ORIGINAL ARTICLE

Incidence of respiratory sensitisation and allergy to enzymes among employees in an enzyme producing plant and the relation to exposure and host factors

A I Larsen, C R Johnsen, J Frickmann, S Mikkelsen

Occup Environ Med 2007;**64**:763–768. doi: 10.1136/oem.2005.025304

Objectives: Belonging to the group of high molecular weight respiratory sensitisers, microbial enzymes have been reported as a well known cause of occupational allergy, typically manifesting itself as rhinitis and/or asthma. High exposure to such high molecular weight sensitisers, and possibly also peak exposures, implies a higher risk than low exposure, but the exact relation between exposure, sensitisation and clinical allergy remains to be clarified. The authors sought to estimate the risk of respiratory enzyme allergy in an enzyme producing plant and to assess the relation between exposure indices and allergy.

Methods: Retrospective follow-up study based upon data gathered from health surveillance since 1970. 1207 employees from production and laboratories were included. The level of enzyme exposure in the relevant departments was estimated retrospectively into five exposure levels based on 10-fold increments/decrements of the threshold limit value and other exposure information. The risk was estimated in an exponential regression survival model fitted with constant intensity for subperiods of time using maximum likelihood estimation.

Results: During the first three years of a person's employment, the enzyme sensitisation and allergy incidence rates were 0.13 and 0.03 per person-year at risk, respectively. In the fitted models, exposure class did not correlate with the outcome variables. The risk of sensitisation decreased along the three decades, whereas the risk of allergy remained unchanged. The risk of sensitisation and allergy was doubled among smokers. Pre-employment atopy was only associated with sensitisation risk.

Conclusion: Sensitisation to enzymes decreased during the study period, possibly reflecting improvements in the working environment. A similar decrease could not be demonstrated for allergy to enzymes. Neither of the two outcomes correlated with exposure estimates, possibly because of the low precision of the estimates.

See end of article for authors' affiliations

Correspondence to:
Dr A I Larsen, Medical
Centre, Novozymes DK,
Krogshøjvej 36, DK-2880
Bagsvaerd, Denmark; ail@
novozymes.com

Accepted 3 May 2007

For decades enzymes have been recognised as a well known cause of occupational respiratory allergy, typically manifesting itself as rhinitis and/or asthma. Although protease fermented on *Bacillus subtilis* (subtilisin) was the first established detergent enzyme allergen, it is now believed that most types of enzymes—proteases, lipases, amylases, cellulases, etc—have the sensitising capacity of the bacteria or fungus by which they are expressed.

Thus studies of both cases and epidemics have been published among employees in the enzyme producing industry and in trades where enzymes are used—for example, among bakers and in the detergent industry.¹ Prevention and surveillance of enzyme allergy is still an important issue.^{2–3}

Enzymes belong to the group of high molecular weight sensitisers, having the typical characteristics of these. It is also assumed that high exposure to high molecular weight sensitisers, and possibly peak exposures, implies a higher risk than low exposure, but the exact relation between exposure, sensitisation and clinical allergy remains to be clarified.⁴ Also the impact of tobacco smoking and atopy on subsequent development of (occupational) allergy and/or sensitisation seems to vary.¹ Selection bias and other types of confounding, insufficient size of study groups and, not least, difficulties concerning the estimation of relevant exposure, are among the challenging methodological problems in the field.

In the present study, we aim to estimate the risk of respiratory allergy and sensitisation in a survival model, where occupational enzyme exposure intensity and time are accounted for together with calendar year, pre-employment allergy, smoking, gender and age at employment.

METHODS

The present study is an extension of our previous study.⁵ Compared with the first study, the procedures for exposure assessment, as well as the statistical methods, have been further developed in the present study. In contrast to the previous study, groups which are difficult to classify in relation to exposure (service personnel, including craftsmen and cleaning personnel) were excluded from the present study. Finally, the observation period was extended to 2002.

