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Application of porous foams for size-selective measurements of airborne wheat allergen

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Background: Exposure to airborne wheat allergen is a well-known cause of bakers' allergy and asthma. Airborne wheat allergen can be measured by enzyme immunoassays (EIAs) in extracts of inhalable dust samples, but only limited knowledge is available on the size distribution of wheat allergen-carrying particles. Recently, a new sampling medium, porous polyurethane foam, has been introduced for the size-selective sampling of airborne dust in various occupational settings. We investigated the applicability of these foams for size-selective wheat allergen measurements.

Methods: Personal and stationary measurements were performed in a flour mill, using respirable and thoracic foams inserted into the conventional IOM inhalable sampler, together with PTFE (Teflon) filters. Foams and filters were eluted and wheat allergen levels determined by human IgG4 inhibition EIA.

Results: Wheat allergen levels could be determined in both filter and foam eluates. Inhalable dust levels from filters and foams ranged from 1.4 to 53 mg m^{-3}, and wheat allergen levels from 15 to 580 μg m^{-3}. The allergen was mainly borne on particles with D_{ae} (particle aerodynamic diameter) > 10 μm and particles with 4 μm < D_{ae} ≤ 10 μm, accounting for 54.5–77.5% and 18.9–43.2% of the total allergen yield, respectively. Less than 4% of airborne wheat allergen was carried by particles smaller than 4 μm (respirable fraction).

Conclusions: Measurement of wheat allergen in dust fractions trapped in respirable and thoracic foams is technically feasible. Both wheat flour dust and wheat allergen are mainly concentrated in larger particle-size fractions (extrathoracic and tracheobronchial).

Keywords: airborne wheat allergen; bakers' asthma; respirable foams; size-selective measurements; thoracic foams

INTRODUCTION

Exposure to wheat flour dust is a well-known cause of respiratory health problems in bakers and flour mill workers. Reports show that 3–21% of bakers suffer from work-related rhinitis, often associated with conjunctivitis, and 0.5–7% are affected by asthma, the most severe form of bakers' allergy (Houba *et al*., 1998). The latter is supposed to be induced mainly by allergen-carrying dust particles of small size, capable of reaching the lower parts of respiratory tract. In order to assess potential association between size of airborne wheat allergen-carrying particles and occurrence of specific respiratory symptoms in exposed workers, particle size-selective sampling of wheat flour dust and consecutive determination of wheat allergen levels should be performed. Such measurements have, thus far, only incidentally been reported (Sandiford *et al*., 1994).

In current occupational hygiene practice, particle size-selective sampling can be performed with various types of cyclones and impactors. Using mechanisms of inertial separation, impactors divide dust into several particle-size fractions collected onto conventional rigid collection substrates (filters or porous metal plates), with cut-off values usually between 0.005 and 50 μm (Berner *et al*., 1979; Vanderpool *et al*., 1987). The main disadvantage of impactors is that they are mostly suitable only for ambient (stationary) air sampling and a very limited number of studies have reported their use for wheat allergens (Sandiford *et al*., 1994). There has also been little work to validate cyclone samplers for airborne allergens.

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Porous polyurethane foams are new media for particle size-selective dust sampling. The foam matrix, made of open cells and cell-connecting filaments, captures and retains dust particles, with aerosol penetration mainly driven by impaction and sedimentation forces (Vincent *et al*., 1993). Particles with an aerodynamic diameter larger than the foam cell diameter are captured within the matrix, while smaller particles are not retained. If the loaded foam matrix is treated e.g. by elution (Kenny *et al*., 1999) or acid digestion (Möhlmann *et al*., 2002), captured particles can be released and analysed with suitable analytical procedures.

Size-selective properties and applicability of foams in occupational settings have been investigated in a number of studies during the last decade (Aitken *et al*., 1993; Kenny *et al*., 1998, 1999, 2001b). The authors suggested that foams, as particle-size selectors inserted into the standard IOM personal inhalable sampler, can provide an easy and low-cost method for measurements of three major dust fractions: inhalable (particle aerodynamic diameter $D_{ae} \leqslant 100$ μm), thoracic ($D_{ae} \leqslant 10$ μm) and respirable ($D_{ae} \leqslant 4$ μm), defined by international sampling conventions (CEN, 1993; ISO, 1995; ACGIH, 1998). Unlike massive cyclones and impactors, the IOM inhalable dust sampler with inserted foam is, due to its lightness, suitable for stationary as well as personal measurements.

