997;10:2612–29.
13. Vandenplas O, Cartier A, Malo JL. Occupational challenge tests. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein D, editors. *Asthma in the workplace*. New York: Taylor & Francis; 2006. p. 227–52.
14. Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R. Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. *Thorax* 1996;51:472–8.
15. Vandenplas O, D'Alpaos V, Heymans J, Jamart J, Thimpont J, Huaux F, et al. Sputum eosinophilia: an early marker of bronchial response to occupational agents. *Allergy* 2009;64: 754–61.
16. European Community for Coal and Steel. Standardization of lung function tests. *Bull Eur Physiopathol Respir* 1983; 19(Suppl. 5):45–51.
17. American Thoracic Society. Standardization of spirometry. 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–36.
18. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235–43.
19. Dufour MH, Lemièr C, Prince P, Boulet LP. Comparative airway response to high- versus low-molecular weight agents in occupational asthma. *Eur Respir J* 2009;33:734–9.
20. Vandenplas O, Cartier A, Ghezzi H, Cloutier Y, Malo JL. Response to isocyanates: effect of concentration, duration of exposure, and dose. *Am Rev Respir Dis* 1993;147:1287–90.
21. Nguyen B, Weytjens K, Cloutier Y, Ghezzi H, Malo JL. Determinants of the bronchial response to high molecular weight occupational agents in a dry aerosol form. *Eur Respir J* 1998; 12:885–8.
22. Cloutier Y, Lagier F, Lemieux R, Blais MC, St-Arnaud C, Cartier A, et al. New methodology for specific inhalation challenges with occupational agents in powder form. *Eur Respir J* 1989;2:769–77.
23. Vandenplas O, Malo JL, Cartier A, Perreault G, Cloutier Y. Closed-circuit methodology for inhalation challenge tests with isocyanates. *Am Rev Respir Dis* 1992;145:582–7.
24. Melillo G, Bonini S, Cocco G, Davies RJ, de Monchy JG, Frolund L, et al. EAACI provocation tests with allergens. Report prepared by the European academy of allergology and clinical immunology subcommittee on provocation tests with allergens. *Allergy* 1997;52:1–35.
25. Boulet LP, Gauvreau G, Boulay ME, O'Byrne P, Cockcroft DW. The allergen bronchoprovocation model: an important tool for the investigation of new asthma anti-inflammatory therapies. *Allergy* 2007;62:1101–10.
26. Park JW, Kim CW, Kim KS, Choi SY, Kang DB, Ko SH, et al. Role of skin prick test and serological measurement of specific IgE in the diagnosis of occupational asthma resulting from exposure to vinyl sulphone reactive dyes. *Occup Environ Med* 2001;58: 411–6.
27. Moscato G, Pignatti P, Yacoub MR, Romano C, Spezia S, Perfetti L. Occupational asthma and occupational rhinitis in hairdressers. *Chest* 2005;128:3590–8.
28. van Kampen V, Merget R, Rabstein S, Sander I, Bruening T, Broding HC, et al. Comparison of wheat and rye flour solutions for skin prick testing: a multi-centre study (Stad 1). *Clin Exp Allergy* 2009;39:1896–902.
29. Cloutier Y, Lagier F, Cartier A, Malo JL. Validation of an exposure system to particles for the diagnosis of occupational asthma. *Chest* 1992;102:402–7.
30. Choudat D, Fabries JF, Martin JC, Villette C, Tabka F, Dessanges JF, et al. Quantification of the dose of inhaled flour: relation with nonspecific bronchial and immunological reactivities. *Eur Respir J* 1999;14:328–34.
31. Caron S, Boileau JC, Malo JL, Leblond S. New methodology for specific inhalation challenges with occupational agents. *Respir Res* 2010;11:72.
32. Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;135:264–7.
33. Cockcroft DW, Davis BE, Boulet LP, Deschesnes F, Gauvreau GM, O'Byrne PM, et al. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. *Allergy* 2005;60:56–9.
34. Merget R, Dierkes A, Rueckmann A, Bergmann EM, Schultze-Werninghaus G. Absence of relationship between degree of nonspecific and specific bronchial responsiveness in occupational asthma due to platinum salts. *Eur Respir J* 1996;9: 211–6.
35. Quirce S, Fernandez-Nieto M, Escudero C, Cuesta J, de Las Heras M, Sastre J. Bronchial responsiveness to bakery-derived allergens is strongly dependent on specific skin sensitivity. *Allergy* 2006;61:1202–8.
36. Malo JL, Cardinal S, Ghezzi H, L'Archevêque J, Castellanos L, Maghni K. Association of bronchial reactivity to occupational agents with methacholine reactivity, sputum cells and immunoglobulin E-mediated reactivity. *Clin Exp Allergy* 2011;41: 497–504.
37. Maestrelli P, Boschetto P, Fabbri LM, Mapp CE. Mechanisms of occupational asthma. *J Allergy Clin Immunol* 2009;123: 531–42. quiz 43–4.
38. Malo JL, Ghezzi H, L'Archevêque J, Cartier A. Late asthmatic reactions to occupational sensitizing agents: frequency of changes in nonspecific bronchial responsiveness and of response to inhaled beta 2-adrenergic agent. *J Allergy Clin Immunol* 1990;85:834–42.
39. Talini D, Novelli F, Bacci E, Dente FL, De Santis M, Di Franco A, et al. Comparison between airway responses to high versus low molecular weight compounds in occupational asthma. *J Allergy* 2011;2011:781470.
40. Sastre J, Fernandez-Nieto M, Novalbos A, De Las Heras M, Cuesta J, Quirce S. Need for monitoring nonspecific bronchial hyperresponsiveness before and after isocyanate inhalation challenge. *Chest* 2003;123:1276–9.
41. Lemièr C, Chaboilliez S, Trudeau C, Taha R, Maghni K, Martin JG, et al. Characterization of airway inflammation after repeated exposures to occupational agents. *J Allergy Clin Immunol* 2000;106:1163–70.