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Wheat Antigen Content of Inhalable Dust in Bakeries: Modeling and an Inter-Study Comparison

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The purposes of this study were (a) to compare wheat antigen content of inhalable bakery dust in Canada and the Netherlands and, (b) to evaluate the validity of dust exposure measurement as a surrogate of specific antigen exposure. Wheat antigen exposure data from the studies of Canadian and Dutch bakeries were used to explore the correlation between dust and antigen levels, and identify factors explaining variability of the antigen content of bakery dust. Direct comparison and pooling of the data were possible because the same antigen assay was used in both studies. Wheat antigen exposure samples totaling 544 were available, all originating from personal full-shift monitoring of bakery workers. The wheat antigen content of bakery dust varied greatly between the two studies and with different products within each study. Bakery dust from the Canadian study had a much higher wheat antigen content. Therefore, the inter-study difference in the wheat antigen content of bakery dust was by far the most significant in undermining the validity of the use of dust levels as a surrogate measures of wheat antigen exposure. The production of croissants, wheat bread and buns, puff pastry, bagels, and cinnamon buns was associated with increased wheat antigen content of bakery dust, while in rye bread production, bakery dust with less wheat antigen content was emitted. Although we can, in part, account for the pattern of variability in the wheat antigen content of bakery dust (explaining 49% of it), we concluded that the effort involved in modeling it accurately would probably exceed that expended in direct measurement of the antigen level. Therefore, for the exposure assessments carried out for the purpose of managing occupational risks of aeroallergen exposure in bakeries, we recommend the use of analytical techniques that can directly measure antigen exposure.

Keywords Bakery, Exposure Assessment, Exposure Modeling, Flour, Occupational Exposure, Wheat Antigen

The measurement of exposure to specific flour aeroallergens in occupational settings presents a considerable challenge because bakery dust is a complex mixture of particles and macromolecules, many of which have allergenic potential. Until recently, no analytical methods of measuring exposure to specific allergens were available. However, the adaptation of classical immunological techniques, such as the enzyme-linked immunosorbent assay for use in industrial hygiene^(1–4) has enabled researchers to overcome some of these difficulties. The currently labor-intensive and costly nature of these analytical techniques raises the question of whether measurement of dust levels is sufficient as a surrogate exposure measure.

The relationship between total particle levels and specific allergen exposure has been explored by various researchers. Tee et al.⁽³⁾ observed a linear relationship between gravimetric dust measurements in the range of 5 mg/m³ to 10 mg/m³ and flour allergen concentrations. The flour allergens in that study were assayed in radioallergosorbent inhibition assay using IgE antibodies from serum of bakery workers as a detection system and the extract of mixed flour as an inhibitor. Similarly, Nieuwenhuijsen et al.⁽⁵⁾ found a strong correlation between personal average work-shift wheat allergen and inhalable dust exposure levels (Spearman correlation coefficient of 0.86). Nevertheless, in an earlier article the same authors⁽⁶⁾ reported that the strength of the relationship varied greatly among locations within a bakery. Studies by Nieuwenhuijsen et al. used inhibition immunoassay with polyclonal rabbit anti-wheat IgG antibodies as the detection system. Houba et al.⁽¹⁾ found that wheat antigen content of bakery dust (assayed in inhibition immunoassay using polyclonal anti-wheat IgG antibodies derived from sera of bakery workers) varied among job titles within a production line.

The specific objectives of this investigation were (a) to examine how the wheat antigen content of inhalable bakery dust

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varies between different types of production in the two different countries and, (b) to evaluate the appropriateness of using inhalable dust as a surrogate of antigen exposure among bakery workers.

