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An Exploratory Quantitative Risk Assessment for High Molecular Weight Sensitizers: Wheat Flour

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Objective: Quantitative risk assessments have been made for wheat dust and allergen exposure and wheat sensitization using classical epidemiological approaches based on simple categorizations in exposure groups. Such analyses suggest the existence of an exposure threshold level for wheat specific sensitization and were used as input in recently conducted risk assessments for wheat flour by the American Conference of Governmental Industrial Hygienists and the Dutch Expert Committee on Occupational Standards. More advanced statistical analyses were applied using generalized additive modeling and smoothed plots to evaluate the shape of the exposure response relationship in greater detail and evaluate the presence of exposure thresholds.

Methods: Data were used from a recently conducted epidemiological study in bakery workers. Information was available on wheat sensitization (IgE antibodies), inhalable dust levels and wheat allergen levels. Initial analyses were based on simple exposure categorizations for inhalable dust and allergen exposure. A more detailed analysis using non-parametric generalized additive models (GAM models) and smoothing plots allowed inspection of the presence of an exposure threshold of evaluation of 'no' or 'lowest observed effect levels' (NOELs, LOELs) using exposure data on the individual level.

Results: All analyses showed an increasing sensitization risk with increasing exposure. The classical epidemiological analyses gave evidence for the existence of an exposure threshold or 'no observed effect level (NOEL)' for specific wheat sensitization between 0.5 and 1 mg/m^3 of inhalable dust. The more advanced analyses did not suggest any evidence for the existence of an exposure threshold. However, estimates of a LOEL obtained by considering an arbitrary increase in sensitization risk between 1.5 and 2 as undesirable, were close to the NOEL from the classical analyses and would therefore not lead to an essentially different exposure limit. The criterion of an increase in wheat sensitization risk was based on the risk in non-wheat dust exposed populations.

Conclusion: Exposure response modeling using different classical epidemiological approaches and advanced statistical methods resulted in health based LOEL or NOEL estimates within a relatively close range. But when sensitization accompanied by asthma or rhinitis symptoms was considered as critical endpoint, steeper exposure–response relationships were observed which would lead to lower LOEL values.

Keywords: sensitizers; flour; quantitative risk assessment; wheat

INTRODUCTION

Until recently, health based exposure standard setting for high molecular weight sensitizers seemed impossible because of the absence of studies revealing exposure response relationships (Tikkainen *et al*., 1996). However, over the past decade, several more recent studies have described exposure response

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relationships for high molecular weight sensitizers and IgE mediated work-related sensitization or work related symptoms. The most prominent relationships have been described in bakery workers exposed to fungal α-amylase (a dough improver) and wheat allergens, and laboratory animal workers exposed to Rat Urinary Proteins (Cullinan *et al*., 1994, Heederik *et al*., 1999b; Houba *et al*., 1996a, 1998; Musk *et al*., 1989). The risk for developing sensitization appeared to increase strongly with increasing allergen exposure levels, usually with a steeper relationship for atopics compared to non-atopics (Heederik *et al*., 1999b; Houba *et al*., 1998). Studies in bakery workers had the primary aim to unravel the role of allergen exposure in the development of work related sensitization or respiratory symptoms, and were not intended to study different exposure response models for risk assessment purposes and standard setting. As a result, most of the studies in bakery workers cannot easily be used for risk assessment purposes. Some limitations are the absence of quantitative exposure data, use of different dust sampling equipment, use of too crude exposure categorizations for a quantitative risk analysis and evaluation of the risk at lower exposure levels, and the use of different measures of association (Odds Ratio versus Prevalence Ratio). Three studies in workers in the flour milling and baking industry could potentially be used for quantitative risk assessment for wheat dust and allergens (Cullinan *et al*., 1994; Houba *et al*., 1998; Musk *et al*., 1989), but the results have not been presented in an informative way for risk assessment purposes. We re-analyzed the data from the largest of these studies (Houba *et al*., 1998), and evaluated exposure response relationships for inhalable dust levels as marker for wheat dust levels, and allergen exposure. This study has been referred to by ACGIH to underpin their proposed TLV of 0.5 mg/m^3 TWA (ACGIH, 1999). In the new analyses, a wider range of exposure proxies was evaluated such as current exposure, cumulative exposure to inhalable dust and allergens, and duration of exposure. Results from this more refined analysis have been used by the Dutch Expert Committee for Occupational Exposure Standards (DECOS) (DECOS, 2001). In both evaluations by ACGIH and DECOS, a classical exposure grouping was used in the exposure–response analysis. Since both evaluations by ACGIH and DECOS used simple exposure categorizations for the exposure–response analysis, details with regard to shape of the exposure response curve and presence or absence of an exposure threshold might have been obscured. We therefore re-analyzed the data again to evaluate the shape of the exposure response relationship in greater detail and explored the existence of an exposure threshold for sensitization by using Generalized Additive Modeling approaches.