The aim of this feasibility study was to investigate whether this new approach of size-selective sampling could be applied to measure airborne wheat allergen. Two types of foams ('respirable' and 'thoracic'), incorporated in the standard IOM inhalable sampler, were used for the size-selective measurements of wheat flour dust at the workplace, followed by elution of the foams and determination of wheat allergen levels in the eluates.

MATERIALS AND METHODS

Sampling site

The sampling took place in the packing area of a flour mill, where flour from silos was transferred into bags. During filling of the bags, workers had to hang bags underneath a spout that conveyed flour, and to guide tops of the filled bags through a sewing machine.

Sampling equipment

A conventional IOM personal inhalable dust sampler, widely applied for measuring inhalable dust in work environments (Mark and Vincent, 1986), was used for collection of inhalable dust (IOM) and for dual-fraction size selective sampling with respirable (IOM-RF) or thoracic foams (IOM-TF). Two types of foams were applied: respirable and thoracic (SKC Ltd, Dorset, UK), with nominal porosity of 90 and 45 pores per inch (p.p.i.), and D_{50} cut-off values of 4 and 10 μm, respectively. Actual porosities of applied foam batches were determined by the Institute for Occupational Medicine (Edinburgh, UK), and appeared to range from 70 to 90 p.p.i. for respirable foams and from 35 to 50 p.p.i. for thoracic foams.

Foams were inserted into the cassettes of the IOM personal inhalable sampler (Fig. 1), in front of the Teflon filters (diameter 25 mm, pore size 1 μm; Millipore Ltd, Watford, UK). Respirable and thoracic dust fractions were thus collected on the filters behind respirable and thoracic foams, while foams themselves retained larger, 'extrarespirable' or 'extrathoracic' particle-size fractions, respectively (Kenny *et al*., 1999, 2001b).

Sampling strategy

Stationary side-by-side sampling experiments (Fig. 2) were performed on 10 occasions in the vicinity of the flour-packing spout, by attaching the referent IOM sampler with either IOM-RF (6 occasions) or IOM-TF sampler (4 occasions) to the stationary mannequin - CALTOOL (Kromhout H,

Fig. 1. IOM sampling head with a filter and either a thoracic foam (second from the right, lower row) or a respirable foam (first from the right, lower row).

Fig. 2. Stationary side-by-side sampling: conventional IOM sampler with a filter (left) and dual-fraction IOM sampler with a filter and a respirable foam (right), attached to the CALTOOL.

Witschger O, Koch W *et al.*, manuscript in preparation). Personal IOM-RF ($n = 7$) and IOM-TF samples ($n = 7$) were collected by positioning IOM-RF or IOM-TF sampler in the worker's breathing zone. Gilair-5 pumps were used to obtain the flow rate of 2 l min^{-1} through the samplers. Duration of stationary and personal sampling ranged from 2 to 4.5 h.

Determination of dust and wheat allergen levels

Before and after sampling, foam and filters were equilibrated and weighed in a temperature and humidity controlled environment. Wheat allergen and other soluble proteins were extracted by eluting respirable and thoracic foams with 3 and 2 ml, respectively, of extraction fluid (PBS + 0.05% Tween–20). In order to obtain a concentrated allergen extract, the volumes of extraction fluid were set at the smallest feasible amount to completely soak the foam. The procedure for foam extraction was as follows: after placement into regular polypropylene tubes, the foams were immersed in the extraction fluid until completely soaked, then squeezed and soaked five times, using a pair of clean tweezers, and sonicated for 2 min. This procedure was repeated twice. The eluate was finally squeezed out of the foams and centrifuged at 5000 *g* for 15 min. The supernatant was removed and stored in aliquots at −20°C until allergen analyses. IOM filters, eluted with 2.5 ml of the extraction fluid, underwent two cycles of vortexing and sonication, with subsequent centrifugation at 5000 *g*, as described previously by Houba *et al.* (1996). Wheat allergen content of filter and foam eluates was determined with a human IgG4 inhibition EIA (Houba *et al.*, 1996). Sensitivity and accuracy of the assay (LOD = 20–50 ng ml^{-1}, CV inter-day = 19%) were comparable to those reported in the previous study.

Wheat allergen concentrations were determined in inhalable, respirable and thoracic fractions, collected by IOM, IOM-RF and IOM-TF samplers. Inhalable wheat allergen concentration (μg m^{-3}) of the referent IOM sampler was calculated from a wheat allergen load (μg) on the IOM filter: $C = m/Qt$, where Q is the 2 l min^{-1} flow rate and t is the sampling time. The IOM-RF inhalable wheat allergen concentration was calculated as the sum of allergen concentrations found on the IOM-RF filter and corresponding respirable foam. Similarly, the IOM-TF inhalable wheat allergen concentration was calculated as the sum of allergen concentrations on the IOM-TF filter and the thoracic foam. Respirable wheat allergen concentrations were determined from wheat allergen loads on the IOM-RF filters, and thoracic concentrations from wheat allergen loads on the IOM-TF filters. Extrathoracic wheat allergen concentrations were determined from allergen loads measured in extracts of thoracic foams. Similar calculations were applied to determine dust concentrations in the different fractions.

