

REVIEW ARTICLE

Relevance of sensitization to occupational allergy and asthma in the detergent industry

David Basketter¹, Ninna Berg², Francis H. Kruszewski³, Katherine Sarlo⁴, and Beth Concoby⁵

¹DABMEB Consultancy Ltd, Sharnbrook, Bedfordshire, UK, ²Novozymes A/S, Bagsvaerd, Denmark,

³American Cleaning Institute, Washington DC, USA, ⁴Procter & Gamble, Cincinnati, OH, USA, and

⁵Genencor, A Danisco Division, Palo Alto, CA, USA

Abstract

There exists considerable historic experience of the relationship between exposure and both the induction of sensitization and the elicitation of respiratory symptoms from industrial enzymes of bacterial and fungal origin used in a wide variety of detergent products. The detergent industry in particular has substantial experience of how the control of exposure leads to limitation of sensitization with low risk of symptoms. However, the experience also shows that there are substantial gaps in knowledge, even when the potential occupational allergy problem is firmly under control, and also that the relationship between exposure and sensitization can be hard to establish. The latter aspect includes a poor appreciation of how peak exposures and low levels of exposure over time contribute to sensitization. Furthermore, while a minority of workers develop specific IgE, essentially none appear to have symptoms, a situation which appears to contradict the allergy dogma that, once sensitized, an individual will react to much lower levels of exposure. For enzymes, the expression of symptoms occurs at similar or higher levels than those that cause induction. In spite of some knowledge gaps, medical surveillance programs and constant air monitoring provide the tools for successful management of enzymes in the occupational setting. Ultimately, the knowledge gained from the occupational setting facilitates the completion of safety assessments for consumer exposure to detergent enzymes. Such assessments have been proven to be correct by the decades of safe use both occupationally and in consumer products.

Keywords: IgE allergy, enzymes, detergents, occupational allergy, risk assessment

Introduction

The bacterial and fungal enzymes that are widely used industrially are well-recognized occupational respiratory allergens. This derives from the historical experience in factories involved in the original manufacturing of detergent products containing enzymes (Flindt et al., 1969; Pepys et al., 1969; Juniper et al., 1977; Zachariae et al., 1981). However, to a great extent it derives even more from the appreciation of how stringent the occupational exposure limits need to be (Sarlo, 2003), how effective the occupational hygiene needs to be (Johnson et al., 2012), and how important it is to back this up with regular health monitoring of the workforce (Kirchner et al., 2012). It is worth noting that all of this experience is based on the clear distinction between the detectable induction of

enzyme specific IgE in an individual compared to the expression of the clinical symptoms of allergic disease, which are related but separate events that must be managed when working with occupational allergens. In the detergent industry, measurement of the specific allergic IgE antibody response and management against this response provides a clear point of control in the successful (and long-term) prevention of occupational asthma to enzymes.

Surveillance of detergent workers has led us to draw certain conclusions regarding the relationship between exposure, induction of IgE antibody, and elicitation of symptoms. When enzymes were first introduced into detergents, the products were dusty and the exposures were high (hundreds of ng/m³). The peak exposures that

Address for Correspondence: Dr David A. Basketter, DABMEB Consultancy Ltd, Sharnbrook, Bedfordshire MK44 1PR, UK.

Tel: 44(0)1234782944. Fax: 44(0)1234782944. E-mail: dabmebconsultancytld@me.com

(Received 12 December 2011; revised 09 January 2012; accepted 09 January 2012)

naturally arose during daily operations in the factory were even higher ($\mu\text{g}/\text{m}^3$ range). This led to high rates of sensitization amongst the workers, with a proportion developing allergy and asthma symptoms. The introduction of exposure control tools led to a drastic fall in exposure, such that nominal levels of enzyme today are $< 15\text{--}20\text{ ng}/\text{m}^3$. Peak exposures occur less frequently and are low ($< 100\text{ ng}/\text{m}^3$). This has led to the elimination of symptoms among IgE positive workers and low rates of new sensitization in the workforce (Sarlo, 2003; Johnson et al., 2012; Kirchner et al., 2012).

