

# Inhalable $\beta(1\rightarrow3)$ glucans as a non-allergenic exposure factor in Dutch bakeries

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## ABSTRACT

**Objectives:** To obtain an overview of inhalable  $\beta(1\rightarrow3)$ glucans levels in Dutch industrial bakeries and explore possible associations with reported respiratory health effects in bakery workers.

**Methods:**  $\beta(1\rightarrow3)$ glucan levels were analysed in 186 personal inhalable dust measurements obtained from a random population of bakery workers. Association between respiratory health effects and exposure to  $\beta(1\rightarrow3)$ glucan was explored in a population of industrial bakery workers participating in a Health Surveillance System for flour processing sectors. Based on their job, bakery workers were assigned to low or high exposure categories given the average job exposure estimates obtained from the measurement study.

**Results:** Bread bakers and dough makers had the highest exposures to  $\beta(1\rightarrow3)$ glucans (GM 1.48  $\mu\text{g}/\text{m}^3$  and 1.37  $\mu\text{g}/\text{m}^3$  respectively). Strong correlations were found between airborne levels of inhalable dust and  $\beta(1\rightarrow3)$ glucans, and between  $\beta(1\rightarrow3)$ glucans and wheat allergens (Pearson correlation coefficients were 0.74 and 0.68 respectively). No significant associations could be identified between  $\beta(1\rightarrow3)$ glucan exposure and work-related respiratory symptoms.

**Conclusion:** This study has shown that bakery workers are exposed to inhalable  $\beta(1\rightarrow3)$ glucan levels comparable with exposure levels found in other occupational settings. More refined exposure assessment is necessary to fully understand the role of  $\beta(1\rightarrow3)$ glucan exposure on respiratory health in bakery workers.

Respiratory symptoms are the most prevalent work-related symptoms among bakery workers (rates of 14–29%).<sup>1</sup> Parts of the work-related symptoms are caused by type-1 hypersensitivity reactions against wheat allergens and/or fungal  $\alpha$ -amylase (or other enzymes) present in the flour dust. Houba *et al*<sup>2</sup> showed that approximately 70% of the Dutch bakery workers with work-related symptoms were not sensitised to wheat allergens and/or  $\alpha$ -amylase. These symptoms have been suggested to be the result of exposure to unidentified allergens, or by non-specific inflammatory or irritative responses to flour dust exposure.<sup>2</sup>

These non-specific responses could be related to exposure to  $\beta(1\rightarrow3)$ glucans,<sup>2</sup> which can be found in fungi, yeast, some bacteria and plant material, including wheat flour.<sup>3</sup> The effect of  $\beta(1\rightarrow3)$ glucans on respiratory health is reviewed by Douwes.<sup>4</sup> An association between airborne  $\beta(1\rightarrow3)$ glucan exposure and airway inflammation and respiratory symptoms is suggested in this study.

To our knowledge, personal inhalable  $\beta(1\rightarrow3)$ glucan exposure in bakeries has not been

studied before. In this paper we describe the results of a study in a cross-section of Dutch industrial bakery workers looking at their exposure to  $\beta(1\rightarrow3)$ glucans. Furthermore we explored if occupational exposure to  $\beta(1\rightarrow3)$ glucan might be a cause of respiratory symptoms in non-sensitised workers taking part in a Dutch health surveillance scheme.

## MATERIALS AND METHODS

### Exposure assessment

The exposure survey was carried out in 2005 and comprised 129 randomly selected workers from 13 Dutch industrial bakeries. Fifty seven workers were monitored on two days, resulting in a total of 186 personal inhalable dust samples being collected. Personal inhalable dust samples were collected during full-shift periods of 6–8 h in the workers breathing zone as described previously.<sup>5</sup> During the personal exposure measurements information on key exposure determinants (for example, function, tasks and working habits) was recorded for each individual worker.

Dust, wheat allergens and  $\beta(1\rightarrow3)$ glucan extraction and analysis were performed as described previously.<sup>3,5</sup> The limit of detection (LOD) for dust was 0.18 mg; 10 samples (5%) were below this LOD. The LOD for wheat allergens was 0.23  $\mu\text{g}/\text{ml}$ ; 29 (16%) samples were below this LOD. The LOD for  $\beta(1\rightarrow3)$ glucans was 0.30  $\mu\text{g}/\text{ml}$ ; 95 (54%) samples were below this LOD. Values below detection limit were replaced by two thirds of the LOD. The assay for  $\beta(1\rightarrow3)$ glucans had a mean reproducibility of 28.3% (median 26.9%).

