

Nasal Air Sampling for Measuring Inhaled Wheat Allergen in Bakeries With and Without Facemask Use

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Objective: Occupational asthma caused by flour is common in bakers. We applied novel intranasal air samplers (INAS) to assess wheat allergen exposure and evaluate respiratory protection in bakeries.

Methods: Two models of INAS (INAS-M1 and INAS-M2) were compared with simultaneous personal air sampling of inhalable dust, both with and without facemasks. Wheat allergen levels were measured using a sensitive sandwich enzyme immunoassay. Allergenic particles were immunostained for microscopic visualization. **Results:** Personal air sampling correlated well with INAS-M1 ($r = 0.89$) and INAS-M2 ($r = 0.75$). INAS-M2 collected particles more effectively than INAS-M1. Facemasks reduced inhalation of wheat allergen by 96% and 93% measured using INAS-M1 and INAS-M2, respectively. **Conclusions:** Nasal air sampling can complement personal air sampling to measure short-term exposure and evaluate respiratory protection. To prevent baker's asthma, facemasks may be an effective solution in addition to improving workplaces. (J Occup Environ Med. 2006;48:948–954)

Respiratory disorders caused by exposure to agents at work are a serious occupational health problem. Inhalation of cereal flours such as wheat may cause baker's asthma due to allergen-specific IgE-mediated sensitization and subsequent allergic reactions in the airways of bakery workers.^{1,2} Numerous studies show a high prevalence of occupational asthma and rhinitis at these workplaces and a high risk of sensitization. The prevalence of baker's asthma is usually approximately 5% to 10%, and 20% to 30% of bakers may be sensitized to occupational allergens.¹

In the last decade, considerable progress has been made in finding the determinants of the disease. Although no exclusively dominating major allergens have been identified, it appears that many of the proteins from wheat eluted in aqueous buffers are potentially allergenic or have IgE-binding activity.³

Epidemiologic dose-response studies have clearly demonstrated that the levels of wheat allergen exposure are a highly significant determinant of the risk of developing wheat sensitization and occupational allergy.^{1,4} Therefore, any measures that effectively reduce wheat flour dust exposure, including technical improvements of equipment or products, other work practices, or the use of respiratory protection, should significantly contribute to a lower risk of occupational allergy in the bakery trade. Even a partial reduction of the risk, eg, to 40% to 60%, may be highly important from an occupational health point of view given

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DOI: 10.1097/01.jom.0000232533.04238.3e

the large numbers of individuals working in bakeries and flour mills. In Sweden, around 15,400 persons working in 1600 companies are occupationally exposed to flour dust.

Although technical or logistic measures that prevent or reduce emission are the first choice as an occupational health practice, the use of personal respiratory protection such as facemasks may provide a useful secondary method to effectively reduce personal exposure.⁵ In one report, in which exposure was not measured, two flour-allergic patients with occupational asthma returned to work and used disposable dust respirators for years without having asthma attacks.⁶ Studies evaluating respiratory protection are often performed using test manikins or simulated exposures or respiratory fit test systems for fumes or particles.^{7,8} This may not reflect real work exposure. Furthermore, allergens may be present in varying proportions in work dust.⁹ There are no studies of actual allergen exposure during work in bakeries using respiratory protection.

Collection of aeroallergens on filters, mounted in the breathing zone of subjects carrying portable air pumps, is commonly used to measure personal exposure to several occupational large-molecular-weight allergens such as those from laboratory animals,^{10,11} latex,¹² and flour.⁹ Although this may be considered the gold standard, such methods are not suitable for evaluation of respiratory protective equipment during work, because this would require sampling behind the facemask. Intranasal air samplers (Woolcock Institute of Medical Research [WIMR], Camperdown, NSW, Australia) have previously been used to detect inhaled cat,¹³ mite,^{14,15} and cockroach¹⁶ allergens in houses, and latex⁵ and rodent¹⁷ allergens in work situations. Because nasal air sampling could provide a method to measure personal exposure behind a facemask during work, we developed and adapted the technique¹⁸ to assess exposure to inhaled wheat allergen in bakeries. Two nasal sampler mod-

els were evaluated and compared with simultaneous personal air sampling using pumps. The nasal air samplers were then used to evaluate the protection offered by respiratory facemasks in this occupational environment.

