

AUTHORS

Joanne Elms^aPaul Beckett^aPeter Griffin^aPaul Evans^bCraig Sams^aMartin Roff^aAndrew D. Curran^a

^aHealth and Safety Laboratory,
Broad Lane, Sheffield, S3 7HQ,
UK; e-mail:

joanne.elms@hsl.gov.uk;

^bHealth and Safety Executive,
Bootle, Merseyside, L20 3QZ,
UK

Job Categories and Their Effect on Exposure to Fungal Alpha-Amylase and Inhalable Dust in the U.K. Baking Industry

Enzymes in flour improver, in particular fungal alpha-amylase, are known to be a significant cause of respiratory allergy in the baking industry. This study measured total inhalable dust and fungal alpha-amylase exposures in U.K. bakeries, mills, and a flour improver production and packing facility and determined whether assignment of job description could identify individuals with the highest exposures to fungal alpha-amylase and inhalable dust. A total of 117 personal samples were taken for workers in 19 bakeries, 2 mills, and a flour improver production and packing facility and were analyzed using a monoclonal based immunoassay. Occupational hygiene surveys were undertaken for each site to assign job description and identify individuals who worked directly with flour improvers. Analysis of exposure data identified that mixers and weighers from large bakeries had the highest exposures to both inhalable dust and fungal alpha-amylase among the different categories of bakery workers ($p < .01$). Currently, the maximum exposure limit for flour dust in the United Kingdom is 10 mg/m³ (8-hour time-weighted average reference period). In this study 25% of the total dust results for bakers exceeded 10 mg/m³, and interestingly, 63% of the individuals with exposure levels exceeding 10 mg/m³ were weighers and mixers. Individuals who worked directly with flour improvers were exposed to higher levels of both inhalable dust and fungal alpha-amylase ($p < .01$) than those who were not directly handling these products. Before sensitive immunoassays were utilized for the detection of specific inhalable allergens, gravimetric analysis was often used as a surrogate. There was a weak relationship between inhalable dust and fungal alpha-amylase exposures; however, inhalable dust levels could not be used to predict amylase exposures, which highlights the importance of measuring both inhalable dust and fungal alpha-amylase exposures.

Keywords: bakery workers, enzymes, exposure, fungal alpha-amylase

Enzymes are found naturally in wheat and cereals at low levels. Their use during the bread making process can materially change the structure and flavor of the final product. Heterologous enzymes from fungal sources have been introduced to improve process control and product quality. Fungal alpha-amylase is one such enzyme that is blended with the flour at the milling stage and also added in the form of flour improvers in the bakery. It is derived from *Aspergillus oryzae* and acts by breaking down starch to low-molecular weight sugars such as maltose

and glucose. This is transformed by yeast to CO₂ gas, causing the dough to rise. The enzyme also contributes to the production of dextrines, which makes the bread lighter and gives it a longer shelf-life. The potential of fungal alpha-amylase to cause asthma in bakers was first described by Flindt.⁽¹⁾ Several authors have focused on sensitization to fungal alpha-amylase,^(2–5) with Baur et al.^(6,7) demonstrating that up to 34% of bakers with respiratory symptoms were sensitized to fungal alpha-amylase, often with no demonstrable reactivity to wheat allergens. In a serological study of some 200 workers

in small bakeries, 15% of workers had specific IgE antibodies to fungal alpha-amylase.⁽⁸⁾ In a separate study 22% of bakers skin tested were found to have an immediate skin reaction to alpha-amylase, again confirming it as an important cause of occupational sensitization.⁽⁹⁾ The purpose of this study was to measure exposure to inhalable dust and fungal alpha-amylase in 19 bakeries, 2 mills, and a flour improver packing facility using a previously described method.⁽¹⁰⁾ Additionally, following assignment of job categories to all study participants, the authors investigated the exposure levels of workers in specific job categories to fungal alpha-amylase and inhalable dust.