There are those who argue that the induction of IgE antibody represents an adverse effect (ACGIH, 2004; Health Council of the Netherlands, 2008). In our view, whilst this represents an apparently simple concept, the reality is that it would seek to replace the proven good practice of preventing symptoms with an unattainable goal of eliminating sensitization. This would be unlikely to contribute meaningfully to assisting or encouraging others adequately to control the risks high molecular weight allergens present to human health (Heederik et al., 2002). Given this debate and the extensive occupational history in the detergent industry, it is then reasonable to ask not only for some detail of the industry approach to the elimination of enzyme-induced asthma, but also what it tells us concerning the relationships between hazards and risks, dose and response for both the induction and the elicitation of respiratory sensitization. Furthermore, what information does it provide concerning the theory vs the practice of the management of risk for both the workforce and for consumers? In this review, these topics are dissected and examined, knowledge gaps identified, and the realities of making the pragmatic decisions that have successfully protected workers and consumers from a significant health hazard are demonstrated.

Exposure and induction of IgE sensitization and elicitation of allergic asthma

Due to the nature of the exposure in the manufacturing environment and the limitations of diagnostic tests that can be conducted on workers, it is very difficult to develop precise exposure-response models for induction of IgE antibody (sensitization) in humans. Work done in animal models demonstrates that low doses of allergen are favorable for induction of IgE antibody, while high doses are favorable for induction of IgG antibody (Vaz et al., 1971; Thorne et al., 1986; Hillebrand et al., 1987; Fletcher and Sarlo, 1997). Recent studies using animal models show that higher doses of allergen induce a regulatory T-cell response leading to a down-regulation of those cellular steps needed to make an IgE antibody (e.g., tolerance) (reviewed in Platts-Mills and Woodfolk, 2011). Consequently, it would be tempting to speculate that maintaining high exposure levels of allergen in the workplace would be more likely to induce tolerance in workers, but the reality is that the immune response to allergens is very complex and not all occupational allergens will behave the same. This is best exemplified

by comparing the detergent enzyme experience with the observations made in cohorts of those working with laboratory animals where there is inter-play between the intensity of exposure and the type of induced immune response. From the detergent enzyme experience we know that the more intense exposures were associated with high rates of sensitization and numerous cases of asthma. As the intensity of exposure dropped, so did the rates of sensitization. In addition, the cases of asthma all but disappeared. In the laboratory animal worker cohorts, a number of individuals developed only the IgE antibody while others generated a T-regulatory-like response with a mixture of IgE and IgG₄ antibodies. Symptoms were strongly associated with those workers with only the IgE response, and less so in those with the IgE and IgG response. Investigators speculate that a tolerance response develops in some laboratory animal workers, but not in detergent workers (Chan-Yeung and Malo, 1999; Jones, 2008).

In the detergent industry, the number of workers that developed enzyme specific IgE antibody dropped dramatically with the introduction of exposure control methods complemented by medical surveillance programs. Regular surveillance of workers for the presence of IgE is detected in a number of ways, typically by serology such as the radio-allergosorbent test (RAST) or by skin prick test (SPT), techniques which are well described elsewhere (Wide et al., 1967; Pepys, 1972). The induction of enzyme-specific IgE is needed for the elicitation of respiratory allergic symptoms (rhinitis, cough, bronchoconstriction, wheezing, asthma) (Chan-Yeung and Malo, 1999; Bernstein, 2007). The IgE must be present in order to have symptoms; therefore, minimizing the number of people with the IgE greatly reduces the risk of individuals with symptoms. One large detergent manufacturer has shown over the years that adherence to an enzyme exposure guideline of $15\text{ ng}/\text{m}^3$ led to maintaining a low yearly incidence of sensitization, i.e., $< 3\%$ per year. This was also associated with a very low risk of symptoms amongst the sensitized workforce (Schweigert et al., 2000; Sarlo and Kirchner, 2002; Sarlo, 2003). When the rates exceed 3% per year, then the risk for symptoms also goes up. Investigation into why workers became sensitized usually revealed a breakdown in exposure control leading to personal exposures higher than typically experienced. Sensitization then becomes a reflection of exposure, and that is why specific IgE measurement represents such a useful point of control for the detergent industry. It is also clear from this industry's experience that the presence of IgE does not mean that clinical symptoms inevitably will follow. Workers with enzyme-specific IgE antibody can continue to work in the detergent manufacturing environment and not develop symptoms (Cathcart et al., 1997; Schweigert et al., 2000; Sarlo and Kirchner, 2002). The existing exposure conditions are below threshold for elicitation of symptoms and it is our opinion that the exposure threshold for induction of IgE may not be very much lower than

the levels of exposure currently found in well-controlled occupational environments.