RESULTS

Airborne wheat allergen was detected and determined in the eluates of both respirable and thoracic foams, indicating that the allergen can be recovered from the foam matrix and measured with the IgG4 inhibition EIA method. Dust and wheat allergen levels obtained by the stationary, foam-based IOM-RF and IOM-TF sampling methods were compared with those from the side-by-side conventional IOM sampler (Table 1). For the dust concentrations <5 mg m^{-3}, inhalable dust levels measured by IOM-RF and IOM-TF matched well dust levels obtained by the referent IOM sampler. At very high dust concentrations (33.2 and 53.0 mg m^{-3}), the IOM-RF sampler showed a tendency to undersample. We were, however, not able to assess the sampling performance of IOM-TF at dust levels of this range, since dust concentrations did not exceed 3.1 mg m^{-3} when the IOM-TF sampler was applied. A scatter plot showing correlation between inhalable dust levels found by IOM-RF, IOM-TF and IOM sampling methods is presented in the Fig. 3a.

Total inhalable wheat allergen levels obtained with IOM-RF and IOM-TF showed a good correlation with referent IOM values (Spearman's correlation coefficients 0.94 and 0.80, respectively), but were generally higher, while dust levels were comparable or even lower than referent dust levels. This was observed especially with the thoracic foams (Fig. 3b). The differences in inhalable dust and wheat allergen levels obtained with the dual-fraction samplers and the referent sampler were not found to be statistically significant ($P > 0.05$), as analysed by the Wilcoxon signed rank test.

Particle size-selective measurements with IOM-RF and IOM-TF clearly showed a predominance of dust and wheat allergens in larger fractions (Table 1). For both stationary and personal measurements, the thoracic fraction ($D_{ae} \leqslant 10$ μm) contributed 23–30% to the total inhalable dust, and the respirable fraction ($D_{ae} \leqslant 4$ μm) only ~5%. The largest proportion of the dust particles (>70%) was found in the extrathoracic ($D_{ae} > 10$ μm) fraction. Similarly, wheat allergens were mainly found in the extrathoracic fraction with 54.5% (stationary samples) and 77.5% (personal samples) of the total inhalable wheat allergen, and only 2.3–3.6% in the respirable fraction. The relative size of the finest, respirable fraction of the dust showed a tendency to decrease with increasing dust concentrations (Fig. 4a), starting already at the moderate dust levels of 1–2 mg m^{-3}. A much less pronounced declining trend was observed for the thoracic fraction. Wheat allergen had a similar particle size-distribution pattern (Fig. 4b).

The relative allergen content of dust showed no clear dependence on particle size. Concentration of the wheat allergen, collected by the personal and

Table 1. Dust and wheat allergen levels in different fractions collected by side-by-side stationary measurements with the conventional IOM sampler (IOM) and: (a) IOM sampler with filter and a respirable foam (IOM-RF), (b) IOM sampler with a filter and a thoracic foam (IOM-TF).

(a) Exp. No.	IOM		IOM-RF			
	Inhalable fraction		Inhalable fraction		Respirable fraction	
	Dust (mg m^{-3})	Wheat (µg m^{-3})	Dust (mg m^{-3})	Wheat (µg m^{-3})	Dust (mg m^{-3})	Wheat (µg m^{-3})
1	2.20	42.70	2.00	59.24	0.13	1.06
2	3.29	45.34	3.65	92.36	0.19	1.35
3	1.42	30.82	1.56	37.68	0.20	1.79
4	33.23	414.02	24.67	286.58	0.23	0.88
5	1.92	38.86	1.57	33.61	0.14	0.80
6	53.03	219.72	27.92	270.66	0.25	8.04

(b) Exp. No.	IOM		IOM-TF			
	Inhalable fraction		Inhalable fraction		Thoracic fraction	
	Dust (mg m^{-3})	Wheat (µg m^{-3})	Dust (mg m^{-3})	Wheat (µg m^{-3})	Dust (mg m^{-3})	Wheat (µg m^{-3})
7	1.59	35.20	1.84	59.41	0.62	28.15
8	2.17	26.47	1.88	40.67	0.52	21.18
9	2.12	28.95	1.52	31.45	0.45	12.20
10	3.11	70.66	3.76	99.59	1.17	43.58