METHODS

Bakery Details

Nineteen U.K. bakeries, 2 mills, and 1 flour improver production and packing facility were sampled during this survey. Facilities were randomly selected from lists of current and former members of bakery and milling associations who were willing to participate in the study. Additionally, workers were selected randomly from different areas of the bakery where flour products were used and asked if they could be monitored. It was assumed that during unsampled periods the exposure was the same as during the sampled period, except when the workers were on a break away from the factory area. The bakeries ranged in size from a small craft bakery with one baker to large industrial bakeries with more than 600 employees. Each bakery was visited once in the summer of 1999, and a total of 117 workers were sampled. The bakeries were subdivided into small craft bakeries (<20 employees), medium-sized bakeries (20–100 employees), or large industrial-type bakeries with work forces of more than 100 employees. Occupational hygiene reports were produced for each of the bakeries and mills and the flour production and packing facility to determine which job task each individual undertook and whether he or she directly handled flour improver. The bakery production workers were classified into seven main categories: mixers; weighers/sievers; packers; bakers (with mixed duties); oven workers; pastry makers; and confectionery producers. The mill workers were subdivided into three categories: millers, mill-packers, and laboratory staff. A miscellaneous group that included cleaners and drivers from the bakeries and mills was also identified. Job tasks performed by the workers were recorded after direct observation of their activities. Four individuals were sampled from the flour improver production and packing facility—a packer and three enzyme weighers and mixers.

Sampling Methods

Personal long-term samples were collected in the workers' breathing zone using IOM sampling heads with glass fiber filters (GF/A, 1.6mm, Millipore) at a calibrated flow rate of 2 L/min. Field blanks were included for each sampling visit. The filters were weighed twice in a preconditioned room before and after sampling was undertaken and the personal dust exposure (milligrams per cubic meter) calculated. Fungal alpha-amylase allergens were recovered by elution of the filters into 2 mL PBS/0.1% Tween 20 overnight by end-over-end mixing. The filters and supernatant were squeezed through a 2-mL syringe and centrifuged at 600 g for 5 min to remove any fine particle matter. The supernatant was removed and stored at –20°C until analysis. The limits of detection of the assays were calculated from the analysis of 10 laboratory blank filters (mean + 2 STD).

Monoclonal Enzyme-Linked Immunosorbent Assay for the Determination of Airborne Fungal Alpha-Amylase

The inhalable fungal alpha-amylase levels were quantified using a monoclonal enzyme-linked immunoassay previously developed in the Health and Safety Laboratory.⁽¹⁰⁾ The assay had an interassay variation of 12.0% at 1360 pg/mL and an intraassay variation of 4.9% at 1340 pg/mL (n=20). The assay had a limit of detection of 200 pg/mL.

Inhibition assays demonstrated that the assay was specific to fungal alpha-amylase and was not affected by cereal alpha-amylases, which occur naturally in flour. The assay also demonstrated no cross reactivity with hemicellulase, which is another commonly used enzyme in the bakery industry.

Statistical Analysis of Data

Data were analyzed using Systat (SPSS, Chicago, Ill.). The distribution of exposure was examined for normality using probability plots and transformed using square root for amylase and inverse square root for dust. These were more appropriate than a simple lognormal transformation, which resulted in extreme outliers. Generalized linear modeling (GLM) was used to analyze transformed data to determine whether there were any significant differences in exposure to total inhalable dust and fungal alpha-amylase as classified by bakery size and job description. Unpaired t-test with Welch correction and two-tailed analysis was used to determine whether direct contact with flour improver affected exposure.

RESULTS

Exposure Levels of Inhalable Dust and Fungal Alpha-Amylase for Bakeries and Mills