There is a secondary body of knowledge deriving from *in vivo* models of respiratory allergy to enzymes that may help to fill the knowledge gaps surrounding exposure, sensitization, and symptoms. To a large extent this comes from experimental studies in the guinea pig. Generally, such testing was designed simply to evaluate the relative allergenicity of enzymes and thus is of limited value for the evaluation of exposure response relationships. However, work which explored the dose response, including an examination of whether repeated low doses were as effective at inducing respiratory sensitization as the same quantity given as a few higher dose exposures (Thorne et al., 1986; Hillebrand et al., 1987). These studies showed an exposure-response relationship between the increase in enzyme aerosol concentrations and an increase in antibody response with pulmonary responses occurring at the higher aerosol concentrations. The repeated low level exposures delivered over weeks were able to induce the generation of enzyme-specific antibody, but were inadequate to elicit pulmonary responses. Elicitation of pulmonary responses was only achieved by much higher levels of exposure. Similar observations have been made in the guinea pig intratracheal instillation test where symptoms became apparent at high doses of enzyme allergen (Vaz et al., 1971). Furthermore, early work in the mouse also indicated that high protein doses were more likely to give rise to anaphylactic antibody production (Fletcher and Sarlo, 1997).

Thus, the results in the animal models tend to reflect the experience in humans; that the formation of relevant antibody occurs more readily than does the elicitation of clinical symptoms and that clinical symptoms become apparent with higher exposure conditions. Investigators have observed that once symptoms (i.e., asthma) are triggered, then it becomes easier to elicit symptoms with lower exposure conditions. In humans, allergic asthma is accompanied by bronchial hyper-reactivity, a condition where the airways respond to lower doses of triggers such as pharmacologic airway constrictors (Johnson and Chan-Yeung, 1999; Berend et al., 2008). The development of hyper-reactive airways following the initial high exposures may be one reason why lower exposure levels of allergen trigger symptoms. It is then possible also to speculate that the maturation of the immune response to the allergen may be optimal, with higher exposures thereby generating more IgE of potential greater binding affinity to the allergen, making it easier for lower levels of allergen to trigger the symptomatic response. Additional investigative work can be done to help better understand the mechanisms behind the relationships between high and low exposure and elicitation of symptoms.

The observation that detergent workers with enzyme-specific IgE antibody can continue to work without expressing clinical symptoms (Juniper et al., 1977; Cathcart et al., 1997; Schweigert et al., 2000; Sarlo, 2003) is in contradistinction to what is often regarded as an

identifying feature of allergy, that once sensitization has been induced, allergy symptoms can be elicited at very much lower levels of exposure. Such is indeed the case in other areas of respiratory allergy, for example in the platinum refining industries, where once occupational sensitization has been detected, then symptoms are commonly present and often can only be limited further by thorough avoidance of all but the very lowest levels of exposure (Linnett and Hughes, 1999; Merget et al., 2000). In the case of detergent enzyme respiratory allergy, sensitization exists in the absence of symptoms and elicitation requires breakdowns in exposure controls, leading to higher exposure. Symptoms (along with higher rates of sensitization) become apparent when there is a breakdown in exposure control (Cullinan et al., 2000; Vanhanen et al., 2000; Brant et al., 2004). Under these conditions, the triggering exposures for symptoms are more intense than those needed for sensitization and, once symptomatic, an individual may start to respond to lower levels of exposure. If this were not the case, it is hard to explain how it has been possible for decades to maintain an asymptomatic workforce with a point prevalence of perhaps 10% or more sensitization. Interestingly, surveys related to the form of delayed type hypersensitivity in the skin known as contact allergy tend to show the same inversion noted for detergent enzymes, that there are more individuals with a positive diagnostic test than there are who are expressing the symptoms of allergic contact dermatitis (Coenraads et al., 2006; Rietschel and Fowler, 2008). Although there are distinct differences between these types of allergy, perhaps there is commonality in the situation that, where induction occurs via repeated low dose exposure, then elicitation often demands a relatively high dose.