MATERIALS AND METHODS

Bakers, Bakeries, and Exposure Measurements

Canadian Study

Ninety-six bakery workers, employed in seven different bakeries (two large and five small ones), participated in the study between October 1995, and April 1996. An attempt was made to obtain a representative sample of working conditions in the bakeries. Two side-by-side, full-shift inhalable dust samples were obtained by a seven-hole sampler (JS Holdings Ltd., United Kingdom) from each study participant on a single occasion. Air was drawn through 25-mm-diameter Teflon filters with PVC support and 0.45 μm pore size (Gelman Sciences #66148) at a calibrated flow rate of 2 L/min. by means of a portable pump. Inhalable dust was measured gravimetrically. Wheat antigen in each dust sample was determined, as will be described in detail. Averages of the side-by-side measurements were used as estimates of personal full-shift exposures. There were no systematic differences in inhalable dust and wheat antigen levels between side-by-side samples,^(7,8) thus no information was lost due to the averaging procedure. Detailed descriptions of the relationships among the exposure measures, production characteristics, and tasks have been published elsewhere.^(7,8)

Dutch Study

Two hundred and thirty bakery workers, employed in 5 large industrial bakeries and 16 small bakeries participated in the study between April 1991, and November 1994. Based on job description and type of bakery, 22 occupational titles were identified. Within each occupational title, randomly selected bakers were asked to participate in personal full-shift air sampling. PAS-6 sampling heads (Wageningen University, the Netherlands) were used to collect the inhalable fraction of dust onto Teflon filters (Millipore; pore size 1.0 μm) by drawing air through the sampling head at 2 L/min. by means of a portable pump. Inhalable dust was determined gravimetrically, and wheat antigen content of the inhalable dust was determined via an immunoassay (see following section for details). Details of the sampling methods, production characteristics, and job descriptions can be found elsewhere.⁽⁹⁾

Wheat Antigen Assay

For wheat antigen, inhibition enzyme-linked immunosorbent assay (ELISA) was used. Each filter (including field blanks) was extracted in phosphate buffered saline. The extracts were frozen until they were assayed for wheat antigen content. Pooled sera

from 59 bakery workers with high IgG₄-titers for wheat flour extract were used as anti-wheat antigen antibodies.⁽¹⁾ In immunoblotting experiments most allergens recognized by this IgG₄-serum pool were also bound by IgE-sera from the same individuals.⁽¹⁾ No cross-reactivity was observed between anti-wheat antigen sera and other ingredients in baking mixtures (baker's yeast, fungal α -amylase, egg) or mold extracts.⁽¹⁾ The same serum pool was used in both studies. A detailed description of these analytical methods has been published elsewhere.^(1,8) The key differences in the way assays were performed were that (a) ELISA plates used in Canada had higher protein binding capacity and (b) a wider range of concentrations in the lower section of the standard curves was used in the Dutch analyses. Costar No. 9018 ELISA plates (Cambridge, Massachusetts) were used in the Canadian study and Greiner No. 655092 ELISA plates (Nuertingen, Germany) were used in the Dutch study. The Costar ELISA plates, according to the manufacturer's specifications, have a higher protein binding capacity than Greiner ELISA plates.

For an inter-study comparison between wheat antigen assays, 10 water soluble extracts were analyzed for wheat antigen in both laboratories. The extracts were derived from the dust samples representing the entire range of inhalable dust concentrations and types of production encountered in the Canadian study.

To estimate the effect of using different ELISA plates on the wheat antigen assay, a test was performed in which a series of twofold dilutions (0.0095 to 40 $\mu\text{g/ml}$) of the wheat antigen standard was assayed concurrently on either high- or low-capacity protein binding plates. The test was performed twice.

We compared the affinity of the detection antibodies derived from sera of the Dutch bakery workers for wheat antigen occurring in Canadian commercial flour with their affinity for the wheat antigen found in the Netherlands. To do that, extracts of the Canadian whole wheat flour (which had no fungal α -amylase added) were prepared in the following manner. Five hundred mg of wheat flour was weighed and placed in a 50 ml centrifuge tube with 5 ml 0.15 M phosphate buffered saline (PBS, pH 7.4, Sigma P4417, Sigma Chemical, St. Louis, Missouri), the same buffer used for sample extraction. The tube was vortexed for two minutes, sonicated for two minutes, vortexed for five minutes, and sonicated for a final two minutes. The flour slurry was centrifuged at 5000 $\times g$ for 15 minutes and the resultant supernatant was filtered through a 0.22 μm syringe filter. The filtered extract was then dialyzed against several changes of distilled water over an eight-hour period. The dialyzed antigen was stored frozen until use. The Canadian wheat antigen, unlike the Dutch one, was not freeze-dried prior to use. Protein content of the flour extract was estimated using BioRad Protein Assay 500-0006 (BioRad Laboratories, Mississauga, Ontario) and was diluted to 5 $\mu\text{g/ml}$ for the coating antigen and to 20 $\mu\text{g/ml}$ for the highest standard for the calibration curve. The resultant Canadian wheat antigen extract and the supplied