Materials and Methods

Permission for this study was obtained from the ethics committee at Karolinska Institutet. All sampling was performed with the informed consent of the participants.

Development of Nasal Air Sampling Method to Assess Wheat Allergen Exposure

Nasal Air Sampling. Two models of intranasal air samplers (INAS-M1 and INAS-M2) were tested. INAS-M1 collects inhaled particles by impaction onto an adhesive strip (Fig. 1A),¹⁹ and allergens can be detected either by elution and enzyme immunoassay (EIA) or by immunostaining using the halogen assay (WIMR).²⁰ The inner surfaces of the INAS-M2 are coated with a liquid adhesive (WIMR), and captured allergens are eluted and analyzed using EIA (Fig. 1B).

Personal Air Sampling. In parallel with nasal air sampling, inhalable airborne dust was collected on 25-mm polytetrafluoroethylene (PTFE) filters (pore size 1.0 μm ; Millipore, Sundbyberg, Sweden) in Institute of Occupational Medicine (IOM) sampling heads²¹ (SKC Inc., Valley View, PA)

fastened in the breathing zone and connected to portable air pumps (SKC) run at 2 L/minute airflow.

Optimization of the Elution Procedure. To assess the optimal elution procedure,¹⁷ INAS ($n = 172$ nostril samples) and personal air samples ($n = 81$) were collected in a medium-sized bakery during sampling periods of 20 minutes. To obtain parallel samples, yet not disturb production, samples were collected by persons in close proximity to, but not performing, bakery activities. INAS pairs were defined as the two nostril parts from the same sampler, whereas personal air sample pairs were collected simultaneously by two persons standing next to each other.

The standard elution method was to place each air sample filter in 1 mL and nostril sample (adhesive tape for INAS-M1 or silicone holder + inner cup for INAS-M2) in 0.5 mL phosphate-buffered saline (PBS), 0.5% Tween 20, 0.15% Kathon CG (Rohmand Haas, Hydrosupra Kemiservice, Helsingborg, Sweden), respectively, and rotated for 2 hours. The eluate was retrieved and bovine serum albumin (BSA) added to 1% final concentration. Extracts were stored at -20°C . The modifications tested were 1) 1 hour instead of 2 hours of incubation, 2) 0.1% Tween instead of 0.5%, 3) H_2O instead of PBS, 4) addition of sucrose to the eluate to 0.2%, and 5) elution of only the inner cup of INAS-M2.

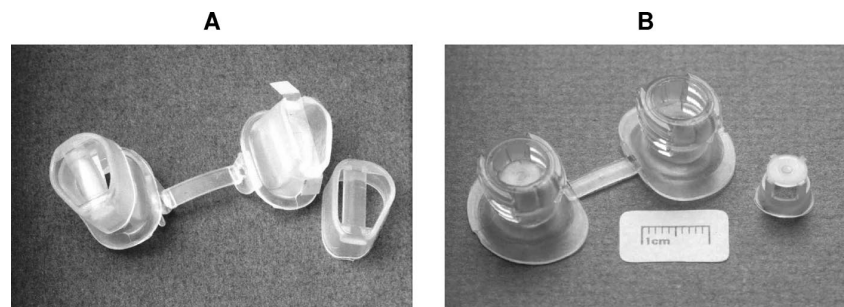


Fig. 1. (A) Intranasal air sampler model INAS-M1. Inhaled dust particles are impacted on the adhesive strip within the sampler. A soft silicone cover holds the adhesive strip in place and increases comfort. (B) INAS-M2 has a hard cup inside a soft silicone holder. The inner surfaces are coated with a liquid adhesive for retention of inhaled particles.