METHODS

Population

The data come from a survey carried out between April 1991 and July 1993 which has been described in detail elsewhere (Houba *et al*., 1998). The study comprised 427 production workers from 21 bakeries and the participation rate was 75%. Maintenance workers were excluded from the analyses because of potential exposures to other respiratory hazards (e.g. welding fumes), leaving 393 bakery workers. From 346 workers, venous blood samples were available and have been analyzed for total IgE and specific IgE antibodies.

Questionnaire

All workers completed a short self-administered Dutch version of the internationally accepted BMRC respiratory questionnaire, supplemented with questions on work-related symptoms. Symptoms were considered work related if they were reported by the subject as being provoked by contact with flour or process related products (e.g. baking additives) during work (‘Do you have any of the following allergic symptoms during work, after contact with certain agents at work?’). Work-related rhinitis was defined as the presence of sneezing or running nose (production of nasal secretions) during work. A complete record of smoking habits and job histories was available for each subject.

IgE-antibodies

Sera were stored at −20°C until IgE analysis. Total IgE was measured with a previously described enzyme immunoassay (Doekes *et al*., 1996). Atopy was defined as a total serum IgE above 100 kU/l.. Specific IgE antibodies to wheat flour were measured with a commercial immunoassay (AlaSTAT; DPC, Apeldoorn, The Netherlands) (El Shami and Alaba, 1989). Sera of class 1 or higher (>0.35 kU/l.) were considered positive.

Allergen exposure assessment

In all bakeries together, 546 personal inhalable dust samples were collected in the workers breathing zone during full-shift periods of 6–8 h. Dust levels were measured by weighing in a preconditioned weighing room before and after the measurements. Inhalable dust levels can be regarded as a proxy of wheat dust and even cereal dust levels since wheat is the most often used flour in Dutch bakeries. Wheat allergens were recovered from the filters, and the wheat allergen concentrations were measured in the extract by inhibition immuno-assay (Houba *et al*., 1996b). Wheat allergen exposure varied considerably among bakery workers, depending on the job of the bakery worker (e.g. dough maker or packer) and the type of the bakery. Based on these two characteristics, 22

occupational titles could be distinguished and each bakery worker was classified into one of these occupational titles. The 22 job titles were collapsed into broader exposure categories: three categories by current exposure, three categories by past exposure, and five categories by cumulative exposure as detailed-described below. This was done because it was expected that although a refined grouping might lead to removal of potential overlap between exposure categories, the disadvantage would be that estimates of average exposure in a category would be on fewer measurements. The following exposure classifications were used:

- Current average inhalable dust and wheat allergen exposure. The mean dust and wheat allergen exposure gradually increased over all 22 occupational titles. Three exposure groups with a distinctly different average exposure were formed with approximately equal numbers of bakery workers. The group with the highest dust or wheat allergen exposure levels, consisted mostly of dough makers from the industrialized bakeries including all workers from small traditional bakeries. The group with intermediate dust or wheat allergen exposure consisted of all-round staff, oven staff and production managers. All other workers were classified into the low exposure group. Other more detailed grouping structures were considered but were not feasible because of strongly overlapping exposure distributions.
- Past exposure in three categories, measured as the average exposure of the level in the highest exposed occupational title category ever worked in over the complete work history.
- Cumulative inhalable dust and wheat allergen exposure, calculated as the sum of the products of job (arithmetic exposures) and duration of exposure in that particular job title, grouped into five equally sized categories with cut-off points of 1.7, 12, 40 and 120 μg/m^3/yr for wheat allergen exposure and 3, 8, 19 and 45 mg/m^3/yr for dust exposure.

Statistical analyses

All statistical analyses involving exposure categories were performed using SAS software (version 6.12). Prevalence ratios (PRs) were calculated by using a proportional hazards model (Cox regression using PROC PHREG) (Thompson *et al*., 1998). The relationship between the PR and average exposure in an exposure category was analyzed using regression analysis as proposed by Rothman (1986), a classical two step approach to obtain a quantitative exposure response relationship using risk estimates from a conventional categorical epidemiological analysis (Checkoway *et al*., 1989). The observations were not, as proposed, weighted by the inverse of the variance of the estimated PRs to account for differences in the width of confidence intervals, since the effect of weighting was marginal. To explore the relationship between dust, allergen exposure and wheat flour sensitization, General Additive Models using Quasi Likelihood estimation, and a log link function available in S-plus version 4.0 were used. These additive models extend a linear (parametric) model by allowing some or all linear functions of the predictor variables (X1, X2, …Xi) to be replaced by arbitrary smooth functions ($f_1(X_1)$, $f_2(X_2)$, … $f_3(X_3)$). f is usually unknown and can be estimated by a scatter plot smoother. The advantage over simple linear modeling is that the shape of an exposure response relationship can be evaluated in greater detail, without applying *a priori* assumptions regarding shape, this at the expense of loss of degrees of freedom. Different plots were produced with LOESS smoothers using fractions of 0.5, 0.6, 0.7, 0.8 and 0.9 of the data. Plots made according to above mentioned specifications yield prevalence ratios for each exposure value over the plotted range. Results from this approach were combined and compared with results from conventional categorical epidemiological analyses. In all analyses, differences with $P<0.05$ (two sided) were considered statistically significant. The results were plotted using Sigma Plot© for Windows 4.0.