Dutch wheat antigen extract were used side-by-side to construct standard curves for the assays of wheat antigen content of serial dilution of the Canadian wheat antigen extract (0.1 $\mu\text{g/ml}$ to 20 $\mu\text{g/ml}$). In all other respects, except for the use of the Canadian wheat antigen in the coating step of the assay, these wheat antigen assays did not differ from the procedure used to ascertain personal wheat antigen exposure.

Statistical Analysis

For data analyses in the Canadian study, values for exposure measurements below the limit of detection were replaced by the analytic limit of detection divided by the square root of 2.⁽¹⁰⁾ In the Dutch study, values below the detection limit were replaced by two-thirds of this limit.⁽⁹⁾

We used a paired t-test to measure the agreement between antigen concentrations measured by Canadian and Dutch laboratories in the same samples. Under the null hypothesis (no difference between laboratories), we expected the mean difference to be zero in a two-tailed test at 0.05 level. The expected and reported concentrations of wheat antigen (in testing the affinity of the detection antibodies) were compared by computing percent-differences, a two-tailed paired t-test (evaluated at 0.05 level), and a correlation coefficient.

The wheat antigen content of bakery dust was calculated for each inhalable dust sample as the ratio of the mass of antigen in a sample to the mass of dust collected during exposure monitoring. The distribution of antigen content of the bakery dust was examined using frequency histograms and cumulative probability plots, to assess the need for transformation prior to statistical modeling. Wheat antigen content of bakery dust followed a skewed distribution, which was normalized by logarithmic transformation. Hence, the natural logarithms of bakery dust antigen content were used in all analyses to stabilize variance. Relationships between dust and wheat antigen were examined graphically for each study. Relationships between products and bakery dust antigen content were examined in one-way random effect ANOVAs constructed separately for each country. Every ANOVA was followed by a multiple range test to identify pairs of significantly different means (0.05 level, Bonferroni test).

Data from both studies was pooled and factors explaining variability in wheat antigen content of bakery dust were examined in multiple linear regression. The goal of the analysis was to examine whether differences in bakery dust antigen content were due to differences in products manufactured in the two countries, or were the result of systematic differences due to other country-related factors. The possibility of combining variables across the studies was explored to maximize the likelihood of detecting associations. Product variables from the two studies were combined if they had similar regression coefficients in multivariate analyses (0.05 level) and the products appeared to be similar. The stepwise procedure ($p \leq 0.05$ to enter the model; $p \leq 0.10$ to remain in the model) was used to select significant

variables. Statistical analyses were performed utilizing SPSS (v. 6.1.2) and Microsoft Excel 7.0 software.

RESULTS

Dust and Wheat Antigen Levels

For wheat antigen, the limit of detection was quantified at 1 $\mu\text{g/m}^3$ in the Canadian study, and at 0.050 $\mu\text{g/m}^3$ in the Dutch study. Twenty percent ($n = 19$) of Canadian and sixteen percent ($n = 70$) of the Dutch wheat antigen samples were below the respective limits of detection. All field blanks in both studies were below the limit of detection. The geometric mean of inhalable bakery dust exposure was 2.1 mg/m^3 (range: 0.1–110 mg/m^3) in the Canadian study and 1.0 mg/m^3 (range: 0.1–33.7 mg/m^3) in the Dutch study. The geometric mean of wheat antigen exposure was 21 $\mu\text{g/m}^3$ (range: <1–1018 $\mu\text{g/m}^3$) in the Canadian study and 0.68 $\mu\text{g/m}^3$ (range: <0.05–1018) in the Dutch study.

The correlation between wheat antigen and inhalable dust levels was the same in both studies (Figure 1). The figure shows that the bakery dust in the Canadian study had a higher antigen content. There appears to be a good and virtually identical correlation between wheat antigen and inhalable dust levels in both studies.