A total of 117 personal samples were taken for workers selected from three sectors of the industry and inhalable dust and fungal alpha-amylase exposures were determined. Two additional samples, however, were compromised during the sampling procedure and were subsequently not used for the analysis. The workers' personal exposure levels to inhalable dust and fungal alpha-amylase (8-hour time-weighted average [TWA]) were shown to be skewed but not lognormally distributed. Median values and ranges were calculated and are shown in Table I. Inhalable dust exposures ranged from below the limit of detection for gravimetric analysis (0.02 mg/m³ for a 500-L sample) to 36.8 mg/m³ for bakeries and from below the limit of detection to 24.9 mg/m³ for the two mills, demonstrating wide variation in occupational exposures. The median exposures for the bakery workers and millers were 5.6 and 12.2 mg/m³, respectively. The inhalable fungal alpha-amylase exposures for bakery workers and millers ranged from below the limit of detection of the assay (<0.42 ng/m³) to 1370 and 11.1 ng/m³, respectively. The median fungal alpha-amylase exposures for bakery workers and millers were 1.9 and 1.5 ng/m³, respectively. Field controls were all below the limit of detection for fungal alpha-amylase.

Flour Improver Production and Packing Facility

The samples from the flour improver production and packing facility were analyzed separately (Table I). The workers were exposed to inhalable dust levels that ranged from 3.8 to 8.5 mg/m³, with a median of 4.4 mg/m³. The exposures to fungal alpha-amylase ranged from 71.2 to 1178 ng/m³ with a median of 455.9 ng/m³, which was higher than the bakeries or mills.

TABLE I. Inhalable Dust and Fungal Alpha-Amylase Exposure for Workers Categorized by the Size of the Bakery

Bakery Size	n	Dust (mg/m ³)			Amylase (ng/m ³)		
		Range	Median	95 Percentile	Range	Median	95 Percentile
Small 1–20	27	bld-27.8	7.4	25.5	bld-173	1.7	59.6
Medium 20–100	28	bld-25.9	4	16	bld-12.1	1.4	9
Large 100+	41	bld-36.8	6.4	24.6	bld-1370	4.22	176.7
Mills	17	bld-24.9	12.2	21.9	bld-11.1	1.5	5.3
All bakeries	96	bld-36.8	5.6	25	bld-1370	1.9	135.4
Improver producers	4	3.8–8.5	4.4	7.9	71.2–1178	455.9	1126.6

Note: bld = below limit of detection, <0.02 mg/m³ for total inhalable dust and <0.42 ng/m³ for fungal alpha-amylase.

Exposure Levels Categorized by Bakery Size and Job Category

The bakeries were categorized as small craft bakeries with between 1–20 workers (eight bakeries); medium bakeries with between 20–100 workers (five bakeries); and large, industrial bakeries with more than 100 workers (six bakeries). The median inhalable dust and fungal alpha-amylase levels were calculated (Table I). The bakeries were stratified by size in this manner to allow comparison of exposure between job tasks in large automated bakeries and smaller bakeries where automation was not used. Table II shows exposure for workers categorized by job grouping only (not by bakery size).

Fungal Alpha-Amylase Exposure

The fungal alpha-amylase exposure data were transformed using the inverse square root $y = -1/\text{SQRT}(x)$. This normalized the data and stabilized the variances for each of the job-within-size subcategories. The data were analyzed by GLM, which showed extremely significant differences between subcategories ($p < .01$), with the two subcategories of “large mixers” and “large weighers” being higher than the rest. Levene’s test ($p = .677$) showed that the variances were not different among the transformed subcategories. The subcategories were split to assign the large mixers and large weighers as the “high-exposure” group, and the rest as “low exposure.” GLM on the high-exposure group proved that large weighers at 76.8 ng/m³ were significantly higher than large mixers at 9.8 ng/m³ ($p = .04$). The GLM on the low-exposure group was not significant at a mean of 1.8 ng/m³ ($p = .677$). Post-hoc analysis using Scheffe’s test showed no significant pairwise differences between the job categories in the low-exposure group. The two groups (high and low exposure) were compared using a pooled variance two-sample t-test, which confirmed the highly significant difference between them ($p < .01$).

Inhalable Dust Exposure

The dust exposure data were transformed using the square root $y = \text{SQRT}(x)$. This normalized the data and helped to stabilize the variances for each of the job-within-size subcategories. GLM showed significant differences between the subcategories ($p < .01$), but no particular subcategories stood out as different from the rest. Instead, the data were analyzed in a two-factor GLM of job and size. Job categories showed significant differences ($p < .01$), with “weighers” and “mixers” again the highest pair. These were only distinguishable from “packers,” the lowest category, in pairwise post-hoc tests using Scheffe’s test ($p = .04$ and $p < .01$, respectively). Size categories of bakeries were not significant ($p = .055$), with no significant pairwise differences. Levene’s test showed no significant differences among group variances ($p = .2$ for job and $p = .33$ for size).