Analysis of Wheat Allergen With Enzyme Immunoassay. Wheat proteins were measured with a polyclonal sandwich EIA using wheat standard extract and rabbit antiwheat antibodies from IRAS. Ammonium sulfate-precipitated antibody at 5 µg/mL in carbonate buffer (pH 9.6) was coated to Maxisorp 96-well plates (Nunc InterMed, Roskilde, Denmark). After blocking 1 hour with PBS, 0.15% Kathon CG, 1% BSA, and washing four times with PBS, 0.05% Tween (PBST), standard dilutions (0.2–20 ng/mL) of wheat allergen and sample extracts in dilution buffer (PBS, 0.05% Tween, 0.15% Kathon CG, 1% BSA) were added in duplicate and incubated for 1.5 hour. Bound wheat protein was detected with subsequent 1-hour incubations with biotinylated antiwheat antibody and streptavidin–alkaline phosphatase (1:500, 3310-10; Mabtech AB, Nacka, Sweden) and finally 30 to 50 minutes with p-nitrophenyl phosphate in 1M diethanolamine, pH 9.8. Absorbance was read at 405–605 nm and analyzed by Softmax 2.6 (Molecular Devices, Sunnyvale, CA) using four-parameter curve fit. Samples with a coefficient of variation >10% between duplicates were reassayed.

Unexposed samples (10 INAS-M1, 3 INAS-M2, 12 air sample filters) eluted together with exposed samples and four control samples from nonbakery environments (house and stable) were included as controls.

Evaluation of Nasal Air Sampling Method to Assess Wheat Allergen Exposure

Evaluation of Intranasal Air Sampler Performance. To assess the reproducibility of the INAS sampling within pairs, nine INAS-M1 nostril pairs eluted using the standard method and analyzed using EIA were compared. To compare nasal air sampling and personal air sam-

pling, wheat allergen exposure measured with EIA as assessed with INAS-M1 ($n = 54$) or INAS-M2 ($n = 29$) sampling was compared with the exposure assessed simultaneously with personal air sampling in the same subject. The relative collection efficacy of the two models of INAS was compared by calculating the ratio between total wheat allergen (in nanograms) collected using INAS-M1 or INAS-M2 and the parallel personal air sample.

Analysis of Wheat Allergen-Carrying Particles by Immunostaining of INAS-M1 Samples. Particles containing wheat allergen were visualized in nine INAS-M1 bakery samples using the halogen assay mainly as described previously.¹⁷ Briefly, adhesive tapes were laminated with 1.0-µm pore size PVDF membrane and eluted overnight in 0.2 M borate buffer. Tapes were immunostained by incubation for 2 hours with rabbit antiwheat antibody (IRAS) in 2% skim milk/PBST at 1 µg/mL followed by polyclonal alkaline phosphatase-labeled goat antirabbit IgG (111-055-003; Jackson Immuno Research Laboratories) at 0.15 µg/mL and finally BCIP/NBT (BioRad Laboratories AB, Sundbyberg, Sweden). The number of stained allergenic particles (with a halo) was counted under 100× magnification. Positive (bakery dust) and negative (blank) laminated adhesive controls and four samples from nonbakery environments were included. Nonrelated antibodies (against mouse allergen) were also included to confirm specificity of the staining method.

Using Intranasal Air Sampling to Measure Task Exposure in Bakeries. Bakers in four bakeries (one small and three medium size) carried INAS-M1 ($n = 13$) in parallel with IOM air samplers ($n = 12$) during different tasks for sampling periods of 23 to 35 minutes to assess and validate task-related exposure measurement using INAS.

Evaluation of Respiratory Protection Provided by Facemasks in the Bakery Environment Using Intranasal Air Sampling

The efficacy of respiratory protection (P2 particle filter facemask, 3M, model 9322 with air vent) was determined using INAS-M1 or INAS-M2 in 22 subject pairs standing side by side during 20-minute sampling periods, one person wearing a facemask and the other without. To verify that exposure was similar within pairs, allergen quantities collected simultaneously using personal air samplers were compared.

Statistics

Calculations were performed using Microsoft Excel, Statview (SAS) and SigmaStat 3.0 (SPSS). When values were not normally distributed, values were log transformed. Ratios between values from parallel nostril or air sample pairs eluted using the standard method were compared with elution variants were tested with one sample analysis and calculated with 95% confidence intervals. Correlation between sample values was tested using linear regression on log-transformed values. A P value <0.05 was considered significant.