In addition to the information on enzymes and respiratory allergy, there exists a very modest amount of material from exposure to other protein allergens. This has been thoroughly reviewed elsewhere (Schweigert et al., 2000). Most notable perhaps is the evidence from seafood and laboratory animal allergies, which suggests that duration of exposure is of some importance, with those workers having long hours of exposure each week being distinctly more likely to develop sensitization. However, evidence also generally is that those with the potential for a higher concentration of exposure are also more likely to become sensitized. So, perhaps it is simply the case that individuals who work longer hours are more likely to have more contact with the allergen and a greater number of episodes of higher-level exposure. Again, drawing from lessons from T-cell-mediated skin sensitization indicates that both frequency and duration have a marked impact on induction, to the point where higher frequency of exposure dominates over the impact of exposure dose (Basketter et al., 2006).

The inter-play between the dose response curves for IgE production, tolerance mechanisms and the elicitation of symptoms produce a complex picture of occupational allergic asthma, even without other variables

such as those associated with exposure (duration, peaks vs troughs, frequency, etc), the presence or absence of concomitant respiratory irritation, or personal susceptibility factors such as smoking or atopy. Under current exposure guidelines, it is difficult to link airborne levels of enzyme to actual individual exposure and induction of IgE antibody. Indeed, the difficulties even in assigning exposed individuals into just a few groups and relating these to IgE induction is eloquently demonstrated by published historic overviews of some decades of experience of occupational asthma (Schweigert et al., 2000; Larsen et al., 2007). This work showed that the exposure class (high, medium, or low) failed to correlate either with the risk of sensitization or of the presence of allergy symptoms. The general level of enzyme in the air, typically taken as a 1-4 h sample, informs very little on the detail of individuals' exposures and can take no account of the short-term variations in the level, nor of the duration of exposure experienced by an individual employee. Efforts to overcome these limitations by refining exposure measures are underway and should prove highly informative in the fullness of time (UK Health and Safety Executive, 2001).

The general review of exposure response relationships for occupational asthma also indicates that respiratory allergy to enzymes follows similar patterns to a number of other respiratory allergens in that there is a lag phase prior to sensitization, typically of several months to a few years (Wide et al., 1967; Chan-Yeung and Malo, 1999). In the earliest days of use of enzyme under those conditions of high exposure, the sensitization and symptoms happened very quickly, within 6-12 months. As exposure drops the lag phase tends to increase, where now sensitization can take years to develop. Sensitization may be occurring, but requires additional boosting exposures over time to get to the point where IgE can be detected. This suggests that repeat low-level exposure is important for the induction of an IgE antibody. Keep in mind that the low level exposure is likely accompanied by some peak exposures, so the peaks, either by themselves or in combination with the low level exposure, may be important to induction of IgE. The most obvious conclusion that can be drawn from the available evidence is that higher exposures are more likely to induce sensitization on a fast timeline, and to do so in a greater proportion of those that are exposed. Furthermore, where exposures are sufficiently high, then clinical symptoms will also start to appear.

IgE induction and control of occupational exposure

It has already been mentioned that the measurement of specific IgE in the potentially exposed workforce represents an important point of control, since development of IgE has become a reflection of how well exposure control guidelines are followed. Breakdown in exposure controls that may not always be detected by air monitoring are likely to impact sensitization (kinetics and rate), so aggressively monitoring sensitization is key to managing