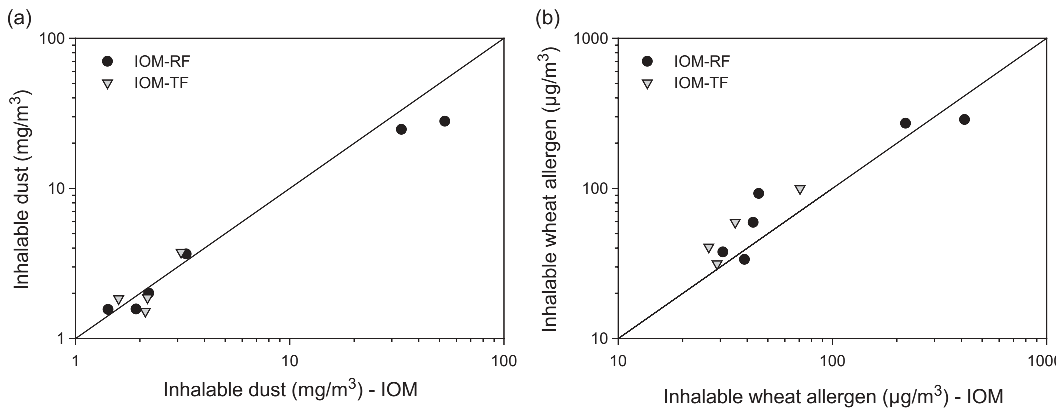

Fig. 3. Comparison of: (a) inhalable dust concentrations and (b) inhalable wheat allergen concentrations, obtained with IOM, IOM-RF and IOM-TF samplers - stationary side-by-side measurements. Line shown in the figure represents a line of unity.

stationary dual-fraction IOM sampling, was on average 1% (w/w) in the respirable fraction, 2–3.8% in the thoracic, 1.6–2% in the extrathoracic, and 1.5–2.5% in the inhalable dust fraction. The relative wheat allergen concentration of the inhalable dust fraction collected by the referent stationary IOM sampling was 1.6%.

DISCUSSION

A number of studies have shown that porous foams can be used as a particle size-selective medium for dust sampling applications in chemical and material processing sectors of industry (Kenny *et al.*, 2001b; Teikari *et al.*, 2003), and for sampling of bioaerosols in the microbial contaminated environments (Kenny *et al.*, 1998, 1999). In this study, we showed that respirable and thoracic foams, inserted in an IOM inhalable sampler, can be used for stationary and personal size-selective measurements of wheat flour dust at the workplace, and for quantitative assessment of wheat allergen levels in size-defined dust fractions. Generally, the inhalable dust levels as measured by an IOM sampler with inserted respirable foam and a filter (IOM-RF) or thoracic foam and a filter (IOM-TF) showed a good agreement with inhalable dust levels of the referent sampler. Sampling was, however, performed at only one workplace, and not all dust concentrations could be covered during these measurements. For larger statistical power

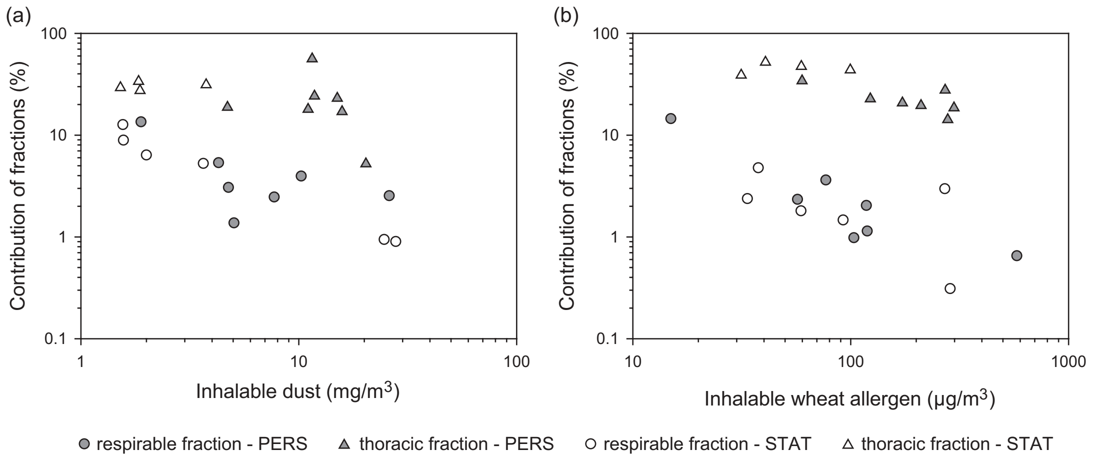

Fig. 4. Contributions (wt %) of respirable and thoracic fractions to: (a) inhalable dust and (b) inhalable wheat allergen—personal (PERS) measurements added to the stationary (STAT) measurements with IOM-RF and IOM-TF.

and more reliable estimation of applicability of IOM-RF and IOM-TF sampling techniques, additional measurements are required at various workplaces, with wider ranges of airborne dust concentrations.