Results

Development of Nasal Air Sampling Methods to Assess Wheat Allergen Exposure

There were no significant differences between the different elution buffers or times for any of the INAS (Table 1) or for the personal air filter sample pairs (data not shown). However, elution of the whole INAS-M2 sampler gave on average a 2.6-fold higher wheat allergen recovery ($P = 0.0016$) compared with elution of only the inner cup. In the further comparisons, therefore, all samples but these were included regardless of elution method.

TABLE 1

Summary of Optimization of Elution Methods for INAS-M1 and INAS-M2*

		Mean Ratio (95% CI)	P
Standard vs 0.1% Tween			
INAS-M1	n = 10 pairs	1.26 (0.93–1.59)	0.11
INAS-M2	n = 10 pairs	1.14 (0.68–1.61)	0.50
Standard vs Aq			
INAS-M1	n = 10 pairs	1.10 (0.85–1.36)	0.39
INAS-M2	n = 10 pairs	1.04 (0.80–1.28)	0.71
Standard vs 1 Hr			
INAS-M1	n = 10 pairs	0.92 (0.84–1.01)	0.06
INAS-M2	n = 10 pairs	1.07 (0.71–1.43)	0.66
Standard vs Sucrose			
INAS-M1	n = 10 pairs	1.07 (0.90–1.24)	0.36
INAS-M2	ND		
Standard vs Cup			
INAS-M1	ND		
INAS-M2	n = 16 pairs	2.64 (1.73–3.56)	0.0016

*The ratio (95% confidence interval) between the values obtained with the standard elution method (phosphate-buffered saline, 0.5% Tween, 0.15% Kathon, 1% bovine serum albumin, 2 hrs of incubation) compared with elution in which the changed factor is indicated.

INAS indicates intranasal air sampler; CI, confidence interval; ND, not done.

Evaluation of Nasal Air Sampling Method to Assess Wheat Allergen Exposure

Evaluation of Sampling Performance. The levels of allergen collected by the two parts of nostril pairs did not differ ($P = 0.91$); mean ratio ($\pm$ standard deviation [SD]) was 1.1 (± 0.3), which allowed comparisons between nostril pairs eluted in different ways.

In the comparisons between parallel personal IOM air sampling and nasal air sampling, the correlation between INAS-M1 and IOM air sampling was $r = 0.89$; $r^2 = 0.79$, standard error of estimate = 0.30 (Fig. 2A), and the correlation between INAS-M2 and IOM air sampling $r = 0.75$; $r^2 = 0.56$, standard error of estimate = 0.31 (Fig. 2B). For sample pairs eluted only using standard elution, $r = 0.89$, $P < 0.0001$ for INAS-M1 and IOM ($n = 34$), and $r = 0.72$, $P = 0.002$ for INAS-M2 and IOM ($n = 15$), respectively.

The INAS-M2 appeared to collect more wheat allergen than the INAS-M1. The mean ($\pm$ SD) ratio (nasal air sampler/IOM air sampler) between total amounts of wheat allergen collected with INAS-M1 compared with the IOM air sampler was 1.5 (± 1.0),

whereas the ratio for INAS-M2 was 4.8 (± 4.0), indicating that the INAS-M2 collected approximately 3.2-fold more inhaled allergen than the INAS-M1.

All nasal sample negative controls were below the detection limit, as were all but two air sample controls (27 and 34 ng/mL, to be compared with a mean 4078 ng of measured wheat allergen/mL in bakery samples). The halogen assay controls showed no halos except for one collected during housecleaning, which also had 4 ng/hour in the EIA.

Analysis of Wheat Allergen-Bearing Particles by Halogen Immunostaining of INAS-M1 Samples. Of the halogen immunostained samples ($n = 9$), analysis was possible for three samples collected during low exposure (Fig. 3), because the rest were overloaded and individual halos could not be distinguished. During cake baking without a mask (Fig. 3B), in total, 150 allergen-bearing particles were collected and counted in the sample compared with only six particles with halos in the sample collected while wearing a P2 filter facemask.

Using Nasal Air Samplers to Measure Task Exposure in Bakeries.

Bakers carried INAS-M1 samplers during work for both EIA and halogen analysis. The distribution of inhaled wheat allergen captured by nasal air sampler (and personal air samplers) differed between work tasks in the bakeries (Fig. 4).