Inter-Study Comparison of Wheat Antigen Assay

There was reasonable agreement between wheat antigen assay results reported by the Canadian laboratory and analyses performed by the Dutch laboratory, as indicated by the results of the two-tailed paired t-test ($p < 0.25$), with the Dutch laboratory reporting, on average, higher wheat antigen concentration (mean difference of 294 $\mu\text{g/ml}$). Rank correlation between the results of the two laboratories was quite high ($r = 0.82$, $p < 0.007$). Nevertheless, it should be noted that estimates derived from the Canadian laboratory appeared to be higher at low antigen concentrations (by a factor of 2), but they are lower at high antigen concentrations (by a factor of about 8, based on two samples).

There appeared to be a consistent shift in inhibition curves constructed with different ELISA plates (Figure 2). This suggested that the use of high-capacity protein binding plates (in the Canadian study) resulted in the observed wheat antigen levels being twice as high as those inferred with the use of low-capacity protein binding plates (in the Dutch study). Curiously, this effect of plates is in the same direction and of the same magnitude as that observed in the comparison of the wheat antigen levels found in the same samples in both laboratories when the antigen concentrations were low (see previous paragraph).

In the test of the affinity of the anti-wheat antibodies for Canadian and Dutch flour, there was very little difference (4%, on average) between the results of wheat antigen assay results performed with either Canadian or Dutch wheat antigen standards. The correlation between the results of the immunoassays was 0.82 and the t-test indicated that there was no statistical pairwise difference in the results of assays ($p < 0.69$, two-tailed).

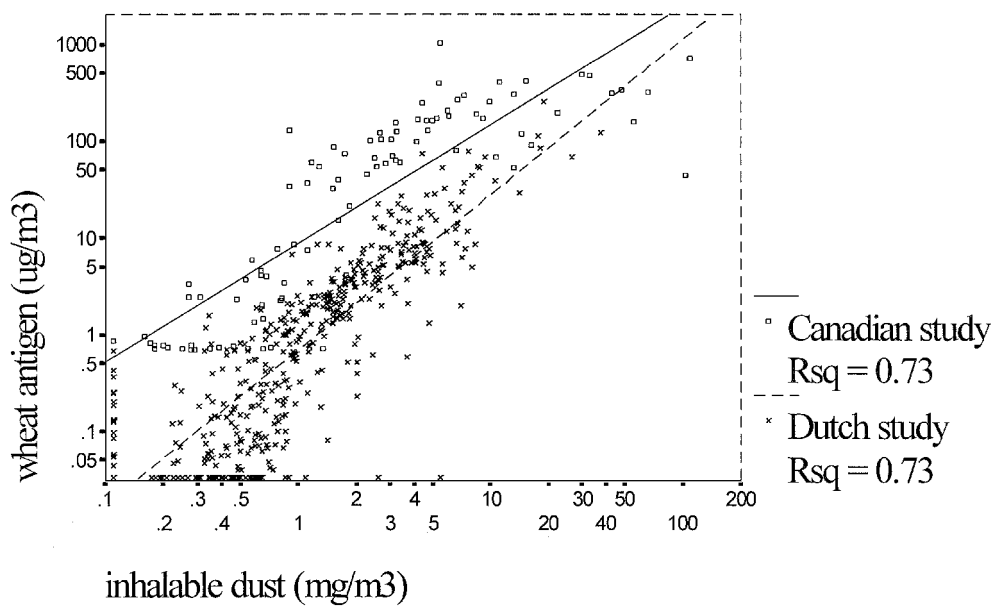


FIGURE 1

Correlation between logarithms of wheat antigen and inhalable dust exposure levels (R_{sq} = coefficient of determination). *Note:* Slopes are not statistically different: slope in Canadian study = 1.22 (standard error = 7.6), slope in Dutch study = 1.60 (standard error = 5.40). Apparent difference in slopes is due to higher limit of detection in the Canadian study.