RESULTS

Population characteristics

Population characteristics are given in Table 1, including an overview of the prevalence of chronic respiratory symptoms, work-related symptoms and results of the IgE-analyses. Wheat flour specific IgE was detected in 36 bakery workers (10%) and 26 (7%) had specific IgE to fungal amylase. Only six workers were sensitized to both wheat flour and α-amylase.

The bakery workers had on average worked 11.7 yr in the baking industry (SD 9.7). This included the years in the baking industry that they were currently employed and other bakeries they had worked in before. When grouped by current exposure, 133 bakery workers had a low, 131 an intermediate and 82 had a high (current) inhalable dust exposure (mg/m^3). If workers were grouped by the highest dust exposure category they had ever worked in, some workers moved to the higher exposure categories and the distribution changed to 103 in the low, 128 in the intermediate and 115 in the high exposure category respectively. A similar phenomenon was observed for current and highest wheat allergen exposure.

In Table 2, results are shown from a conventional analysis for average cumulative exposure per exposure category and wheat sensitization rate per exposure category. The populations could be divided into equal exposure categories for all cumulative exposure groupings. The sensitization rates in the lowest cumulative wheat and dust exposure categories

Table 1. Characteristics of the group of bakery workers (*N*=346)

	Median	SD	Range
Age (yr)	32	10.0	17–61
Years smoked	9.0	10.1	0–43
Pack-years	4.0	8.3	0–43
	N	%	
Smokers	166	48	
Ex-smokers	78	22	
Non-smokers	102	30	
% Male	305	88	
Chronic respiratory symptoms			
Chronic cough	45	13	
Chronic phlegm	25	7	
Shortness of breath	22	6	
Ever wheezing	83	24	
Frequent wheezing	29	8	
Chest tightness	36	10	
Work related respiratory symptoms			
Rhinitis	71	21	
Chest tightness	24	7	
Serology and skin prick test results			
Total IgE >100 kU/l.	87	25	
SPT house dust mite	69	20	
SPT grass pollen	53	15	
SPT birch pollen	16	5	
SPT cat allergens	15	4	
SPT wheat flour	36	10	

(categorized in five groups) were 4.4% (3/69) and 5.9% (4/68) respectively. These figures can be considered as the lowest baseline sensitization rates in this population. The risk ratios for the whole population and those obtained for atopics (not included in table) have been included in Fig. 1(a and b) for inhalable dust exposure. For wheat allergen exposure the curves had the same shape but the point estimates of the PRs were somewhat higher in all analyses.

Multiple regression models suggested that current smoking was positively associated with wheat sensitization (PR=1.7; c.i. 0.9–3.3). The number of cigarettes smoked by (current) smokers was also positively associated to the risk of wheat sensitization (PR=2.8 per 20 cigarettes per day; c.i. 0.9–8.13). However, correction of the relationship between exposure and sensitization for smoking yielded similar point estimates for the effect of the exposure, while the overall model fit hardly improved, and smoking was therefore omitted in further analyses. Age was not associated with wheat sensitization and therefore did not confound exposure sensitization relationships either, and was also not included in the final regression models. No difference in sensitization rate was found between males and females after correction for exposure and atopy (for example, PR 0.88, $P>0.80$ in a model with current wheat exposure).

Results from analyses with current exposure, highest exposure over the total job history to inhalable dust, and wheat allergens also showed increasing risks for developing sensitization at higher exposure levels (not presented). The model fit was somewhat lower for these models compared to models with cumulative exposure, although differences in fit between different models were small. We therefore continued further analyses using parametric models and cumulative exposure to inhalable dust and wheat and used an exposure grouping into five equally sized exposure categories (Table 2).

The role of atopy was further evaluated in more detailed analyses presented in Table 3. The differences for inhalable dust and wheat allergen levels were, as for the models in Table 2, small, probably because of the high correlation between wheat and dust exposure levels after grouping. Two different models were evaluated, a model with atopy as dichotomous variable added to the model with exposure variables (model I) and a model with the interaction between atopy and exposure (model II). The first model assumes that the ratio of prevalence ratios for atopics and non-atopics per exposure category remains constant over the exposure range, and this ratio is equal to $\exp(\beta_{\text{atopy}})$. This model also assumes

Table 2. Average current and cumulative exposure (and standard deviation) by exposure category for wheat allergen and dust exposure