The study was a retrospective follow-up study based upon Occupational Health Service (OHS) data gathered from the regular allergy surveillance programme at Novozymes in Denmark (previously Novo and Novo Nordisk) since 1970. The programme has been modified over the years, but the basic elements have remained unchanged: at the pre-employment examination, the individual is examined in order to determine the presence of atopy and/or type 1 allergy. At regular screenings during the first three years of employment, or at ad hoc examinations initiated by the employee, testing for enzyme allergy is done (see details below). The surveillance programme is not compulsory.

Until 1980 atopics were not allowed in the workplace. Since then, atopics have been accepted in laboratories, and (from 1987) in production. Outwith these two disciplines, some selection against atopics still exists, both as a result of medical advice and perhaps also in connection with other recruitment procedures. The departments included in the programme have altered as a result of changes in production and updated risk

Abbreviation: OHS, Occupational Health Service

assessments. The departments include production units (recovery, formulation), pilot installations and selected laboratories. The surveillance programme covered the first three years of employment (between scheduled examinations there is free access to medical advice and examination in OHS).

Inclusion criteria for being selected for the present study was (a) employment in the company for the first time; (b) employment in a production department and laboratories included in the allergy screening programme; (c) a pre-employment examination performed between 90 days before and not later than 14 days after the date of employment; (d) at least one subsequent examination completed. Only observations within the first three years of employment were included in the present study, as systematic surveillance data only exist for that period. Those who were sensitised for enzymes at the pre-employment examination were excluded from the study.

At the pre-employment examination, information on type 1 allergy symptoms and smoking were recorded. Records of allergy symptoms were diagnosed separately for rhinitis, asthma and urticaria, based on reported symptoms associated with exposure to relevant allergens. Atopy was defined as one or more positive results in standard skin prick test with 10 common inhalant allergens (pollen, animals, dust mites, and moulds) with or without corresponding symptoms. Test for enzyme sensitisation was done if previous enzyme exposure was stated.

Follow-up data concerned allergy symptoms and sensitisation to enzymes. Measurements of specific IgE to enzymes were made from the early 1970s until 1990 by the Radio Allergo Sorbent Test (RAST) described by Wide *et al.*⁶ The cut-off level for positive values was defined as >0.5 sorbent units (SU). Since 1990, measurements were made by the Pharmacia CAP-RAST system with a positive cut-off value of >0.35 kilo units/litre (kU/l).^{7, 8} Comparison of the two test systems in relation to 25 enzymes showed that the results correlated well ($r = 0.7$, $p < 0.0001$, $n = 89$).⁷ Sensitisation to enzymes was defined as detection of measurable IgE antibodies to one or more enzymes, and enzyme allergy was defined as typical symptoms of asthma, rhinitis or urticaria, related to working with enzymes, unless an alternative diagnosis was obvious. In addition to the clinical data, exact dates of employment periods in different departments were extracted from the company personnel files. Causes of leaving were not recorded because an exit examination is not part of the screening programme.

Exposure assessment by department and decade was based on measurements of enzyme concentrations in the air and other exposure information, such as measurement data from other departments with equivalent processes, information on use of masks, modification in ventilation systems, etc. Trained and experienced occupational hygienists employed in Novozymes OHS retrieved and scrutinised available files on exposure measurements and other exposure information from different departments throughout each decade of the observation period. The hygienists were not informed about the sensitisation frequencies in the departments. Measurements of enzyme concentrations in the air in the different departments have not been systematic throughout the decades. In general, their purpose has been to examine if governmental standards for exposure limits for enzymes ($0.06 \mu\text{g}/\text{m}^3$ subtilisin enzyme protein (or equivalent for other enzymes)) were adhered to. Measurements were also made to outline where to use respiratory protectors and to track sources of exposure, in order to control exposures—for example, prompted by cases of enzyme sensitisation and allergy. Measurements do not cover all enzymes and processes and are not representative for the individual's exposure profile over the day. Nevertheless, we used these measurements as an initial indicator of exposure

level. The measurement data from a department were described by the 50% and the upper 90% percentiles for each calendar year, and subsequently classified by decade into a five-point exposure level scale with cut points at 0.006, 0.06, 0.6 and $6.0 \mu\text{g}/\text{m}^3$ subtilisin enzyme protein (or equivalent for other enzymes). This classification was used as a guideline for the final classification, which also considered other exposure information, as outlined above.