### Statistical analyses

The statistical analyses were performed using SAS software (version 8.2, SAS Institute Inc, Cary, NC, USA). Descriptive statistics were used to explore the distribution of the exposure data. Because of the log-normal distribution, exposure data were log-transformed before analysis. Pearson correlations were calculated between exposures. Arithmetic means, geometric means, geometric standard deviations and the ranges of exposure were calculated for each job title. The geometric mean per job title was used as the exposure estimate per job title.

To explore a possible relation between exposure to  $\beta(1\rightarrow3)$ glucans and respiratory symptoms, a small subpopulation of industrial bakers ( $n = 191$ ) from the Dutch Health Surveillance system was assigned an average exposure estimate based on their job performed. From these, individual information was also available on respiratory symptoms (self-administered questionnaire) and on

sensitisation to work-related allergens and atopic status (serology). Frequency tables were used to examine a possible association between  $\beta(1\rightarrow3)$ glucan exposure and respiratory health symptoms.

## RESULTS

### Exposure measurements

Exposure varied among the bakery workers depending on the job performed. Table 1 gives descriptive statistics for exposure to  $\beta(1\rightarrow3)$ glucans, inhalable dust and wheat allergens for each job.

Highest exposures to  $\beta(1\rightarrow3)$ glucans were found for bread bakers (GM 1.48  $\mu\text{g}/\text{m}^3$ ) and dough makers (GM 1.37  $\mu\text{g}/\text{m}^3$ ). Lowest exposure to  $\beta(1\rightarrow3)$ glucans was found for wrappers (GM 0.55  $\mu\text{g}/\text{m}^3$ ). Average job exposure levels to  $\beta(1\rightarrow3)$ glucans showed moderate variation (GSD 1.38–4.79) for different job titles, nevertheless large overlap in exposure ranges were observed. Variation in the average job exposures for wheat allergens were considerable higher compared to variation in  $\beta(1\rightarrow3)$ glucans exposure.

Strong correlations were found between personal exposure levels to  $\beta(1\rightarrow3)$ glucans, and exposure levels of inhalable dust and airborne wheat allergens (Pearson correlation coefficients were 0.74, and 0.68 respectively;  $p < 0.001$ ). The relations for samples with exposure levels above the detection limits are presented in figure 1.

### Exposure and respiratory health symptoms

Categorisation of bakery workers in two  $\beta(1\rightarrow3)$ glucan exposure categories resulted in a low exposure category comprising of 48 workers and a high exposure category comprising of 143 workers. Fifty seven (30%) workers were atopic and 42 (22%) were sensitised to wheat and/or  $\alpha$ -amylase. Symptoms were most prevalent in workers sensitised to wheat allergens and/or  $\alpha$ -amylase followed by atopic workers. Workers sensitised to wheat allergens and/or  $\alpha$ -amylase in the high exposure category had more symptoms than workers in the low exposure category, the same was found for atopic workers. No significant associations were found.