Nasal Air Samplers to Evaluate Personal Respiratory Protection in the Bakery Environment

The disposable P2 facemasks reduced the amount of inhaled wheat allergen by a mean of 96% as determined by INAS-M1 and by a mean of 93% as determined by INAS-M2 (Fig. 5A, B).

The pairs of samples collected on filters with the person-carried pumps, while subjects wore nasal air samplers either with or without facemask, were highly correlated ($r = 0.95$) with very similar exposure levels within pairs, the difference (SD) between log value within pairs = -0.067 (± 0.176).

Discussion

Measuring personal exposure to airborne allergens is instrumental in understanding the association between allergen exposure and allergic disease and in occupational settings when trying to implement safer work practices. One important question to answer is how effectively various strategies (ventilation, personal protective equipment, and so on) reduce exposure in bakers, who have a high risk of developing work-related airway symptoms. We have applied INAS to measure inhaled wheat allergens in bakeries, which has not previously been done. Of the two tested models, the newly developed INAS-M2 had not been used before in the occupational setting.

The very good correlation between left and right nostril sample values allowed comparisons between nostrils from the same sampler. In comparisons between different elution buffers or times, no significant differences were found, suggesting that several methods can be used that effectively release wheat allergen

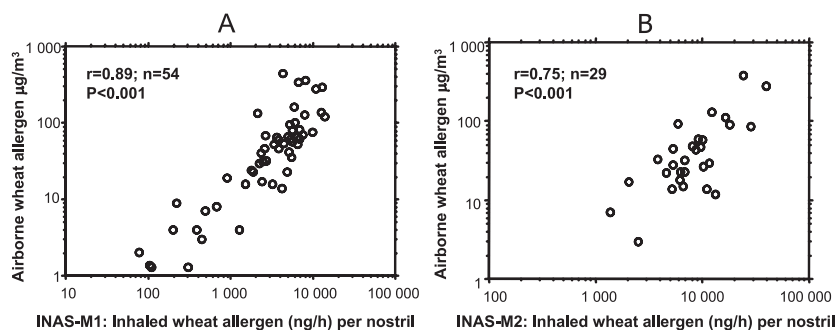


Fig. 2. Correlation between wheat allergen collected simultaneously using personal (IOM) air sampler and mean nostril value of nasal air samplers INAS-M1 (A) and INAS-M2 (whole sampler eluted) (B), respectively.

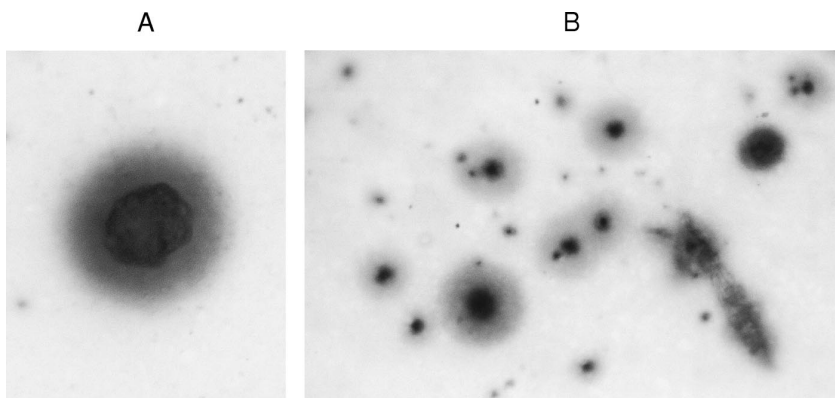


Fig. 3. Halogen immunostaining, microscope images at 100 $\times$ magnification. (A) Nasally inhaled wheat allergen-bearing particle collected on adhesive tape using INAS-M1, laminated with PVDF membrane, and immunostained using halogen assay with polyclonal antibodies against wheat proteins (IRAS). (B) Part of laminated and halogen-stained INAS-M1 sample collected during 30 minutes of cake baking without a mask.