Statistics

An exponential regression survival model, with constant intensity for subperiods of time, was fitted to data. The following eight potential risk factors were included in the full model:

- Gender (female, male)
- Smoking at employment (no, yes)
- Age at employment (<25, 25–30, 30–40, >40)
- Pre-employment atopy for common allergens (no, yes)
- Pre-employment allergic diagnosis for urticaria, rhinitis or asthma (no, yes)
- Duration of employment (0–1 year, 1–2 years, 2–3 years)
- Calendar time (1970–9, 1980–9, 1990–2002)
- Enzyme exposure class for department (1–5).

The model was parameterised as a normal linear analysis of variance model with eight main effects and one interaction effect between exposure class and calendar time. If E_{ij} denotes the vector of effects for person i and subperiod j , then

$$\text{intensity}_{ij} = \exp(\text{intercept} + \sum_k E_{ijk})$$

Applying the exponential function causes the intensity to be a product of the hazard ratios associated with the respective effects, and guarantees that it is always positive, regardless of parameter values. If H_{ijk} denotes the hazard ratio for person i , subperiod j and effect k , then

$$H_{ijk} = \exp(E_{ijk})$$

A maximum likelihood estimate of the model parameters was computed by a ridge-stabilised Newton-Raphson procedure (SAS Institute, SAS/OR version 8). The full model was fitted initially, and then non-significant factors were removed successively, until all remaining factors were significant on a 5% level. Factor testing was done by likelihood ratio tests. The procedure was repeated for sensitisation data and for allergy data. Factors showing significance for either dataset were included in the final model.

Ethics

Ethics committee approval was not required as we used already existing medical data only.

RESULTS

Exposure

An overview of measurement data from which exposure was classified is displayed in table 1. A huge number of measurements were retrieved and assessed. For some departments/decades, however, relatively few or no measurements exist. It is also obvious that uncertainty on the estimated exposure levels is substantial. Laboratories were classified in the lowest exposure group, although no systematic exposure data exist for the period. The measurements indicate a decreasing exposure over decades in the granulation and recovery departments but not in the pilot departments.

Table 1 Distribution of enzyme dust measurements* by department type and decade

Department type	Decade	Departments with employees in cohort, n	Departments with measurements	Measurements, n	50% percentile exposure in $\mu\text{g}/\text{m}^3$, mean (range)	Upper 90% percentile in $\mu\text{g}/\text{m}^3$, mean (range)
Granulation	1970s	1	0	0		
	1980s	2	2	3521	0.03 (0.01–0.05)	0.2 (0.07–0.6)
	1990s	2	2	8351	0.01 (0.001–0.06)	0.1 (0.01–1)
Laboratory	1970s	4	0	0		
	1980s	6	0	0		
	1990s	10	0	0		
Pilot	1970s	1	0	0		
	1980s	2	1	250	4 (2–6)	30 (6–123)
	1990s	3	2	86	5 (0.008–215)	46 (0.1–505)
Recovery	1970s	7	4	3607	0.4 (0.006–1)	1 (0.02–19)
	1980s	8	6	4537	0.1 (0–5)	0.5 (0.05–59)
	1990s	11	8	2837	0.03 (0.0006–4.1)	0.2 (0.01–63)
Total		29	13	23189		

*Measurements from areas where use of respiratory protection is mandatory are included.

Baseline employee data

Baseline data are displayed in table 2. 1207 employees were included in the follow-up study (199 females and 1008 males). In production departments, the majority of workers were males; in laboratory departments, the majority were females. The prevalence of atopy and allergic disease at employment was low (14.7%) due to the selection practice (as explained above).

Follow-up

Median follow up time was 2.7 years. A total of 324 employees, or 26.8%, became sensitised, and 78 employees, or 6.4%, developed an enzyme-related allergy diagnosis (table 3). All of these were sensitised, hence the ratio of enzyme allergy to sensitisation was 0.24. The corresponding incidence rates were 0.13 and 0.03 per person-year at risk, respectively. The distribution of allergy diagnosis is illustrated in figure 1.