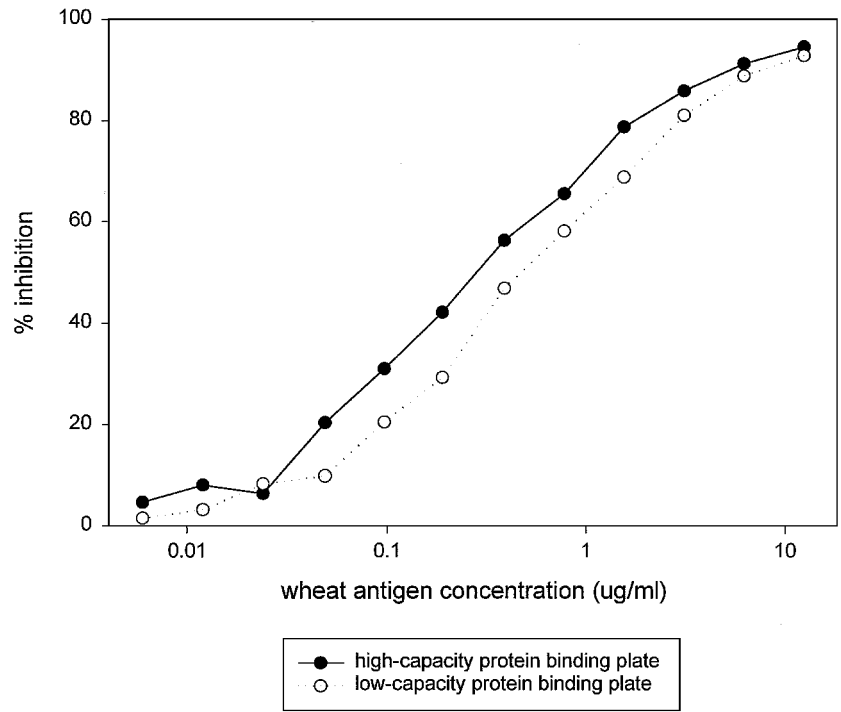


FIGURE 2

Standard curves for the wheat antigen assays constructed while using two different ELISA plates. *Note 1:* Percent (%) inhibition reflects the change in optical density of colorimetric reaction in the well of an ELISA plate attributable to wheat antigen in the unknown. *Note 2:* High-capacity protein binding plate = Costar No. 9018 (used in the Canadian study); low-capacity protein binding plate = Greiner No. 655092 (used in the Dutch study).

TABLE I
Wheat antigen content of bakery dust in different types of production^A

Products	<i>n</i>	Median wheat antigen content of bakery dust (μg wheat antigen/mg dust) (95% CI)
Canadian study		
Croissants	8	37 ^{B,C} (29–48)
Puff pastry	14	15 (7–30)
Cinnamon buns	6	14 (3–61)
Bagels	9	14 (7–27)
Crumpets	6	7 (3–16)
Wheat bread and buns	17	8 ^B (3–17)
Cakes and pastries	25	8 ^C (5–12)
Dutch study		
Wheat bread	244	1.1 ^D (0.9–1.3)
Rusk	76	0.5 (0.3–0.6)
Pastry	92	0.5 (0.4–0.7)
Rye bread	25	0.1 ^D (0.07–0.2)

^AResults of two separate one-way ANOVAs: one for each study. 95% CI = 95% confidence interval.

^{B,C}Statistically different pairs of values in Canadian study (0.05 level, Bonferroni test).

^DStatistically different pair of values in Dutch study (0.05 level, Bonferroni test).

Country and Product Effects on the Wheat Antigen Content of Bakery Dust

There appears to be a relationship between the bakery dust content of antigens and products manufactured within each study (Table I). Products such as wheat bread in the Dutch study and croissants in the Canadian study, were associated with the emission of bakery dust with a higher wheat antigen content (two- to tenfold difference).

The results of the analyses of combined exposure data from the two studies are presented in Table II. Variables describing the production of wheat bread and buns were pooled across the two studies, because they appeared to have the same effect (i.e., identical regression coefficients in multivariate analyses). We observed a large (twenty-two-fold) difference in the wheat antigen content of bakery dust between the two studies, even after correcting for differences in the type of products manufactured. The role of various product types in determining wheat antigen content of bakery dust, suggested by the results presented in Table I, was confirmed and further clarified by the combined analyses. Namely, higher than average wheat antigen content of bakery dust was observed in the production of croissants, wheat bread and buns, puff pastry, bagels, and cinnamon buns.

