

ALLERGIES IN THE WORKPLACE

SENSITISATION TO MAIZE IN THE WET-MILLING INDUSTRY

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ABSTRACT

Aims. The aims of this study in the South African maize wet-milling industry were to determine the extent of skin-prick reactions to extracts of common allergens (atopy) and to maize products in mill employees, to test for specific IgE to grain weevils and storage mites and to evaluate whether positive tests of sensitisation were associated with work-related symptoms.

Methods. A cross-sectional study was carried out in four maize mills and the company head office. Workers were then followed up at 6-monthly intervals over a period of 18 months. Examinations included symptoms, employment and exposure histories. Skin-prick tests were done: (i) for atopy using commercially available common allergens; (ii) with a commercial extract of *Zea mays* to determine positivity to maize pollen; and (iii) with in-house extracts of three mill products to determine sensitisation to maize products. Tests for IgE antibodies to grain weevils and storage mites were done using ImmunoCAP.

Results. Our results showed that a high proportion of employees were atopic (over a third in most categories). Positive skin-prick tests to in-house maize extracts were common (24% at the cross-sectional study and 19% at follow-up), but this sensitisation to maize allergens was not always associated with work-related symptoms. Approximately 25% of subjects were sensitised to grain weevils or storage mites and this may be a factor in some symptomatic employees.

The grain milling industry in South Africa employs a large number of workers who process maize products. Employees may be exposed daily to numerous inhalant allergens that can elicit an immune response. In addition to the actual grain, allergens may include fungi, insects, rodent hair, urine and droppings, grain weevils, bacteria and their toxins. Sensitisation to grain dust can therefore be due to several components. The increased risk of respiratory disease resulting from exposure to grain dust – asthma, asthma-like syndrome (non-allergic bronchial constriction) and chronic airflow limitation, as well as conjunctivitis and dermati-

tis, are well documented.¹⁻³ Organic toxic dust syndrome and allergic alveolitis and the possibility of prolonged exposure causing permanent irreversible lung damage have been reported.¹⁻⁴ However, very little has been published on the respiratory health effects of exposure to maize dust in particular and the allergenic properties of specific maize proteins.

Wet milling is a process whereby maize is cleaned and softened in tanks with a water-based solution, to release starch. The water is then drawn off and the maize is milled to produce a variety of products. In the South African maize-milling industry pre-employment medical examinations may include skin-prick tests to establish existing sensitisation to common allergens and maize. The test series typically used includes a commercially available extract of *Zea mays* (ZM) (maize pollen) but not extracts of actual products to which the workers may be exposed. As a result, the National Institute for Occupational Health (NIOH) was approached to evaluate a set of commercial extracts of common allergens and extracts of actual maize products to be prepared by the NIOH (designated in-house extracts). The role of these tests in predicting subsequent sensitisation, and the diagnosis, management and monitoring of exposed workers regarding maize-related respiratory disease were the primary concerns.

The objectives of the study reported here were:

- to determine the extent of positive skin-prick tests to common aeroallergens (an index of atopy)
- to determine the extent of positive skin-prick test responses to maize allergen extracts prepared from materials to which workers were exposed
- to identify sensitisation to storage mites and grain weevils that may be present in the work environment, by testing for the presence of specific IgE in workers and
- to determine whether maize-sensitised workers had work-related symptoms.

Materials and methods

The study population comprised employees of a wet-milling company producing maize products, working in the company head office (HO), three mills in Gauteng (designated Mill A, Mill B and Mill C), and one mill in the Cape (Mill D). The study had two phases: firstly, a cross-sectional study and secondly, a cohort (follow-up) study which included cross-sectional study participants and additional existing workers who agreed to follow-up examinations.

The cross-sectional study comprised all consenting workers appointed by the company between January 1997 and January 2000 who were available on the days of the examinations. Data for the cross-sectional study were derived from this first examination and from records of the original pre-employment skin-prick tests where these were available. Eighty-four employees took part in the cross-sectional study: 14 from HO and 22, 13, 18 and 17 from mills A, B, C and D respectively.