The occupational hygiene survey enabled further subdivision of bakers and millers to distinguish between those individuals who directly handled flour improver and those individuals who may or may not have been in close proximity to flour improvers but were not in direct contact with them (Table III). These subgroups were transformed as before and analyzed using an unpaired t-test. This revealed that the workers who directly handled flour improver exhibited significantly higher exposures to inhalable dust ($p < .01$) and fungal alpha-amylase ($p < .01$) than those workers who had no direct contact with flour improver.

Correlation of Dust and Amylase Levels in the Bakeries and Mills

Pearson correlations of the transformed dust and fungal alpha-amylase data showed a degree of association between the two variables that could account for only between 10 and 30% of the variation of the data. The Pearson correlation coefficients for the

TABLE II. Inhalable Dust and Fungal Alpha-Amylase Exposure for Workers Categorized by Job Grouping

Job Category	n	Dust (mg/m ³)			Amylase (ng/m ³)		
		Range	Median	95 Percentile	Range	Median	95 Percentile
Mixer	29	1.0–36.8	7.6	25.2	bld-123.7	3.2	98.6
Weigher	13	2.4–26.3	11.4	26.1	bld-1370	29.1	864.7
Packer	9	bld-2.2	0.8	2.1	bld-2.1	1.4	1.9
Baker	21	bld-27.8	6.3	14.3	bld-185	1.6	12.1
Confectionery	5	0.6–8.2	4	7.5	bld	bld	bld
Pastries	3	2.5–13.3	11.2	13.1	bld-1.5	1.1	1.5
Ovens	8	bld-18.0	2.1	14.5	bld-3.2	1.5	3.2
Miller	8	bld-21.1	6.1	19.2	bld-11.1	1.3	8.4
Mill laboratory	2	5.3–8.2	6.8	8.1	bld-2.2	1.7	2.2
Mill packer	4	5.7–25.0	16.2	23.7	bld-1.7	1.6	1.7
Miscellaneous	11	bld-23.1	1.9	18.9	bld-24.6	1.4	14.3

Note: bld = below level of detection, <0.02 mg/m³ for total inhalable dust and <0.42 ng/m³ for fungal alpha-amylase.

TABLE III. Exposure Data for Workers Who Directly Handle Flour Improver

Handles Flour Improver?	n	Dust (mg/m ³)			Amylase (ng/m ³)		
		Range	Median	95 Percentile	Range	Median	95 Percentile
Yes	43	bld-36.8	7.9	27.2	bld-1370	5.3	176
No	69	bld-25.0	4.8	17.4	bld-24.6	1.4	4

Note: bld = below level of detection, <0.02 mg/m³ for total inhalable dust and <0.42 ng/m³ for fungal alpha-amylase.

bakeries and mills were: small bakeries ($r=.65$), medium bakeries ($r=.38$), large bakeries ($r=.62$), and mills ($r=.50$).

DISCUSSION

From the statistical analysis of 117 personal samples for total dust and fungal alpha-amylase, it was demonstrated that the large bakeries, as a group, exhibited higher median fungal alpha-amylase exposures than the medium-sized bakeries and mills. This difference, however, is accounted for by the fact that a proportion of workers in the industrial bakeries, mainly weighers and sievers, were exposed to higher levels of inhalable fungal alpha-amylase than those individuals who worked further down the production line, which skewed the results. Additionally in this survey, the authors concentrated on those individuals working with flour products. The median fungal alpha-amylase exposure for bakery workers was 1.9 ng/m³. This can be compared with the exposure levels demonstrated by Burstyn et al.⁽¹¹⁾ who measured fungal alpha-amylase levels in 96 bakery workers (mean amylase exposure 22.0 ng/m³), although this study included only small and medium-sized bakeries. Additionally, care must be taken when comparing results derived using different immunoassays; but unfortunately, to date there are no standardized methods for amylase measurement.