	Cumulative wheat exposure		Cumulative dust exposure	
	Average (SD) [$\mu g/m^3/yr$]	PR (c.i.)	Average (SD) [$mg/m^3/yr$]	PR (c.i.)
1.low	0.7 (0.5)	1 –	1.5 (0.8)	1 –
2.	5.3 (3.0)	1.6 (0.4–14.0)	5.4 (1.7)	1.3 (0.3–4.7)
3.	22.4 (8.0)	1.7 (0.4–7.1)	12.0 (3.0)	1.2 (0.3–4.5)
4.	70.6 (2.1)	3.7 (1.0–13.3)	29.3 (8.2)	2.4 (0.8–7.7)
5.	224 (109)	3.9 (1.1–13.9)	81.9 (39.3)	3.0 (1.0–9.2)
−2 log likelihood		411.9		414.2

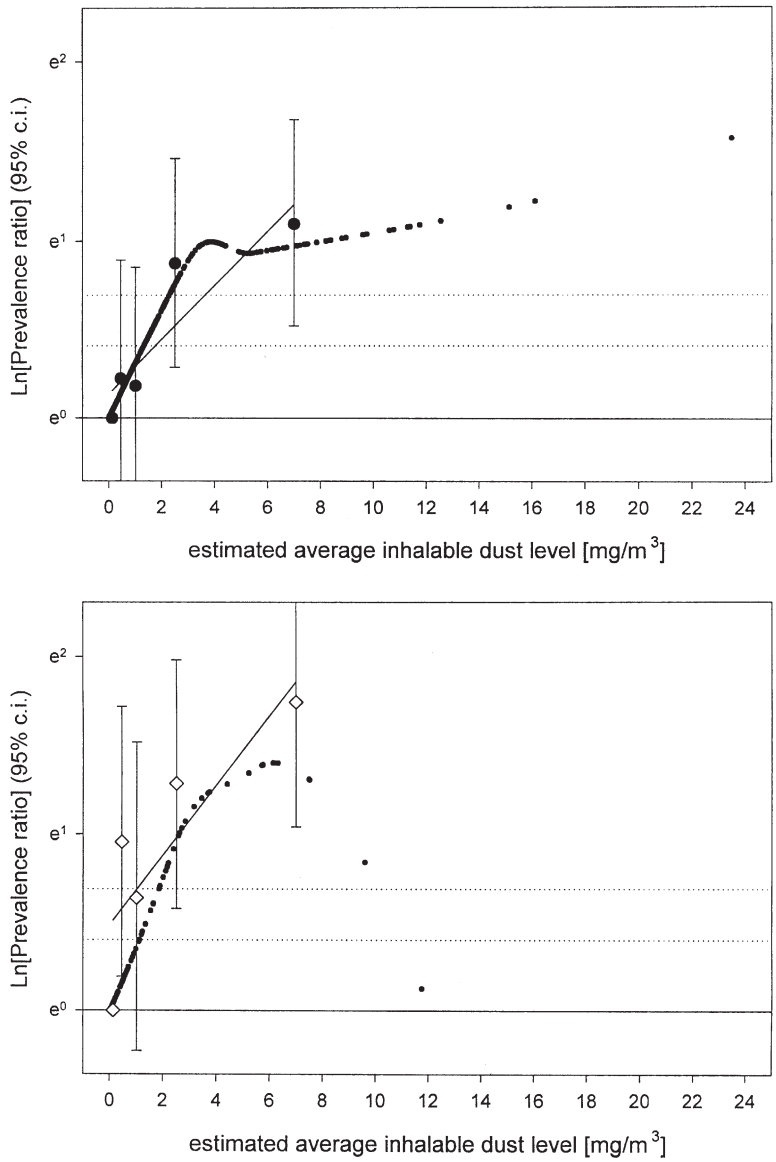

Fig. 1. (a and b) Exposure response relationships for estimated average inhalable dust exposure with wheat specific sensitization (IgE>0.35 kU/l.) for conventional categorical analyses (straight lines) and smoothed non-parametric exposure–response curves, for the whole population (a) and atopics only (b).

that the difference in sensitization risk increases with increasing exposure. So, there is no interaction on a multiplicative (ratio) scale, but there is interaction on an additive (risk difference) scale. For this model the coefficient of atopy was 0.70 (for both wheat and dust exposure), which implied that for the lowest exposed (atopics in the reference category) a sensitization rate of exp(0.70)=2 was estimated. The second model allowed the effect for atopy to vary over different exposure categories (interaction on the multiplicative scale). This model performed slightly better in terms of overall fit, and for wheat exposure, atopics in the highest exposure category had a lower sensitization risk than lower exposed atopics. However, the increase in overall fit was very small. The model with atopy as a single term would have the attractiveness of a simpler model that seems to describe the data almost equally as well as any other model. This model would have the disadvantage that it assumes that a systematic elevated risk exists for atopics, even in the absence of exposure, since the PR for each exposure category, even the baseline exposure category will have a PR value above 1. Comparison with results from model II suggests that at lower exposures the risk is overestimated by model I. Therefore, the results of the model with atopy as an interaction term describe the data in an optimal way.