Contrary to inhalable dust levels, inhalable wheat allergen levels obtained with the three different sampling methods showed some discrepancies. Although not proved to be statistically significant, higher wheat allergen yields were observed with the foam-based sampling methods, especially with IOM-TF. This might be due to a higher extraction efficiency of wheat allergen from foams than from filters. In preliminary experiments with a number of foams and filters, we indeed observed that after the second extraction of filters up to 20–25% of allergen could be additionally released and measured in the extracts, while in the second extracts of foams hardly any additional allergen release was detected (data not shown).

A reported problem of foams as size-selective sampling media are inter-batch variations in porosities that can cause changes in D_{50} cut-off values (Kenny *et al*., 2001a). In our study, differences between nominal (specified by the manufacturer) and actual foam porosities varied up to 20 p.p.i. for respirable foams, and up to 10 p.p.i. for thoracic foams. Similarly, differences mostly not exceeding 10 p.p.i. were found in the study by Kenny *et al.* (2001a), both for respirable and thoracic foams. Although we did not investigate the effects of the mentioned variations on real cut-off values of foams, it is very unlikely that variations of this magnitude would considerably change the outcomes of our study.

The results of dual-fraction size-selective measurements with IOM-RF and IOM-TF demonstrated that both dust and wheat allergen predominate in larger particle-size fractions. Wheat allergenic proteins in the flour mill were mainly borne on particles with $D_{ae} > 10$ µm and particles with 4 µm $< D_{ae} \leqslant 10$ µm, accounting for 54.5–77.5% and 18.9–43.2% of the total allergen yield, respectively. Less than 4% of airborne wheat allergen was carried by particles smaller than 4 µm. Similarly, Sandiford *et al*. (1994) reported a predominance of wheat allergen at larger particle sizes.

The contribution of thoracic and, particularly, respirable fraction appeared to decrease with increasing dust and wheat allergen levels. There are two possible explanations for this observation. The first is that respirable or thoracic foams at increased dust levels undergo changes in penetrations characteristics, causing a decrease in actual D_{50} value ('loading effects'). As a consequence, the foam retains too many particles of the smaller particle-size fraction (respirable or thoracic), and the amounts collected on the backup filter are underestimated (Chung *et al*., 1997; Chen *et al*., 1998; Kenny *et al*., 2001b). The second and more likely explanation is that during flour-handling tasks, relatively large quantities of the coarse flour dust particles become airborne, while at lower dust levels smaller particles with a much longer half-life in the air predominate. Therefore, at high dust levels the contribution of coarse particles to the total inhalable dust weight is relatively high. Similar observations have been described by Sandiford *et al*. (1994). Using an eight-stage personal cascade impactor, they found that at high dust levels in a flour mill (GM total dust = 15 mg m^{-3}) particles with $\leqslant$ 6.0 µm diameter contributed with only 20% to the total inhalable dust weight, while in a less dusty area of a bakery (GM total dust = 2 mg m^{-3}) the contribution of these particles was 52%. This implicates that the relative distribution of wheat allergenic particles over specific compartments of a worker's respiratory tract might be different at more or less dusty conditions at the workplace. Since larger particles predominate over the whole range of flour dust concentrations, the upper parts of the respiratory tract will be the primary target organs for the deposition of wheat allergenic particles. There is always, however, a small fraction of fine particles ($D_{ae} \leqslant 4$ μm) capable of reaching the lower parts of respiratory tract, and this fraction might be larger at low dust levels.

CONCLUSIONS

We have demonstrated that dual-fraction size-selective sampling method using porous polyurethane foams can be applied for assessment of wheat allergen levels in health-relevant dust fractions. This method is inexpensive, easily applicable and suitable for personal measurements, which offers an important advantage for large-scale studies of personal exposure. This small-scaled feasibility study showed that wheat allergen can be recovered from the foam matrix and allergen levels determined with an immunoassay. Validation of the novel size-selective sampling technique with larger number of samples from various workplaces is still needed, which should take into account inherent foam-related problems, such as batch-to-batch differences and effects of particle mass loading on penetration characteristics of foams.

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