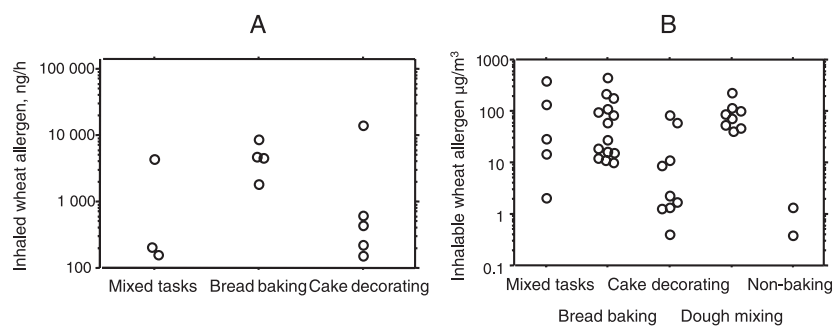


Fig. 4. Inhaled wheat allergen (nanograms per hour) collected using nasal air samplers (A) and inhalable wheat allergen (ng/m^3) collected using person-carried pumps (B) by personnel with different work tasks. One nasal air sample collected while wearing a facemask and decorating a cake (5 pg/hour) is not shown in A.

from the adhesive. We also tested whether eluting only the inner cup of bakery samples was possible, as indicated by preliminary data from other environments, or would result in loss of material. However, elution of the whole INAS-M2 sampler

unit resulted in on average 2.6-fold higher recovery of wheat allergen, ie, inhaled allergen was dispersed within the whole sampler. Thus, for bakeries and other dusty workplaces, elution of the whole unit should be recommended.

We found that for wheat allergen, the correlations between results of personal IOM air sampling and both INAS-M1 ($r = 0.89$) and INAS-M2 ($r = 0.75$) were high. The r values can, however, not be directly compared, because the samples were collected over slightly different exposure distributions. A previous study in animal facilities showed a similar correlation between personal air sampling and INAS-M1, $r = 0.8$.¹⁷ The IOM sampling heads are designed to follow the inhalable convention for health-relevant particle size fractions²¹ at a specified flow rate. The INAS collect particles aspired by human inhalation, fluctuating in rate, collecting larger particles ($>10 \mu\text{m}$) with high efficiency, but especially the INAS-M1 collects particles $<5 \mu\text{m}$ with less efficiency, depending on flow rate. The good correlation between methods may reflect the size distribution of allergenic particles: wheat allergens in bakeries are mainly found on larger particles.^{22,23} The clinical relevance of the sampled particles was shown when the INAS-M2 was recently tested as a nasal filter to capture inhaled pollen and was found to reduce symptom expression in pollen allergic subjects with rhinitis.²⁴

For maximum comparability and power, the sampling design required parallel sampling of two persons exposed to as similar levels as possible. It was not realistic to request that bakers should repeatedly leave their workstations to work in pairs next to a colleague for short sampling times. Therefore, to enable, for instance, the evaluation of using particle face-masks, while not disturbing production in the bakeries, most of the samples were collected by persons standing side by side, in close proximity to the bakers, but not actively baking. A wide range of exposure levels was sampled in this manner that was not significantly different from that of samples collected during bakery tasks (data not shown) and thus representative for the real work-

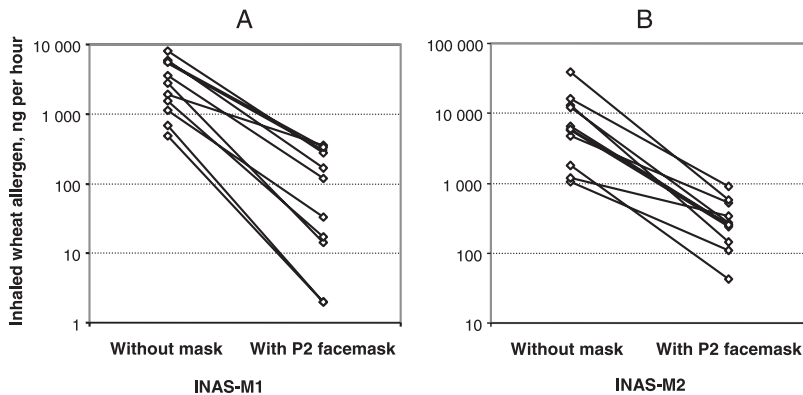


Fig. 5. Parallel sampling of subject pairs side by side in bakeries without or with disposable P2 facemasks (model 9322, 3M). The masks reduced the amount of inhaled wheat allergen by a mean 96% ($P = 0.001$) for INAS-M1 (A) and by a mean 93% ($P = 0.016$) for INAS-M2 (B).

related exposure and allowing generalization of the results.