A smoothed plot (fraction of data 0.9) is given for inhalable dust, in Fig. 1(a and b) for the whole population (atopics and non-atopics) and atopics only

Table 3. Different exposure response models for atopics and non-atopics between cumulative wheat sensitization and exposure to inhalable dust and wheat allergens in 346 bakery workers

Exposure category	Cumulative wheat allergen exposure				Cumulative dust exposure			
	Non-atopics		Atopics		Non-atopics		Atopics	
	PR	c.i.	PR	c.i.	PR	c.i.	PR	c.i.
MODEL I								
(exposure+atopy)								
1. Low	1	–	2.0	0.5–8.7	1	–	2.1	0.5–8.3
2.	1.7	0.4–7.1	3.4	0.7–16.6	1.2	0.3–4.5	2.5	0.6–11.1
3.	1.7	0.4–7.3	3.5	0.7–16.6	1.2	0.3–4.5	2.5	0.6–11.1
4.	3.7	1.0–13.1	7.4	1.8–30.9	2.4	0.8–7.7	5.0	1.3–18.9
5. High	4.3	1.2–15.5	8.7	2.0–38.0	3.2	1.0–9.9	6.6	1.7–25.5
−2 log likelihood	408.1	410.2						
MODEL 2								
(exposure+atopy+exposure.atopy)								
1.Low	1	–	1	–				
2.	1.7	0.4–7.6	1.4	0.1–13.0	0.7	0.1–3.9	2.6	0.6–11.4
3.	1.4	0.3–6.7	2.7	0.5–16.2	1.0	0.2–4.3	1.9	0.3–10.3
4.	2.0	0.4–8.7	7.7	2.0–30.0	2.0	0.6–7.1	3.6	0.9–14.3
5. High	4.0	1.1–14.4	3.8	0.6–23.0	2.4	0.7–7.9	5.7	1.4–22.7
−2 log likelihood	406.3	408.1						

without correction for confounding factors. A similar plot for the relation between wheat allergen exposure and wheat specific sensitization is given in Fig. 2. The underlying model for these plots had the lowest deviance and thus described the data most accurately. In this figure, relationships between exposure and sensitization including symptoms for asthma or rhinitis were included as well. The plots show a clearly increasing sensitization risk, with an increasing exposure for the whole population and for atopics only. There is no indication of the existence of a threshold for wheat sensitization risk in any of the plots. For atopics, the sensitization risk leveled off at higher exposure levels and decreased at even higher levels (average exposures of approximately 4 mg/m^3 inhalable dust or 10 μg/m^3 wheat allergens). The esti-

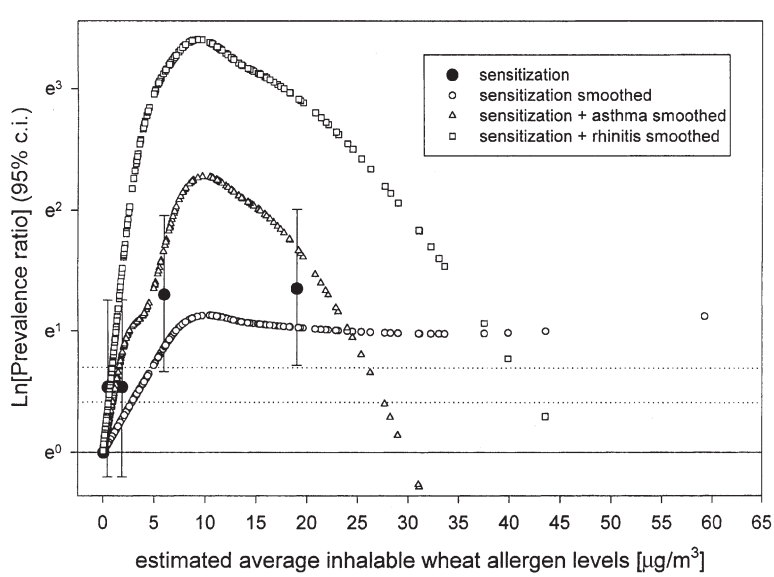

Fig. 2. Exposure response relationships for estimated average inhalable wheat allergen exposure with wheat specific sensitization (IgE>0.35 kU/l.) for the conventional categorical analysis (●) and smoothed non-parametric exposure–response curve (○), for the whole population and for sensitization accompanied by rhinitis symptoms(□) or asthmatic symptoms (△).

mated average exposures were obtained by dividing the cumulative exposures by the duration of exposure. Overall steeper slopes were observed for the atopics compared to the non-atopics. However, differences between atopics and non-atopics were small, especially at lower exposure levels. Comparison of the grouped analysis, linear regression on the basis of the results from the grouped analysis and the non-parametric modeling gave some interesting insights. Since the exposure distribution was categorized, and few atopics had exposures above approximately 4 mg/m^3 of dust or 10 $\mu g/ m^3$ of wheat, the observation of a lower risk for higher exposed atopics did not strongly influence the results of the grouped analysis (the risk estimated for the highest exposed category is probably somewhat underestimated). This effect did result in some leverage in the conventional regression analysis based on the exposure grouping (straight lines in plots). Point estimates for atopics and by wheat exposure seemed less stable and deviated more from the smoothed non-parametric estimates, probably because the grouped analysis was relatively sensitive to the cut-points chosen for the exposure categories.