Currently, the maximum exposure limit for flour dust in the United Kingdom⁽¹²⁾ is 10 mg/m³ (8-hour TWA reference period). In this study 25% of the total dust results for bakers exceeded 10 mg/m³, which was higher than that reported by Jeffrey et al.⁽⁸⁾ who surveyed small bakeries with fewer than 50 employees. Of these results 63% of the individuals with exposure levels exceeding 10 mg/m³ were from weighers and mixers. Interestingly, these two groups were also exposed to higher mean fungal alpha-amylase levels than the other groups of bakery workers examined. This trend was also identified by Nieuwenhuijsen et al.⁽¹³⁾ (although this team used a polyclonal antibody in their immunoassay validated by Houba et al.⁽¹⁴⁾) and Vanhanen et al.⁽¹⁵⁾ who found that the highest levels of dust and fungal alpha-amylase in dough making areas of the bakery. Additionally, Burstyn et al.⁽¹¹⁾ suggested that individuals involved in tasks such as weighing and pouring had increased flour antigen exposure. Unfortunately, this survey gained no information regarding work-related respiratory symptoms or sensitization to fungal alpha-amylase, so the authors cannot make any assumptions regarding potential exposure response relationships. However, it is important to note that previous authors^(13,16) have described an association between sensitization to amylase (measured as a positive skin prick test) and exposure, and it would have been of interest to determine whether this relationship applied to the weighers and sievers in this study.

It can be postulated from this data that occupational exposures to both inhalable dust and fungal alpha-amylase in the bakeries may be reduced if those individuals who carry out weighing and mixing duties are targeted for control measures. Properly functioning local exhaust ventilation on scales and sieves, and seals fitted on mixing machines, can all be used to help reduce exposure

levels.^(17,18) Many flour improvers also contain "dampening agents," which are often soya or starch based and may help prevent the formation of dust clouds. The switch to these types of flour improver products or the paste-type products (which were observed being used in some of the smaller bakeries) may reduce exposure to fungal alpha-amylase. As predicted, those individuals who directly handled flour improvers by weighing or sieving activities had median exposures to total dust and fungal alpha-amylase that were significantly higher than those who had no direct contact with flour improvers. This category of workers can be identified as those workers who are potentially exposed to high levels of fungal alpha-amylase. The occupational hygiene reports also highlighted situations in which empty bags of flour improver were manually folded for disposal, which visibly generated dust clouds. It is the authors' opinion that good working practices may also contribute to the reduction of fungal alpha-amylase exposures.

Four individuals were sampled in the flour improver production and packing facility. Although none of the workers was exposed to total inhalable dust levels above 10 mg/m³, as a group they demonstrated the highest mean inhalable fungal alpha-amylase exposure of any of the groups identified in this survey. This exposure indicates that further research is necessary to determine median exposures to fungal alpha-amylase in various flour improver production facilities, because this group may be exposed to higher levels than those in either bakeries or mills.

Previous studies quantified fungal alpha-amylase exposure in bakeries using polyclonal rabbit anti-alpha-amylase antibodies⁽¹⁶⁾ or enzymic methods⁽¹⁹⁾ and more recently monoclonal based assays.^(11,20) To allow for comparisons between studies to be accurately determined, it would be useful to compare the assay from the current study with other methods in the fashion of Lillienberg et al.⁽²¹⁾

This study demonstrated that workers' exposure to dust does not necessarily define their exposure to fungal alpha-amylase, and this suggests that fungal alpha-amylase measurement is a useful tool for occupational hygienists.