Figures 2 and 3 show the “survival” of the population in relation to sensitisation and allergy, respectively, by Kaplan-Meier curves stratified on decade of employment. For sensitisation, “survival” seems to improve over the decades, whereas the pattern is less distinct for allergy development, although the decay seems to be slower for persons employed after 1990.

To estimate the independent significance of occupational and personal potential risk factors, multivariate survival models

were set up for both endpoints. Table 4 displays the final models for sensitisation and allergy, respectively. Exposure class was not associated with either of the two endpoints. Employment decade appeared as a clear risk factor for sensitisation (decreasing risk during the study period), but not for allergy. Furthermore, duration of employment was an inverse risk factor, consistent with the initial steeper part of the Kaplan-Meier survival curves (figs 2 and 3).

Both smoking and atopy were risk factors for sensitisation, but only smoking for enzyme allergy. Age, gender and exposure class by decade interactions were excluded from the final models as they had no significant effects and did not influence the effects of the other covariates in the models.

DISCUSSION

Although the present study was relatively large and profits from follow-up data and satisfactory completeness, there are some important limitations. First of all, the recruited population was the object of formal and informal selection suppressing the numbers of atopics recruited to the population. Hence, the crude risk of sensitisation and allergy might well be underestimated. Furthermore, those with a severe degree of atopy were probably under-represented in heavier-exposed environments, thus underestimating the impact of atopy also. Likewise, as strict formal selection procedures have been

Table 2 Description of population at the time of employment

	1970s	1980s	1990s	Total
Total	219	564	424	1207
Exposure class				
1 – Lowest exposure	45 (21%)	94 (17%)	78 (18%)	217
2	8 (4%)	23 (4%)	16 (4%)	47
3	56 (26%)	146 (26%)	135 (32%)	337
4	103 (47%)	281 (50%)	188 (44%)	572
5 – Highest exposure	7 (3%)	20 (4%)	7 (2%)	34
Allergy status at time of employment				
Negative SPT and no allergy symptoms	206 (94%)	490 (87%)	333 (79%)	1029
Atopy without symptoms	11 (5%)	57 (10%)	63 (15%)	131
Clinical allergy	2 (1%)	17 (3%)	28 (7%)	47
Smoking at time of employment				
No	77 (35%)	262 (46%)	277 (65%)	616
Yes	142 (65%)	302 (54%)	147 (35%)	591
Department type				
Granulation	26 (12%)	97 (17%)	72 (17%)	195
Laboratory	53 (24%)	107 (19%)	75 (18%)	235
Pilot plant	7 (3%)	30 (5%)	31 (7%)	68
Recovery	133 (61%)	330 (59%)	246 (58%)	709

SPT, skin prick test.

Figure 1 Distribution of allergy diagnosis.

loosened in line with improvements in working conditions, the separate effects of atopy and exposure over calendar time may be difficult to disentangle.

Another issue is that the population has been the object for intervention through follow-up and not just observation. Strengthened personal protection—for example, by increased use of masks or cease of exposure through job change—probably modified the natural process of sensitisation leading to allergy. These limitations could not be rectified through statistical analyses. Our health data, collected over an extended time period, were inevitably influenced by varying clinical practice, but overall we consider them to be quite reliable and valid.

Figure 2 "Survival" in relation to sensitisation.

In contrast, the methods applied to classify the relevant exposure may be seriously questioned. Firstly, exposure measurements were not made in a systematic way to be representative of department exposure levels. Secondly, exposure may vary from person to person in the same department. Thirdly, we do not know if peak exposures are more important than average exposure, and peak exposures may occur in departments with a low average exposure. Furthermore, the significance of mono-enzyme exposure in contrast to a multi-product exposure situation is unknown.