A cohort of 75 workers was assembled for follow-up, which included 57 of the 84 cross-sectional study participants, and 18 of 25 other currently employed workers who had not been included in the original

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cross-sectional study but who wished to take part. Workers were excluded from the cohort study if they had positive skin-prick tests to in-house allergens at their first examination; they were deemed maize-sensitised in terms of the study definition and it was therefore inappropriate to follow them up. Study participants underwent a medical examination on entry into the study and were re-examined and tested for sensitisation at 6-monthly intervals for a maximum of 18 months. Subjects who became sensitised (as defined by positive skin-prick tests to in-house allergens, see below) were no longer followed up, i.e. were censored. Participants were grouped by levels of exposure to low, medium or high levels of maize dust, assigned according to job descriptions. Low exposure indicated unlikely occupational contact with maize, medium exposure some indirect contact with maize, and high exposure, direct contact with maize. The same criteria were used for all four mills and HO.

A standard respiratory questionnaire was adapted to include some questions related to working history in the maize industry and exposure to maize or maize-related products at home. Experienced employees of the NIOH administered the questionnaires. Skin-prick tests were carried out by the standard 'prick-through' method. Reactions were read after 10 minutes. A wheal and flare, with induration of at least 3 mm greater than the negative control, was regarded as positive and measured in mm.

In-house allergen extracts from maize kernels, animal feed and gluten (three potential allergens derived from maize, to which the workers were most likely to be exposed) were freshly prepared before each series of tests. The products were ground to a powder and the protein extracted in phosphate-buffered saline (PBS) by mixing at 4°C overnight. The extracts were centrifuged and filtered and the protein content adjusted to 1 mg protein/ml of PBS⁵ using the standard BIORAD method and stored at 4°C until use. For the purposes of this study, the allergens were grouped as commercial extracts, in-house extracts and ZM.

A blood sample was collected at each medical examination to use for detection of IgE specific for allergens from a mix of two types of storage mites (*Tyrophagus putrescentiae* and *Lepidoglyphus destructor*) and grain weevils (*Sitophilus granarius*) using standard methodology and ImmunoCAP tests using the UNICAP system. Results were expressed as positive when values exceeded 35 kU/l.

At each interview, participants were classed as symptomatic for work-related allergy if at that interview they reported symptoms of asthma, hay fever, conjunctivitis or rhinitis related to work.

Atopy was defined by a positive skin-prick test to one or more of a panel of common allergens. Commercial maize allergen was used at the time of the initial skin-prick test.

Subsequent sensitisation to maize was demonstrated by a positive skin-prick test to one or more of the three in-house maize allergens.

Results

Eighty-four consenting employees were included in the cross-sectional study. Differences in the distribution of age, gender and smoking habits among workers in the different mills were insignificant (not shown). Exposure varied more (Table I), as the distribution of employees in the three exposure groups differed among mills and particularly HO. To accommodate these variations subsequent results are stratified by exposure group rather than by mills.

Workers' skin-prick test results are shown in Table II. For reference purposes, the total number of employees per category is shown in the left column and then (from left to right) the number of employees sensitised to each of the allergen groups, as previously described. The first two columns show results of the skin-prick tests of 50 workers for atopy at their initial pre-employment examination. Comparison within the relevant categories showed that at pre-employment, the prevalence of atopy was somewhat higher in smokers than non-smokers (38% vs 14%).