REFERENCES

1. Flindt, J.H.: Allergy to α -amylase and papain. *Lancet*. i:1407-1408 (1979).
2. Brisman, J., and L. Belin: Clinical and immunological responses to occupational exposure to α -amylase in the baking industry. *Brit. J. Ind. Med.* 48:604-608 (1991).
3. Quirce, S., M. Cuevas, M.L. Díez-Gómez, et al.: Respiratory allergy to *Aspergillus*-derived enzymes in bakers' asthma. *J. Allergy Clin. Immunol.* 90:970-978 (1992).
4. Carmona, J.G.B., S.J. Picón, and M.G. Sotillos: Occupational asthma in bakeries caused by sensitivity to α , Ω amylase. *Allergy* 46:274-276 (1991).
5. Sandiford, C.P., R.D. Tee, and A.J. Newman Taylor: The role of cereal and fungal amylases in cereal flour hypersensitivity. *Clin. Exp. Allergy* 24:549-557 (1994).

6. Baur, X., G. Fruhmann, B. Haug, B. Rasche, and W. Weiss: Role of *Aspergillus* amylase in baker's asthma. *Lancet* 1:43–44 (1986).
7. Baur, X., W. Sauer, and W. Weiss: Baking additives as new allergens in baker's asthma. *Respiration* 54:70–72 (1988).
8. Jeffrey, P., P. Griffin, M. Gibson, and A.D. Curran: Small bakeries—a cross-sectional study of respiratory symptoms, sensitisation and dust exposure. *Occup. Med.* 49:237–241 (1999).
9. Morren, M.A., V. Janssens, A. Doms-Gossens, et al.: Alpha-amylase, a flour additive: An important cause of protein contact dermatitis in bakers. *J. Am. Dermatol.* 29:723–728 (1993).
10. Elms, J., S. Denniss, M. Smith, et al.: Development and validation of a monoclonal based immunoassay for the measurement of fungal alpha amylase. A focus on peak exposures. *Ann. Occup. Hyg.* 45:89–95 (2001).
11. Burstyn, I., K. Teschke, K. Bartlett, and S.M. Kennedy: Determinants of wheat antigen and fungal α -amylase exposure in bakeries. *Am. Ind. Hyg. Assoc. J.* 59:313–320 (1998).
12. Health and Safety Executive: *Occupational Exposure Limits 2002* (EH40/2002). London: HSE Books, 2002.
13. Nieuwenhuijsen, M.J., D. Heederik, G. Doekes, K.M. Venables, and A.J. Newman Taylor: Exposure-response relations of α -amylase sensitisation in British bakeries and flour mills. *Occup. Environ. Med.* 56:197–201 (1999).
14. Houba, R., P. van Run, G. Doekes, D. Heederik, and J. Spithoven: Airborne levels of α -amylase allergens in bakeries. *J. Allergy Clin. Immunol.* 99:286–292 (1997).
15. Vanhanen, M., T. Tuomi, H. Hokkanen, et al.: Enzyme exposure and enzyme sensitisation in the baking industry. *Occup. Environ. Med.* 53:670–676 (1996).
16. Houba, R., D.J.J. Heederik, G. Doekes, and P.E.M. van Run: Exposure-sensitisation relationship for α -amylase allergens in the baking industry. *Am. J. Respir. Crit. Care Med.* 154:130–136 (1996).
17. Health and Safety Executive: *COSHH Essentials. Easy Steps to Control Chemicals*. London: HSE Books, 1999.
18. Health and Safety in Bakeries Liaison Committee: *Guidance on Dust Control and Health Surveillance in Bakeries*. London: Health and Safety in Bakeries Liaison Committee, 1998.
19. Brisman, J., and L. Belin: Clinical and immunological responses to occupational exposure to α -amylase in the baking industry. *Brit. J. Ind. Med.* 48:604–608 (1991).
20. Sander, I., C. Neuhaus-Schröder, G. Borowitzki, X. Baur, and M. Raulf-Heimsoth: Development of a two-site enzyme-linked immunosorbent assay for α -amylase from *Aspergillus oryzae* based on monoclonal antibodies. *J. Immunol. Methods* 210:93–101 (1997).
21. Lillienberg, L., X. Baur, G. Doekes, et al.: Comparison of four methods to assess fungal alpha-amylase in flour dust. *Ann. Occup. Hyg.* 44:427–433 (2000).