The estimated incidence rates for sensitisation and allergy were 13.1 and 2.9 per 100 person-years at risk, respectively. No standard of reference relevant for the actual setting could be

Table 3 Person-years at risk and crude incidence rates

	Sensitisation			Diagnosed allergy*		
	Person-years at risk	Count of incidences	Incidence rate	Person-years at risk	Count of incidences	Incidence rate
Total	2466	324	0.13	2704	78	0.03
Exposure class						
1 – Lowest exposure	417	58	0.14	454	20	0.04
2	91	11	0.12	97	1	0.01
3	721	104	0.14	799	19	0.02
4	1155	140	0.12	1265	37	0.03
5 – Highest exposure	82	11	0.13	89	1	0.01
Decade						
1970s	318	62	0.20	368	11	0.03
1980s	1162	206	0.18	1324	46	0.04
1990s	987	56	0.06	1011	21	0.02
Employment duration (years)						
<1	1117	136	0.12	1141	40	0.04
1–2	845	113	0.13	953	27	0.03
>2	504	75	0.15	609	11	0.02
Allergy status at time of employment						
Negative SPT and no allergy symptoms	2146	273	0.13	2342	65	0.03
Atopy without symptoms	240	38	0.16	271	12	0.04
Clinical allergy	80	13	0.16	92	1	0.01
Smoking at time of employment						
No	1302	121	0.09	1372	24	0.02
Yes	1165	203	0.17	1332	54	0.04
Gender						
Female	394	42	0.11	417	16	0.04
Male	2072	282	0.14	2287	62	0.03
Employment age (years)						
<25	638	84	0.13	704	15	0.02
25–30	632	67	0.11	672	20	0.03
30–40	717	95	0.13	783	28	0.04
>40	480	78	0.16	545	15	0.03

SPT, skin prick test.

*The distribution of allergy diagnosis is shown in figure 1.

Figure 3 "Survival" in relation to enzyme allergy.

found in the literature. Although data were available from other exposure settings, data were sparse from follow-up studies of an acceptable study size and participation rate. Risk estimates around 10 per 100 person-years for sensitisation and 2–5 for occupational allergy are however typical.⁹

Regarding risk factors, it was not possible to demonstrate any sensible association between exposure class and the two outcomes (tables 2 and 3). This may be the result of misclassification of the (relevant) exposure as outlined above. Nevertheless, it still seems odd that even in the laboratories the risk of sensitisation and allergy was at the same level as in the production areas (data not shown). The laboratories in particular, however, have been characterised by earlier and more consequent decrease of pre-employment selection. Furthermore, it is our impression that the attention to allergy symptoms is higher among the predominantly female laboratory technicians than among the predominantly male production workers.

"Decade of employment" might be another meaningful indicator for exposure, assuming that the working environment has been improved during the 30 years of observation, as a result of more adequate personal protection and better hygiene. Although the exposure certainly differs between departments, the assumption that general working conditions have improved in the company during the three decades seems to be sensible. The reduction in risk of sensitisation over the years is consistent

with a hypothesis of an exposure-response relation. The decrease in risk of sensitisation cannot be explained by differences between test systems as mentioned above. In contrast, the risk of allergy seemed unrelated to decade of employment. However, if the exposure has significantly decreased during the study period, and thereby also the risk of sensitisation and allergy, this might be negated or hidden by the gradually milder selection practice introduced over the same period and by an increased attention to allergy symptoms over the years, increasing the probability of allergy detection. Another explanation is that the exposure-response curve is less steep for allergy than for sensitisation in the relevant range of exposure.

Our study confirmed the view that smoking is a risk factor for sensitisation as well as for allergy. Other studies conflict with this observation. Pre-employment atopy appeared to increase the risk of sensitisation and possibly of allergy, but not significantly so in our study.

In conclusion, our data confirmed that enzymes expressed by microorganisms can induce sensitisation and allergy. Sensitisation to enzymes decreased during the study period, possibly reflecting improvements in the working environment. A similar decrease could not be demonstrated for allergy to enzymes. Neither of the two outcomes correlated with exposure

Main messages

- The study confirmed that microbial enzymes can induce sensitisation and allergy at low exposure levels.
- Sensitisation decreased during the study period, most likely due to improved working conditions
- A similar time trend was not found for allergy caused by enzymes, possibly because of differential workforce selection over time.
- We found no evidence of an exposure-response relation, probably to the result of exposure misclassification.