Table I. Exposure levels by mill and head office (HO)

	N	Exposure levels		
		High (%)	Medium (%)	Low (%)
Mill A	22	5 (23)	9 (41)	8 (36)
Mill B	13	1 (8)	7 (54)	5 (38)
Mill C	18	11 (61)	0 (0)	7 (39)
Mill D	17	5 (29)	5 (29)	7 (41)
HO	14	0 (0)	3 (21)	11 (79)
Total	84	22 (26)	24 (29)	38 (45)

Table II. Atopy pre-employment and atopy and sensitisation at cross-sectional study for all study sites

Category	Pre-employment examination		Cross-sectional					
	Atopy		Sensitisation					
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Total	50	14 (28)	84	36 (43)	84	15 (18)	84	20 (24)
Duration								
≥ 2 years			41	22 (54)	41	6 (15)	41	*13 (32)
< 2 years			39	13 (33)	39	8 (23)	39	5 (13)
Smoking								
Yes	13	5 (38)	20	11 (55)	20	4 (20)	20	*8 (40)
No	37	9 (14)	64	25 (39)	64	11 (17)	64	12 (19)
Exposure								
High			22	9 (41)	22	3 (14)	22	4 (18)
Medium			24	11 (46)	24	4 (17)	24	4 (17)
Low			38	16 (42)	38	8 (21)	38	8 (21)

ZM – Zea mays, IH – in-house allergen extracts

*Statistically significant (p < 0.05).

At the cross-sectional examination, work service of 2 years or more, and being a smoker, each appeared to increase the likelihood of being sensitised to in-house allergens, but multivariate analysis (logistic regression) showed neither to be independently statistically significant (data not presented here). Thirty-six employees were classed as atopic, having one or more positive responses to the common allergens widely used for atopy screening, and 15 were sensitised to ZM.

The follow-up cohort was made up of 75 eligible employees who had not been sensitised to in-house allergens at their first examination (Table III). Thirty-six of the original 50 tested for atopy at their initial pre-employment examination went forward into the follow-up study; they are listed by category on the left. The last column of Table III also shows the numbers of participants, by category, who became sensitised to in-house allergens in the follow-up study. Thirteen of the 14 (93%) were sensitised at 6-months follow-up and the remaining one (7%) at 12 months. There were no significant differences in the numbers of employees who became sensitised in any category.

ImmunoCAP tests to storage mites and grain weevils were carried out on 70 of the 84 employees at the cross-sectional study. IgE specific for storage mites was found in 8, and IgE specific for grain weevils in 4 of the subjects. All of the 75 subjects included in the follow-up were tested at least once for IgE to both pests. As shown in Table IV, 18 of the 75 were positive for storage mites and these included all 13 of the grain-weevil-positive subjects. The proportion of positive cases varied considerably between mills.

Workers who were smokers or had 2 or more years' service were more likely to report symptoms (Table V). In bivariate analysis, there were no significant associations between symptoms and the level of exposure, sensitisation to ZM or in-house

allergens. Specific IgE to grain weevils was detected in the serum of proportionately more subjects with symptoms than without, but in this small number the association was not statistically significant.

Table IV. Specific IgE against storage mites and grain weevils by study site

Mill	N	SM	SM + GW
A	17	5 (29)	3 (18)
B	10	1 (10)	0 (0)
C	19	4 (21)	3 (16)
D	16	7 (44)	6 (38)
HO	13	1 (8)	1 (8)
Total	75	18 (24)	13 (17)

SM – storage mite, GW – grain weevil.

Table V. Cross-sectional study: association between work-related symptoms, exposure characteristics and sensitisation

Category	Symptoms		POR	95% CI
	Yes	No		
	(N = 33)	(N = 49)		
Characteristic				
Smoker	12 (36)	7 (14)	3.43	1.05 – 11.75
> 2 years service	21 (64)	18 (40)	3.01	1.10 – 8.38
Exposure medium or high	17 (51)	28 (57)	0.79	0.29 – 2.12
Positive skin-prick test to				
In-house allergen	7 (21)	13 (27)	0.75	0.22 – 2.36
<i>Zea mays</i> commercial allergen	7 (21)	8 (16)	1.38	0.38 – 4.93
Common allergens (atopic)	16 (48)	18 (37)	1.62	0.60 - 4.37
IgE positive	N = 28	N = 50		
Grain weevils	3 (11)	1 (3)	4.80	0.36 – 257.08
Storage mites	5 (18)	8 (20)	0.92	0.21 – 3.57

Percentages are given in parentheses. POR – prevalence odds ratio, CI – confidence interval.