The analyses with symptoms (asthma or rhinitis) and sensitization as critical endpoint showed a steeper exposure relationship than for sensitization only. This seems due to the fact that few sensitized individuals are symptomatic at lower exposure levels, while almost all sensitized individuals are symptomatic at intermediate allergen exposure levels. The increase in risk with increasing exposure is more pronounced for rhinitis symptoms compared to asthma symptoms. A strong reduction in risk was observed at high exposure levels. Similar plots were obtained for dust exposure, with less steep increases and decreases of risk with increasing exposure. A breakdown by atopics status did yield similar patters for atopics and non-atopics with a tendency for steeper increases and descreases of risk for atopics compared to non-atopics.

Further analyses showed that the difference between a simple parametric model (with the exposure as a continuous variable) and the non-parametric additive model was small for sensitization as critical endpoint, suggesting that the exposure–sensitization curve could be described in a satisfactory way by simple parametric models. This seems counterintuitive since especially the non-parametric analysis for atopics suggested leveling off of the risk at higher exposures, possibly due to the presence of a healthy worker effect. However, the number of data points at higher exposure levels is low and these observations have little influence in a parametric analysis with cumulative exposure as a continuous variable. Generalized additive models were superior in terms of model fits compared to parametric models for sensitization accompanied by symptoms.

In all previous analyses, sensitization was defined as an IgE titer above 0.35 kU/L. If a more rigid definition of sensitization was applied (anti-wheat IgE-titer of 0.7 kU/l.), the exposure–response relationship shifted somewhat to the right, and elevated risks were only observed in the highest exposure category. The same happened when sensitization in combination with the presence of work-related symptoms was used as endpoint in the analyses (rhinitis, asthma). The latter could be seen as an approach to model the risk of symptomatic wheat sensitization or allergy. Also, analyses limited to workers with relatively short work history in this industry ($\leq$4 yr) did not yield different results.

DISCUSSION

Very few explicit risk analyses have been described for high molecular weight sensitizers. As a result, few exposure standards have been proposed for these agents despite their wide spread occurrence in the work environment. A TLV of 0.06 $\mu g/m^3$ exists for one particular high molecular weight sensitizer; bacterial subtilisin, a protease that is used in the detergent industry (ACGIH, 1980). However, the underlying risk assessment is not well described in quantitative terms, and the rationale for the TLV seems to be more strongly driven by analytical limitations. An evaluation by the Nordic Expert Group for Criteria Documentation indicated that the TLV for subtilisin probably does not protect against sensitization (Brisman, 1994).

An earlier recent review of the literature by the Nordic Expert Committee on wheat flour exposure and respiratory disease did not result into a formal risk assessment due to the absence of appropriate quantitative exposure data and exposure response relationships (Tikkainen *et al*., 1996). Recently, the ACGIH proposed a TLV–TWA for flour dust exposure of 0.5 mg/m^3 measured as inhalable dust. The ACGIH used a recently published exposure–response relationship for wheat flour exposure and specific sensitization by Houba *et al*. (1998). The authors of this paper suggested that '…the sensitization risk is minimal below inhalable dust levels of 0.5 mg/m^3…'., using a categorization in three exposure groups. The Dutch Expert Committee on Occupational Standards (DECOS) will most likely use the conventional risk analysis presented in this paper with a more refined exposure response analysis based on five exposure categories. This analysis suggests that a cumulative inhalable dust exposure of around 11.97 $mg/yr/m^3$ as a No Observed Effect Level (NOEL). The long-term average exposure over an 11.7 yr period, can, based on these figures be estimated as 11.97/11.7=1.02, rounded down to 1 mg/m^3.