## DISCUSSION

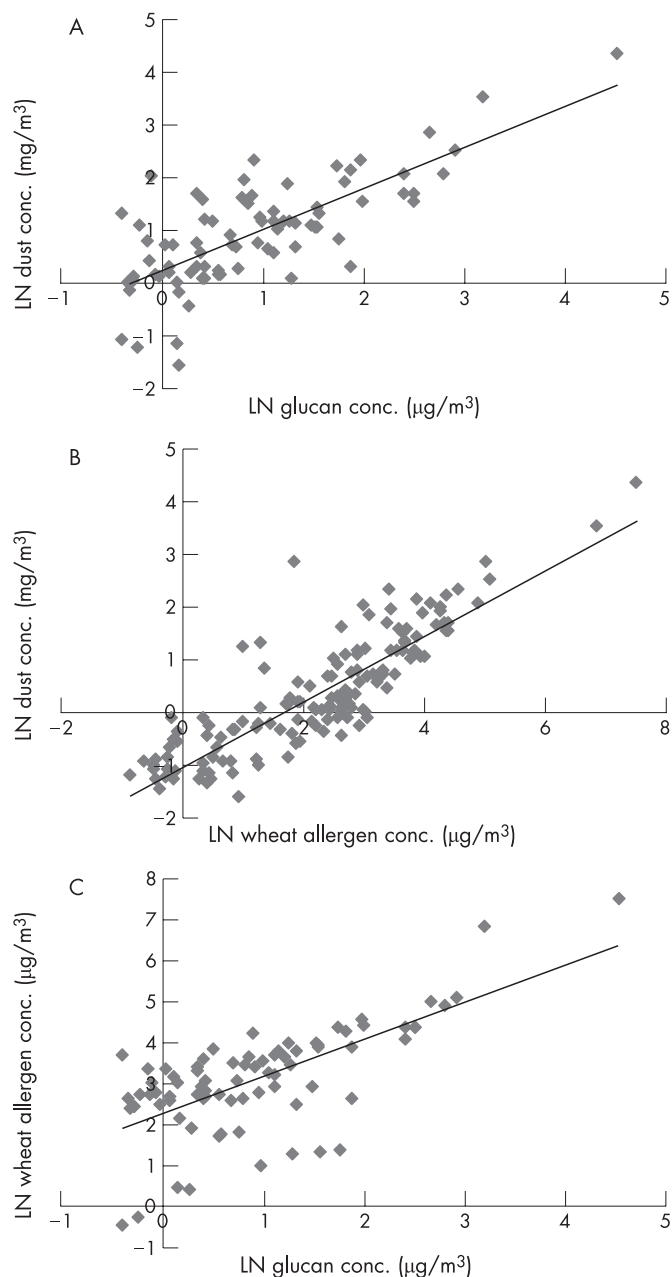
In this study we showed that bakery workers in Dutch industrial bakeries are exposed to considerable levels of  $\beta(1\rightarrow3)$ glucans. The levels and ranges of exposure found in this study are comparable to what is found in other occupational sectors. Studies looking at the general indoor environment found lower exposures to  $\beta(1\rightarrow3)$ glucans (0.1–169  $\text{ng}/\text{m}^3$ ).<sup>4</sup> Nevertheless comparisons of exposure levels between these studies should be interpreted with some caution because results were obtained using different assays (glucan inhibition EIA and the LAL assay) with different sensitivity and specificity. Whereas all previous studies focused on fungal, yeast and bacterial  $\beta(1\rightarrow3)$ glucan exposure, our study is the first to show occupational wheat  $\beta(1\rightarrow3)$ glucan exposure levels. The possible effects of plant  $\beta(1\rightarrow3)$ glucans on respiratory health and underlying mechanisms are not clear,<sup>6</sup> while the effects of  $\beta(1\rightarrow3)$ glucans originating from bacteria, fungi and yeast on respiratory health have been studied more extensively.<sup>4</sup>

In this study we were unable to effectively explore a possible relation between job-related exposure to  $\beta(1\rightarrow3)$ glucans and reported work-related respiratory symptoms. This is primarily the result of some limitations observed in our study. In the first