We found that the INAS-M2 collected 3.2-fold more wheat allergen than INAS-M1. INAS-M2 samplers have a much larger sample collection area than INAS-M1 and are therefore less likely to be overloaded during high exposure activity, losing less allergen. Indeed, overloading of INAS-M1 was shown in the halogen-stained samples during high exposure activities so that individual halos could not be counted. The INAS-M1 have previously mainly been used in domestic situations in which loading is probably less than in occupational environments. The sensitivity of allergen measurement assays may limit the possibility of measuring in low exposure or short-term sampling situations. In the bakery environment, overloading of nasal sampling surfaces is instead a risk, and short sampling times are necessary, especially for visualization of allergenic particles with immunostaining using INAS-M1.

However, using halogen staining of bakery allergens, microscopy photos can illustrate that even simple disposable facemasks significantly reduce inhalation of allergens and particles, and that in high exposure work situations, thousands of allergen-bearing particles might otherwise be inhaled per hour. It is noteworthy that some of the few particles collected behind the mask

were probably too large to have passed through the matrix of the mask's filtering material and rather may have leaked around the edges.

Personal sampling of airborne allergens on filters carried in the subject's breathing zone using person-carried pumps is well-established in occupational exposure assessment. However, the constant flow rate (2 L/minute) is adjusted to collect the inhalable dust fraction and does not reflect exposure when breathing is altered due to change of workload, effects of drafts and thermals, and so on. By using nasal air samplers, personal exposure assessment is possible without expensive, heavy equipment and difficulty.

The samplers have been used previously in several studies both in the workplace and in homes, and have generally been well tolerated. Although our bakers were more reluctant to wear INAS than carry personal air samplers, those who volunteered tolerated the INAS well and had no difficulties following the instructions. The INAS-M2 model is especially comfortable to wear, and neither causes more than little or unnoticeable limitation of nasal airflow.¹⁹ Because sampling is through nasal breathing, limitations are that chronic mouth breathers or persons with nasal blockage (possibly caused by the work environment!) are not suitable for sampling, and related to this, sampling during very heavy

work is unsuitable if subjects switch to oral breathing.

Because the nasal air samplers can be worn behind respiratory protection equipment such as facemasks, unlike IOM or other personal sampling heads, they can be used to estimate the efficacy of personal protective equipment in real work situations. The nasal air samplers showed reduced exposure to wheat allergens by approximately 95% when P2 facemasks were used. This may be an overestimate if the sampling efficiency is reduced at high loads. Another approach to measuring the protective factor of P2-type masks, using optical particle counters, was recently published.²⁵ Here, air was simultaneously sampled in real time both inside and outside masks while these were worn during corn harvesting; the protective factor was similar to that described here.

In conclusion, we found that it was possible to assess short-term exposure to inhaled wheat allergens in bakeries using two types of nasal air samplers (INAS-M1 and INAS-M2), of which INAS-M2 collected allergen more effectively. Although especially INAS-M1 may underestimate the amount of inhaled allergen carried on particles smaller than 5 μm , because the correlation with personal air sampling is good and calibration to air sampling possible, and wheat allergen is found on mainly large particles, this system may be an alternative method for measuring occupational exposure to flour allergens. Polyclonal antibodies could also be used in the halogen assay to visualize wheat allergen-carrying particles. Nasal air samplers, which have been applied successfully to measure both indoor home allergens (notably cat) and latex and rodent allergens, can thus also be applied to measure exposure to wheat allergen in bakeries (especially during short task-related exposure periods of, eg, <30 minutes). When applied to evaluation of facemasks, P2 particle filter disposable facemasks significantly reduced

amounts of inhaled wheat allergen by approximately 95%.

Acknowledgments

The authors thank the participating bakers and bakeries for all their help with the sampling. This study is part of the MOCALEX project (Measurement of Occupational Allergen Exposure) financed by the European Community 5th framework "Quality of Life" program project QLK-CT-2001-00432.

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