There are several principal issues in both risk assessments that need some further discussion:

1. Both risk assessments the one by ACGIH and

DECOS consider sensitization as the critical endpoint. Others have suggested that asthma should be considered as the critical endpoint. The Nordic Expert Group concluded earlier that 'since sensitization is not a disease', and the relationship between sensitization and symptoms is weak and the predictive value of sensitization with respect to development of disease is unclear, it 'appears unrealistic and not sufficiently founded to suggest and OEL to prevent senstization' (Tikkainen *et al*., 1996). Three arguments exist in favor of using sensitization as critical endpoint. First, there is widespread agreement that sensitization is the first step in a disease process that is accompanied by symptoms, bronchial hyper-responsiveness, and airway obstruction when exposure continues (Heederik *et al*., 1999a and Chan-Yeung and Malo, 1999). Second, the more recent studies do suggest a strong correlation between work-related sensitization and symptoms, suggesting that most sensitized workers are symptomatic. In the study by Houba *et al*. (1998), wheat sensitized workers were 2–4 times more often symptomatic compared to non-wheat sensitized workers, depending on the cut-off point for exposure. However, the correlation between sensitization and symptoms is not perfect, and most authors do believe that symptoms can also be caused by non-immune mediated mechanisms (see Houba *et al*., 1998 for an overview). In addition, longitudinal studies are needed to verify the importance of wheat sensitization for subsequent development of respiratory symptoms and airway obstruction. Third, there is some evidence for the existence of and exposure response relationship between symptoms and wheat allergen exposure in wheat sensitized workers (Houba *et al*., 1998). However, taking symptomatic allergy (sensitization in combination with symptoms) as an approximate endpoint for disease in the analysis yielded steeper exposure response relationships at the low end of the exposure range. This suggests that when even stricter defined clinical endpoints will be used, e.g. sensitization in combination with bronchial hyperresponsiveness or peak flow variability, steeper relationships will be obtained as well, and as a result, lower LOEL values.

2. A major problem in risk assessment for wheat allergen exposure is that results had to be expressed in terms of inhalable dust levels. This was necessary because immuno-assays for measurement of wheat allergens levels are not available for hygienists in the field and wheat flour allergen exposure has to be evaluated by measuring inhalable dust levels as a proxy. Two approaches are possible; a risk assessment directly based on inhalable dust levels, or one based on wheat allergen levels that have to be converted back to wheat allergen levels. Both approaches have disadvantages. The slope of the exposure response relationship seems somewhat underestimated when inhalable dust levels are used instead of wheat allergen levels. Dust measurements are a proxy of the wheat allergen exposure, leading to misclassification of the exposure and subsequently leading to underestimation of the slope of the exposure response relationship (Heederik and Attfield, 2000). This would in its turn lead to overestimation of the NOEL of LOEL. A risk analysis based on wheat allergen level is to be preferred, however, conversion of wheat allergen levels would lead to complications as well. The wheat antigens: dust ratio has been estimated to have an average value of 1.450 μg/mg, with a range of 0.4–2.9 μg/mg (Houba *et al*., 1996b). Higher values of the ratio were observed in environments where wheat flour was the major contributor to the dust exposure. Interestingly, the ratio was even larger when exposure measurement series taken in the Netherlands and Canada were compared, most likely because of differences in allergen content of North American and European wheat flour mixtures (Burstyn *et al*., 1999). This conversion could be accounted for in an uncertainty factor. In the future, this problem can be avoided by introduction and use of wheat allergen assays in the field, but it is expected that standardization of the methods applied will be a complicated issue because of the many sources that contribute to differences between outcomes of assays (Hollander *et al*., 1996; Renström *et al*., 1999).
3. The Nordic Expert Group concluded in 1996, that: 'Existing data on exposure–response relationships do not allow the identification of a NOAEL for flour dust. Due to the nature of allergy it is unlikely that the setting of a NOAEL for flour dust will be practicable even in the near future ' (Tikkainen *et al*., 1996). In some text books it is even suggested that exposure response modeling for sensitizers is technically impossible, even when personal exposure data are available (Becklake, 1993; Becklake *et al*., 1999). Although this paradigm no longer seems valid, it continues to exist. Recent publications with quantitative exposure response relationships for wheat and some other allergens including fungal α-amylase (Houba *et al*., 1996a; Nieuwenhuijsen *et al*., 1999) and rat urinary proteins (Heederik *et al*., 1999b) show the potential of well designed epidemiological studies with strong exposure assessment strategies. The discussion now shifts towards the question how data should be analyzed and what rules should be applied in risk analyses when dealing with high molecular weight sensitizers in contrast to approaches applied for other agents such as for instance (genotoxic) carcinogens. In addition, the biological basis for choosing a parti-

cular statistical model or analytical approach is not yet established.

4. This re-analysis of existing data using more advanced statistical tools shows that conventional epidemiological and statistical approaches based on crude exposure categorizations might lead to less clear and sometimes confusing conclusions. Dependent on the critical endpoint, the relationship between exposure and risk is not always described adequately by simple parametric models. Results of conventional analyses are expected to be strongly dependent on cut-off point chosen for different exposure categories and exposure ranges included in specific categories. Simple parametric models could reasonably adequately approximate relationships between exposure and sensitization. For sensitization in combination with the presence of symptoms this was not the case. The decrease in risk at higher exposure levels for symptomatic sensitized workers might be associated with job migration towards lower exposed jobs or out of the backing industry. This observation needs to be confirmed in longitudinal studies.
5. The exposure–response relationship obtained for sensitization accompanied by symptoms is the result of superimposing the exposure response relationship for exposure and symptoms on the relationships between exposure and sensitization. In this study, the steepest relationship was obtained for sensitization in combination with rhinitis symptoms, compared to sensitiztion only or in combination with asthmatic symptoms. This is in agreement with a recent Swedish study (Brisman *et al*., 2000) and might be explained by the fact that wheat dust particulates mainly deposit in the higher airways, mainly leading to allergic rhinitis.