DISCUSSION

Dust and Wheat Antigen Levels

Both dust and antigen exposures were higher in the Canadian study. Differences in the types of products manufactured can, at

TABLE II
Factors influencing wheat antigen content of inhalable bakery dust ($\ln(\mu\text{g}$ wheat antigen/mg inhalable dust): model based on 544 samples from Canadian and Dutch studies

	B ^A (95% CI ^B)	Relative antigen content ^D
<i>Factors/products associated with increasing antigen content</i>		
Canadian study	2.5 (2.1 to 2.8)	22
Croissant	1.8 (0.9 to 2.7)	12
Puff pastry	0.9 (0.2 to 1.6)	5
Cinnamon bun	0.9 (−0.1 to 1.9)	4
Bagel	0.8 (−0.02 to 1.7)	4
Wheat bread and bun	0.7 (0.5 to 0.9)	4
<i>Factors/products associated with decreasing antigen content</i>		
Rye bread	−1.5 (−2.0 to −1.0)	0.4
Dutch study/other products ^C	−0.7 (−0.8 to −0.5)	1
% explained variability in dust antigen content ^E		49

^ARegression coefficient.

^B95% confidence limits of a regression coefficient.

^CConstant term in regression (intercept).

^DEstimated from regression equation as (antigen content associated with a 'factor' if it was present by itself/ 'background' antigen content).

^EEstimated from coefficient of determination (R^2), adjusted for sample size.

least in part, account for this. For example, (a) rye bread production, associated with low exposure levels, was not observed in the Canadian study, and (b) dough brakers, which were observed to lead to high dust levels in Canadian study,⁽⁷⁾ were not used by bakers in the Dutch study. The difference cannot be accounted for by use of different dust sampling heads to collect inhalable fraction of dust in the two studies, because these sampling heads have been demonstrated to have similar collection efficiencies.⁽¹¹⁾ More importantly, however, this comparison of exposure levels allows us to infer that, because a flour antigen exposure-sensitization relationship was observed in the Dutch study,⁽¹²⁾ the risk of sensitization to wheat antigen among Canadian bakers is at least as high.

The correlation between inhalable dust and wheat antigen levels found in both studies was very similar to the one reported by Nieuwenhuijsen et al.,^(5,6) despite the use of different analytical techniques to assay the antigen. Wheat antigen can be expected to be present in flour (because wheat flour was used predominantly in both studies), and thus originates from the principle source of dustiness: suspension of flour in the air. This would account for a strong positive correlation between wheat antigen and inhalable dust levels.

Inter-Study Comparison of Wheat Antigen Assay

According to the results of the analysis of 10 water-soluble extracts in Canadian and Dutch laboratories, there appeared to be no significant and systematic difference in the wheat antigen levels reported by the immunoassay in the two studies. Even if the observed difference was statistically significant, its magnitude cannot account for the inter-study difference in bakery dust antigen content. However, testing the effect of the type of ELISA plates on the performance of the wheat antigen assay indicated that the use of the high-capacity protein binding plates resulted in the detection of twice as much wheat antigen in samples analyzed in the Canadian laboratory. Thus, we can attribute a twofold difference between the studies to differences in the ELISA plates used in the wheat antigen inhibition assay. It remains to be seen if other factors can explain the remaining tenfold inter-study difference in wheat antigen content of bakery dust.

The analytical limit of detection for the inhibition assay performed in Canada is much higher than that in the Netherlands. However, the difference in the analytical limit of detection between the studies was principally caused by the use of different ranges of concentrations to construct standard curves, which were used to estimate the detection limits. Thus, the difference in the detection limits does not indicate a systematic difference in the calibration of the assays. It would also appear that we can expect no other biases in the results of the wheat antigen assay, because there were no differences in the affinity of the anti-wheat antibodies for the wheat antigen present in either Canadian or Dutch flour.

Country and Product Effects on Wheat Antigen Content of Bakery Dust

In general, our findings support an earlier report⁽⁶⁾ that the correlation between dustiness and airborne antigen levels changes depending on the location within the bakery. We found that wheat antigen content of bakery dust varies significantly among production lines engaged in manufacturing different products. The products requiring little or no antigen in the recipe appear to be associated with reduced levels of that allergen in airborne bakery dust. For example, little or no wheat antigen is present in rye bread mixes, which can account for the observation that the associated bakery dust had low relative antigen content. Even though no quantitative data was collected on the properties of wheat flour used in both studies, it appears plausible that products such as croissants and wheat bread were manufactured from flour with elevated wheat antigen content, compared to other wheat flour-based products.