Table III. Atopy pre-employment, atopy and sensitisation during follow-up (all sites)

Category	Pre-employment examination		During follow up					
			Atopy		Sensitisation			
	Atopy		'Ever'		ZM 'ever'		IH	
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Total	36	11 (31)	75	38 (51)	75	19 (25)	75	14 (19)
Duration								
≥ 2 years	14	4 (10)	34	15 (53)	34	8 (24)	34	6 (18)
< 2 years	22	7 (32)	41	20 (49)	41	11 (27)	41	8 (20)
Smoking								
Yes	10	4 (40)	19	13 (68)	19	6 (32)	19	3 (16)
No	26	7 (27)	56	25 (45)	56	13 (23)	56	11 (20)
Exposure								
High	9	2 (22)	18	8 (44)	18	4 (22)	18	5 (28)
Medium	10	3 (30)	24	13 (54)	24	3 (13)	24	2 (8)
Low	17	6 (35)	32	217 (53)	32	12 (38)	32	7 (22)

ZM – *Zea mays*, IH – in-house allergen extract. 'Ever' indicates a positive result during any of the examinations throughout the study.

Discussion

This study showed that a typically large proportion of employees were atopic (over a third in most categories). Positive skin-prick tests to maize allergens were common (about 20% of workers), but were not always associated with symptoms of respiratory disease within the study period. Sensitisation to weevils and storage mites was evident in approximately 20% of subjects.

There are numerous problems associated with the study of sensitisation and occupational allergy in general, many of which apply to this study. For example, actual measurements of maize-dust exposure were not available and an



Maize silos at one of the mills.

indirect marker of exposure (job description) was used for all mills. The small dataset from several different mills, with different products, layout and facilities was grouped together for analysis. Not all eligible workers participated in the study and most subjects were not new recruits to the maize industry but had varying lengths of service. In addition, information on the reasons for employees leaving the industry was not available. It is therefore possible that the work force may be largely a 'survivor population', caused by workers with acute symptoms, who were unable to tolerate their work environment, having left soon after employment, while those without symptoms stayed on, giving rise to a 'healthy worker effect'. Nevertheless, some findings were of interest despite these methodological limitations.

Sensitisation of 24% of workers to in-house extracts of actual products in the cross-sectional study and 19% within 12 months of follow-up confirmed the efficiency

of these extracts in producing a positive skin-prick test and presumably an immune response. But there were many workers with positive skin-prick tests who did not report work-related symptoms and there was not an obvious association between positive skin-prick tests and work-related symptoms, so the interpretation of these positive maize skin-prick tests and their value in monitoring exposed subjects requires further investigation. A relationship between positive skin-prick tests to maize in particular and symptoms in workers has not been shown in the literature to date.

In the study of specific IgE measured by ImmunoCAP detection, IgE specific to grain weevils only occurred together with specific IgE to a mix of two storage mites, but some subjects only had IgE to storage mites. This suggests that either grain weevils share some antigenic features with one of the storage mite species, or subjects have dual exposure in some environments, jobs or areas.⁵

Conclusions

Bearing in mind the limitations of the study, sensitisation to maize allergens was not associated with symptoms of respiratory disease by the end of the study, but sensitisation to grain weevils and storage mites may be a factor in some symptomatic employees.

Acknowledgements

The study team wishes to thank the management and staff of the company, in particular Sr Nicolene de Jager, for their support and co-operation throughout this study.

Declaration of conflict of interest

The authors have no conflict of interest.

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