All applied exposure–response modeling approaches in this study suggested an increasing sensitization risk with increasing dust and allergen exposure. The smoothed plots did not give any evidence for the existence of a threshold. It therefore seems unreasonable to interpret results from the classical exposure grouping approach as indicative for the existence of a threshold between estimated average levels between 0.5 and 1 of mg/m^3 dust and 2 and 6 μg/m^3 wheat allergens. Therefore only a LOEL can be derived from the available data. Alternatively, what is called a 'benchmark' approach is needed for which an acceptable increase in risk needs to be defined, for instance on the basis of information regarding sensitization in non-occupationally exposed individuals. On the other hand, estimates of a LOEL from the more advanced statistical analyses were close to the NOEL and would not lead to an essentially different exposure limit.

A complication in the interpretation of the results of the models is that little is known about background sensitization levels to most high molecular weight agents in occupationally non-exposed individuals. Gautrin *et al*. (1997) found that 1.2 and 4.1% of apprentices in animal health and dental hygiene, respectively, were sensitized to wheat flour, compared with 5% to baker's apprentices. Houba *et al*. (1998) reported skin prick test results with a wheat extract in 416 laboratory animal workers and found a positive test in 2.1% of these workers. When information (Houba *et al*., 1998) on sensitivity and specificity of wheat allergen skin prick test results compared to specific anti-wheat IgE analyses are used to calculate the prevalence of specific IgE against wheat allergens in laboratory animal workers a prevalence of 6.4% is obtained. This is still in the same order of magnitude and only slightly above the sensitization rates found in the lower exposure categories used in the risk analysis (4.4% (3/69) and 5.9% (4/68) in the lowest cumulative wheat and dust exposure categories). Background sensitization can be explained by exposure to wheat allergens in non-occupational environment (domestic, food products), or to cross-reactivity to other allergens, for instance pollens (Gautrin *et al*., 1997). Because of this spread in baseline rate values for wheat sensitization at low exposure levels, a prudent interpretation of increases in sensitization rates in exposed populations compared to low or non-exposed populations seems warranted. A simple approach to assess a Lowest Observed Effect Level (LOEL) is based on defining an unacceptable arbitrary increase in risk. Given the variability in prevalence in non-wheat exposed populations, a cut-point for this increased risk of 1.5 to 2 in comparison to the low exposure categories seems justified. Application of such criteria using either the smoothed plot or the linear intrapolation between the five exposure categories would lead to LOEL values between respectively 1.3–1.8 (PR=1.5) and 2.3–3.6 (PR=2.0) mg/m^3 inhalable dust, with the higher values for the linear interpolations. The risk assessment approaches based on linear regression analysis of the Risk Ratios on the average exposure in each category underestimate the slope of the exposure–response relationship, which in its turn leads to overestimation of a Low Observed Effect Level, therefore results from the smoothed plots are to be preferred.

Models for atopics and non-atopics showed, as expected, generally steeper slopes for atopics. The risk leveled off at higher concentrations. Regression models with an interaction variable for dust and wheat exposure with atopy, as well as the non-parametric smoothed plots, made clear that the sensitization risk in atopics was twice as high as in non-atopics over the whole exposure range. As a result, the difference in sensitization risk is small in absolute terms at lower exposure levels. This observation has important implications for risk assessment purposes since it suggests that if a standard has to protect atopic and non-atopic workers, the factor compensat-

ing for the difference in risk can be relatively small. The use of a correction factor has to be considered for a difference in sensitization risk between atopics and non-atopics. The epidemiological study used for this risk assessment suggested an increased sensitization risk for atopics of a factor of 2 ($e^{0.70}=2$). The risk assessment for the whole population already includes the effect of atopy to some extent. To allow further for this difference in risk a correction factor of 1.4 (weighted for the proportion of atopics in the population) could be applied to compensate for the fact that atopics have an elevated risk.

The risk assessments presented leans heavily on the one cross-sectional study that allows a quantitative risk assessment. Ideally results from more than one exposure–response study should be available, preferably cohort studies. Although the evidence for the presence of a healthy worker effect is not strong and uniform, the presence of this effect cannot be ruled out, especially not among atopics. It is generally accepted that the healthy worker effect lead to underestimation of an exposure–response relationship. This could in its turn lead to underestimation of the slope of the exposure–response relationship and could results in too high estimates for a LOEL or NOEL. Despite these limitations, these results suggest that an exposure standard can be derived. It will be a matter of debate what factors will be needed to compensate for some of the uncertainties in this quantitative risk assessment process.

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