Even though a number of statistically significant associations between the type of product and wheat antigen content of bakery dust were observed, the study variable had by far the most dramatic influence on the antigen content of bakery dust. One possible reason for the observed "country effect" is that there is a difference in the proportion of personnel from different job titles in the two studies. It has been demonstrated by Houba et al.⁽¹⁾ that wheat antigen content of bakery dust varies by up to a factor of 10 among individuals performing different tasks within the same production line or bakery. Therefore, we can infer that up to a tenfold difference in antigen content between the two studies can be accounted for by differences in tasks performed by bakery workers during exposure monitoring. This is a plausible *partial* explanation of the observed differences, considering that the proportion of bakers involved in making dough in the Canadian study appears to be somewhat larger. Unfortunately, classification into job titles, which formed the basis of the Dutch finding,⁽¹⁾ cannot be reproduced in the Canadian study because job titles were not assigned to Canadian bakery workers.

However, we can still test whether this difference in jobs sampled could have resulted in the observed "country effect" in the following manner. Let us assume, as a worst case scenario of discrepancy between the sampled jobs in the two studies, that all bakery workers in the Canadian study were involved in "dough-making." In that case the wheat antigen content of inhalable flour dust for that group, according to Dutch data,⁽¹⁾ should be about 3 $\mu\text{g}/\text{mg}$ or lower (given that it is not likely that all Canadian bakers would have qualified as "dough-makers" in the Dutch study). Nevertheless, according to Table I, wheat antigen content of bakery dust in the Canadian study was higher than that value. Consequently, differences in jobs do not account for the observed contrast in wheat antigen content of bakery dust. Thus, we can conclude that we have no evidence that the observed "country effect" is due to difference in sampling strategies between the two studies.

There are a number of other explanations that may possibly account for the differences between the studies. However, none of these hypotheses can be tested using the data available to us. A possible explanation for this effect may be that flour used in Canadian bakeries has a higher allergen content. It can, upon suspension in air, result in bakery dust with elevated antigen content. A difference in particle size distribution can also result in difference in *measured* allergen content if antigen was more effectively extracted from small particles. Even if extraction efficiency is not a function of particle size, and bulk flour itself had the same antigen content in both studies, the *inhalable* fraction of dust generated from that flour may have a different antigen content if (a) antigen is not equally distributed among particles of different sizes and, (b) flours differ in particle size distribution of the appropriate range. There is some evidence that α -amylase (another flour antigen) is carried predominantly by large dust particles (diameter greater than 5 μm).⁽²⁾ However, wheat antigen content of various size fractions of dust was not determined in either study, hindering further speculation on this issue.

Limitations

We were able to explain only 49 percent of variability in wheat antigen content of bakery dust. This implies that factors other than the product type and study play important roles in determining the antigen content of bakery dust. We were also unable to account satisfactorily for the inter-country difference in wheat antigen content of bakery dust. A better model might also consider the effect of tasks, specific equipment used by bakers, flour composition, the particle size distribution of bakery dust within each product category, and antigen content of different size fractions of bakery dust. Even though some information on flour composition, tasks, and equipment used is available for both studies, the number of observations for each one of these combinations is not sufficient to allow meaningful modeling. In a separate analysis of the Canadian data,⁽¹³⁾ it was possible to construct good predictive models of wheat antigen and α -amylase antigen levels (explaining 74 to 79% of variability), using dust levels and production characteristics as the predictor variables. No such analysis was performed on the Dutch data. However, the models were difficult to interpret, because it was not possible to elaborate on plausible explanations of why some variables, but not others, were significant in the models. These variables might have been significant due to their correlation with factors that have a causal relationship with the specific antigen content of dust. However, the available data revealed little about either the nature of these correlations or the identity of the underlying causal factors. Therefore, the authors could not be certain that the models would be valid outside of the range of conditions encountered in the study.