exposure and preventing disease. The importance of vigilance in the maintenance of these controls has been reinforced by the rare outbreak of occupational enzyme allergy (Cullinan et al., 2000; Vanhanen et al., 2000; Brant et al., 2004). Furthermore, any complacency that everything is now fully understood would be entirely misplaced (Vanhanen et al., 2000). Nevertheless, it is evident that, where occupational controls are fully in place, then the number of workers that are sensitized is low, and sensitized individuals may continue their employment free from any symptoms of allergy (Cathcart et al., 1997; Schweigert et al., 2000; Sarlo and Kirchner, 2002; Sarlo, 2003). Thus, the emphasis is placed on successful control of airborne enzyme below strict limits, regularly monitoring to ensure that this is achieved, and use of a trained workforce that makes use of all the necessary procedures and personal protective equipment (Johnson et al., 2012; Kirchner et al., 2012).

Consumer risks

A fair question that follows from the above, despite the control in the factories, is whether the consumer is placed at any risk? At the outset of the use of enzymes in detergent products, since the risks were not fully understood, this was in fact the case, and adverse reactions in a few consumers were reported, albeit over 3 decades ago (Belin et al., 1970; Bernstein, 1972; Zetterstrom and Wide, 1974). Since that time, changes in product formulations have ensured that consumer exposure to these materials through use of detergent products has remained very low. Testing carried out in a variety of geographic locations, to ensure that a broad range of consumer habits has been taken into account, has shown that adverse reactions do not occur, and neither is there any evidence suggesting that the production of enzyme-specific IgE has been induced (Bannan et al., 1992; Rodriguez et al., 1994; Sekkat et al., 1995; Sarlo et al., 1996; Cormier et al., 1997).

It is not appropriate here to undertake a detailed review of this material. Some has already been reviewed in earlier publications (e.g., Schweigert et al., 2000) or in a more recent industry document (US SDA, 2005). This last mentioned publication is important in that it also provides extensive details and guidance on how to undertake a risk assessment relating to the use of enzymes in a wide range of consumer products. Two things should be taken on board, however. Firstly, that in > 35 years of use since proper controls came into place, and speaking globally, enzyme-containing detergents have not been demonstrated to be associated with the causation of consumer respiratory allergy. Given the duration and the scale of the market, although it does not represent evidence of absence, it is hard to imagine a better standard of absence of evidence. Furthermore, although more limited in scale, as mentioned earlier, there is also evidence of absence too. Finally, the products, and particularly the enzymes they contain, have also been suggested to be associated with skin allergy, but a thorough review

found that this was not the case (Basketter et al., 2008). In the light of these simple facts, it is fair to conclude that, despite their undoubted sensitization hazard, enzymes can be, and are, used safely in consumer detergent products.

Conclusions

Enzymes present an important respiratory sensitization hazard. However, hazards do not translate into harm unless there is sufficient exposure (i.e., risk). So, this risk can be fully controlled if exposure is kept with strict limits. The veracity of this statement is demonstrated by three and half decades of safe occupational and consumer use, with the very rare occupational exceptions providing telling reminders of how important it is to maintain full control in the factory situation (Nicholson et al., 2001; Sarlo and Kirchner, 2002). All of this has been achieved via the use of medical surveillance programs where enzyme-specific IgE is the key occupational control point. This is, of course, set against a backdrop of appropriate exposure controls and regular air monitoring to ensure exposure levels are kept appropriately low. These successes have been made despite the incomplete knowledge of dose-response relationships for induction and/or elicitation of respiratory sensitization. This experience should serve as a guide for others who may use enzymes in a variety of products. For consumers, the minimization of exposure, notably via enzyme encapsulation, to a level that is well below that found in the properly controlled factory situation, has similarly resulted in decades of safe use of enzymes in detergents.

Acknowledgment

The preparation of this manuscript was funded by the American Cleaning Institute, Washington, DC.

Declaration of interest

DB was paid for the preparation of this manuscript; the other authors are employees of the detergent enzyme industry or of its trade association in the US.