**Table 1**  $\beta(1\rightarrow3)$ glucan, inhalable dust and wheat allergen exposure data per job title

| Function           | Glucan ( $\mu\text{g}/\text{m}^3$ ) |      |             |            | Inhalable dust ( $\text{mg}/\text{m}^3$ ) |      |             |            | Wheat allergens ( $\mu\text{g}/\text{m}^3$ ) |      |       |              |              |       |
|--------------------|-------------------------------------|------|-------------|------------|-------------------------------------------|------|-------------|------------|----------------------------------------------|------|-------|--------------|--------------|-------|
|                    | n                                   | AM   | GM (GSD)    | Range      | <LOD                                      | n    | AM          | GM (GSD)   | Range                                        | <LOD | n     | AM           | GM (GSD)     | Range |
| Bread baker        | 20                                  | 2.57 | 1.48 (2.84) | 0.44–12.11 | 7                                         | 2.67 | 1.98 (2.31) | 0.29–7.43  | 0                                            | 19   | 24.26 | 14.97 (3.02) | 1.35–81.06   | 0     |
| Pastry baker       | 9                                   | 2.87 | 1.33 (3.13) | 0.46–16.16 | 4                                         | 2.40 | 1.62 (2.62) | 0.40–8.27  | 0                                            | 9    | 21.94 | 7.05 (4.87)  | 0.61–131.89  | 2     |
| Dough maker        | 62                                  | 3.81 | 1.37 (3.23) | 0.39–90.66 | 24                                        | 4.21 | 1.85 (3.35) | 0.13–82.40 | 2                                            | 64   | 56.60 | 17.42 (3.96) | 0.42–1833.18 | 2     |
| Wrapper            | 30                                  | 0.62 | 0.55 (1.55) | 0.38–1.75  | 27                                        | 0.45 | 0.37 (1.97) | 0.12–1.21  | 4                                            | 30   | 1.09  | 0.53 (3.09)  | 0.07–6.60    | 16    |
| Cleaner            | 7                                   | 0.76 | 0.72 (1.38) | 0.44–1.29  | 5                                         | 0.51 | 0.47 (1.49) | 0.22–0.98  | 1                                            | 7    | 1.10  | 1.03 (1.47)  | 0.63–1.66    | 1     |
| Boss/foreman       | 16                                  | 2.08 | 0.50 (2.95) | 0.39–14.11 | 8                                         | 2.05 | 0.85 (3.28) | 0.14–18.17 | 1                                            | 17   | 17.61 | 4.30 (6.36)  | 0.13–150.75  | 0     |
| Storage worker     | 6                                   | 4.40 | 0.96 (4.79) | 0.47–23.83 | 5                                         | 6.50 | 1.10 (6.33) | 0.16–35.69 | 1                                            | 6    | 59.54 | 8.22 (16.27) | 0.40–949.39  | 0     |
| Maintenance worker | 11                                  | 2.28 | 1.11 (3.20) | 0.42–10.86 | 7                                         | 2.48 | 1.01 (3.94) | 0.16–10.77 | 1                                            | 12   | 13.21 | 3.05 (6.89)  | 0.29–60.56   | 1     |
| Oven operator      | 14                                  | 1.41 | 0.84 (2.60) | 0.40–6.46  | 8                                         | 1.19 | 0.92 (2.23) | 0.20–3.29  | 0                                            | 15   | 8.84  | 5.80 (2.87)  | 0.79–26.74   | 0     |

n, number of measurements in a group; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; <LOD, number of measurements under the limit of detection.



**Figure 1** Relations between log transformed airborne levels of (A) inhalable dust and  $\beta(1\rightarrow3)$ glucans, (B) inhalable dust and wheat allergens, and (C) wheat allergens and  $\beta(1\rightarrow3)$ glucans (C).

place exposure was assigned to an individual according to their job performed, because contrast in exposure was poor (overlapping job exposure distribution) and there was a relatively high potential for misclassification of exposure on an individual level.

Secondly the high correlation with wheat exposure caused difficulties disentangling causal relations between symptoms and exposure. In our opinion the followed approach of looking at non-sensitised individuals with and without symptoms proves a promising way of studying such complex relations between combined exposures and symptoms. Nevertheless this study lacked power in numbers of cases to effectively perform such analysis.

In conclusion this study provides valuable information on the occupational exposure levels of  $\beta(1\rightarrow3)$ glucans among bakery workers. Previous studies have suggested that similar exposure levels did lead to respiratory health effects. In this study we were not able to show such an association for the studied population of bakery workers. We believe that a larger study, with more refined exposure assessment and a larger variation in exposure (for example, adding other occupations like flour mill workers) will provide more contrast in exposure and enable more rigid and detailed analysis to fully understand the role of  $\beta(1\rightarrow3)$ glucan exposure in bakeries on respiratory health.

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## REFERENCES

1. Houba R, Doekes G, Heederik D. Occupational respiratory allergy in bakery workers: a review of the literature. *Am J Ind Med* 1998;**34**:529–46.
2. Houba R. *Occupational respiratory allergy in bakery workers. Relationships with wheat and fungal  $\alpha$ -amylase*. Thesis. Wageningen, the Netherlands: Agricultural University Wageningen, Department of Occupational and Environmental health, Wageningen, 1996.
3. Douwes J, Doekes G, Montijn R, *et al*. Measurement of beta(1 $\rightarrow$ 3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microb* 1996;**62**:3176–82.
4. Douwes J. (1 $\rightarrow$ 3)-beta-D-glucans and respiratory health: a review of the scientific evidence. *Indoor Air* 2005;**15**:160–9.
5. Houba R, Van Run P, Heederik D, *et al*. Wheat antigen exposure assessment for epidemiological studies in bakeries using personal dust sampling and inhibition ELISA. *Clin Exp Allergy* 1996;**26**:154–63.
6. Eduard W, Douwes J, Mehl R, *et al*. Short term exposure to airborne microbial agents during farm work: exposure-response relations with eye and respiratory symptoms. *Occup Environ Med* 2001;**58**:113–18.