Policy implications

- High quality exposure data are needed to further study exposure-response relations.
- Strict exposure control is essential for preventing enzyme sensitisation and allergy.

Table 4 Adjusted hazard ratios for sensitisation and allergy respectively in the exponential regression survival model

Covariate	Outcome: sensitisation	Outcome: allergy
	Hazard ratio (95% CI)	Hazard ratio (95% CI)
Intercept	0.20 (0.14 to 0.29)	0.04 (0.02 to 0.09)
Exposure class (2)	0.82 (0.39 to 1.68)	0.00
Exposure class (3)	1.05 (0.75 to 1.45)	0.43 (0.23 to 0.80)
Exposure class (4)	0.90 (0.63 to 1.28)	0.71 (0.39 to 1.29)
Exposure class (5)	0.82 (0.41 to 1.65)	0.00
Decade (1980s)	0.60 (0.45 to 0.80)	1.41 (0.71 to 2.79)
Decade (1990s)	0.15 (0.10 to 0.23)	0.83 (0.38 to 1.81)
Duration of employment (1–2 years)	0.77 (0.55 to 1.08)	0.48 (0.25 to 0.89)
Duration of employment (2–3 years)	0.89 (0.63 to 1.27)	0.20 (0.07 to 0.55)
Pre-employment atopy without symptoms (yes)	1.89 (1.37 to 2.60)	1.35 (0.71 to 2.57)
Smoking at employment (yes)	2.02 (1.57 to 2.58)	2.19 (1.31 to 3.65)

estimates, possibly because of the low precision of the estimates.

ACKNOWLEDGEMENTS

The authors thank laboratory technician Ditte Andersen for gathering and assessing exposure data.

Authors' affiliations

A I Larsen, C R Johnsen, J Frickmann, Medical Centre, Novozymes A/S, Bagsvaerd, Denmark

C R Johnsen, Medical Department, Roskilde County Hospital, Roskilde, Denmark

S Mikkelsen, Department of Occupational Medicine, Copenhagen University Hospital, Glostrup, Denmark

Competing interests: AI Larsen and J Frickmann are both employed by Novozymes. CR Johnsen does occasional consultancy work for Novozymes.

REFERENCES

- 1 **Bernstein JA**, Gaines Jr WG. Enzymes. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, eds. *Asthma in the workplace*, Second edition. New York: Marcel Dekker, 1999:363–76.
- 2 **Cullinan P**, Harris JM, Newman Taylor AJ, et al. An outbreak of asthma in a modern detergent factory. *Lancet* 2000;**356**:1899–900.
- 3 **Sarlo K**. Control of occupational asthma and allergy in the detergent industry. *Ann Allergy Asthma Immunol* 2003;**90**(Suppl):32–4.
- 4 **Heederik D**, Thorne PS, Doekes G. Health-based occupational exposure limits for high molecular weight sensitizers: How long is the road we must travel? *Ann Occup Hyg* 2002;**46**:439–46.
- 5 **Johnsen CR**, Sorensen TB, Larsen AI, et al. Allergy risk in an enzyme producing plant: a retrospective follow up study. *Occup Environ Med* 1997;**54**:671–5.
- 6 **Wide L**, Bennich H, Johansson SGO. Diagnosis of allergy by an in vitro test for allergen-specific IgE antibodies. *Lancet* 1967;**2**:1105–7.
- 7 **Ewan PW**, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy* 1990;**45**:22–9.
- 8 **Poulsen LK**, Johnsen CR, Lovborg U, et al. A system for in vitro monitoring of occupational sensitization of workers in bioindustrial plants. *J Allergy Clin Immunol* 1995;**207**:1995.
- 9 **Malo JL**, Gautrin D. How often does it occur? Proceedings of the first Jack Pepys occupational asthma symposium. *Am J Crit Care Med* 2003;**167**:452–3.