CONCLUSIONS AND RECOMMENDATIONS

It appears to be rather challenging to model bakery dust wheat antigen content on the basis of the type of product manufactured,

even though different studies found a strong and consistent correlation between wheat antigen and inhalable dust levels. The modeling effort required to produce a model capable of accurately predicting antigen exposure might exceed that involved in measuring antigen levels directly. Therefore, it appears that for the purposes of risk management and standard-setting in bakeries, one cannot rely on dust as an adequate surrogate exposure measure. Measuring allergen levels directly can be simpler and more accurate than inferring them from dustiness and production characteristics. Consequently, we recommend the use of direct measurement of wheat antigen levels in exposure assessment among bakery workers.

Nevertheless, it is possible to derive a good predictive model of specific antigen exposure within a study. One benefit of such an approach is reduced costs, due to the smaller number of specific antigen assays being performed. Samples analyzed for a specific allergen and used in building a predictive model should be selected at random from strata formed by potential predictors of exposure. However, we would advise investigators who choose this approach to store all their dust samples in such a manner that they can be assayed for specific antigen content. This leaves the investigators an option to measure antigen content in all dust samples if the predictive model derived from a subset of samples proves inadequate.

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REFERENCES

1. Houba, R.; van Run, P.; Heederik, D.; et al.: Wheat Antigen Exposure Assessment for Epidemiologic Studies in Bakeries Using Personal Sampling and Inhibition ELISA. *Clin Exp Allergy* 26:154–163 (1996).
2. Houba, R.; van Run, P.; Doekes, G.; et al.: Airborne Levels of α -Amylase Allergens in Bakeries. *J Allergy Clin Immunol* 99:286–292 (1997).
3. Tee, R.D.; Gordon, D.J.; Gordon, S.; et al.: Immune Response to Flour and Dust Mites in a United Kingdom Bakery. *Br J Ind Med* 49:581–587 (1992).
4. Sandiford, C.P.; Nieuwenhuijsen, M.J.; Tee, R.D.; et al.: Measurement of Airborne Proteins Involved in Bakers' Asthma. *Clin Exp Allergy* 24:450–456 (1994).

5. Nieuwenhuijsen, M.J.; Lowson, D.; Venables, K.M.; et al.: Correlation Between Measures of Exposure in a Cohort of Bakery Workers and Flour Millers. *Ann Occup Hyg* 39:291–298 (1995).
6. Nieuwenhuijsen, M.J.; Sandiford, C.P.; Lowson, D.; et al.: Dust and Flour Aeroallergen Exposure in Flour Mills and Bakeries. *Occup Environ Med* 51:584–588 (1994).
7. Burstyn, I.; Teschke, K.; Kennedy, S.M.: Exposure Levels and Determinants of Inhalable Dust Exposure in Bakeries. *Ann Occup Hyg* 41(6):609–624 (1997).
8. Burstyn, I.; Teschke, K.; Bartlett, K.; Kennedy, S.M.: Determinants of Wheat Antigen and Fungal α -Amylase Exposure in Bakeries. *AIHA J* 59:313–320 (1998).
9. Houba, R.; Heederik, D.; Kromhout, H.: Grouping Strategies for Exposure to Inhalable Dust, Wheat Allergens and α -Amylase Allergens in Bakeries. *Ann Occup Hyg* 41:287–296 (1997).
10. Hornung, R.W.; Reed, L.D.: Estimation of Average Concentration in Presence of Nondetectable Values. *Appl Occup Environ Hyg* 5:46–51 (1990).
11. Kenny, L.C.; Aitken, R.; Chalmers, C.; et al.: A Collaborative European Study of Personal Inhalable Aerosol Sampler Performance. *Ann Occup Hyg* 41:135–153 (1997).
12. Houba, R.: Occupational Allergy Among Bakery Workers in Relation to Wheat Flour and α -Amylase Allergen Exposure. In: *Occupational Respiratory Allergy in Bakery Workers—Relationship with Wheat and Fungal α -Amylase Aeroallergen Exposure*, pp. 101–118. Thesis Landbouwniversiteit Wageningen, the Netherlands, ISBN 90-5485-527-4 (1996).
13. Burstyn, I.; Teschke, K.; Bartlett, K.; et al.: Predictive Models of Wheat Antigen and α -Amylase Exposure Levels in Bakeries. In: 1997 American Thoracic Society Conference, Abstract, San Francisco, p. A136.