References

- ACGIH, American Conference of Governmental and Industrial Hygienists. 2004. Enzymes in the Workplace: Strategies for Minimising Health Effect. September 2004.
- Bannan, E. A., Griffith, J. F., Nusair, T. L. and Sauers, L. J. 1992. Skin testing of laundered fabrics in the dermal safety assessment of enzyme-containing detergents. *J Toxicol Cut Ocular Toxicol* 11:327-339.
- Basketter, D. A., English, J. S., Wakelin, S. H. and White, I. R. 2008. Enzymes, detergents and skin: Facts and fantasies. *Br J Dermatol* 158:1177-1181.
- Basketter, D. A., Jefferies, D., Safford, B. J., Gilmour, N. J., Jowsey, I. R., McFadden, J., Chansinghakul, W., Duangdeeden, I. and Kullavanijaya, P. 2006. The impact of exposure variables on the induction of skin sensitization. *Contact Derm* 55: 178-185.
- Belin, L., Hoborn, J., Falsen, E. and André, J. 1970. Enzyme sensitisation in consumers of enzyme-containing washing powder. *Lancet* 2:1153-1157.
- Berend, N., Salome, C. M. and King, G. G. 2008. Mechanisms of airway hyperresponsiveness in asthma. *Respirology* 13:624-631.
- Bernstein, I. L. 1972. Enzyme allergy in populations exposed to long-term, low-level concentrations of household laundry products. *J Allergy Clin Immunol* 49:219-237.
- Bernstein, J. A. 2007. Occupational asthma. In: *Allergy & Asthma - Practical Diagnosis and Management* (Mahmoudi, M., Ed.). New York: McGraw-Hill, pp. 145-155.
- Brant, A., Hole, A., Cannon, J., Helm, J., Swales, C., Welch, J., Taylor, A. N. and Cullinan, P. 2004. Occupational asthma caused by cellulase and lipase in the detergent industry. *Occup Environ Med* 61:793-795.
- Cathcart, M., Nicholson, P., Roberts, D., Bazley, M., Juniper, C., Murray, P. and Randell, M. 1997. Enzyme exposure, smoking and lung function in employees in the detergent industry over 20 years. Medical Subcommittee of the UK Soap and Detergent Industry Association. *Occup Med (Lond)* 47:473-478.
- Chan-Yeung, M. and Malo, J. L. 1999. Natural history of occupational Asthma. In: *Asthma in the Workplace* (Bernstein, I. L., Chan-Yeung, M., Malo, J. L. and Bernsein, D. I., Eds.). New York: Marcel Dekker, pp. 129-144.
- Coenraads, P. J., Diepgen, T., Uter, W., Schnuch, A. and Gefeller, O. 2006. *Epidemiology. In: Contact Dermatitis, 4th Edition* (Frosch P. J., Menné, T. and Lepoittevin, J. P., Eds.). Berlin: Springer, pp. 135-166.
- Cormier, E., Sarlo, K., Scott, L., Vasunia, K., Smith, M., Payne, N. and MacKenzie, D. 1997. Lack of sensitization to laundry enzymes among consumers in the Philippines. Results of an 18-month clinical study. *J Allergy Clin Immunol* 99:579.
- Cullinan, P., Harris, J. M., Newman Taylor, A. J., Hole, A. M., Jones, M., Barnes, F. and Jolliffe, G. 2000. An outbreak of asthma in a modern detergent factory. *Lancet* 356:1899-1900.
- Fletcher, E. R. and Sarlo, K. 1997. Development of allergic antibody in guinea pigs exposed to a no effect dose of enzyme allergen: Effect of peak exposure and development of type 1 allergy. *The Toxicologist* 36:1042(a).
- Flindt, M. L. 1969. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1:1177-1181.
- Health Council of the Netherlands. 2008. *Prevention of Work-related Airway Allergies. Recommended Occupational Exposure Limits and Periodic Screening*. The Hague: Health Council of the Netherlands, Publication #2008/03E.
- Heederik, D., Thorne, P. S. and Doekes, G. 2002. Health-based occupational exposure limits for high molecular weight sensitizers: How long is the road we must travel? *Ann Occup Hyg* 46:439-446.
- Hillebrand, J. A., Thorne, P. S. and Karol, M. H. 1987. Experimental sensitization to subtilisin II. Production of specific antibodies following inhalation exposure of guinea pigs. *Toxicol Appl Pharmacol* 89:449-456.
- Johnson, A. and Chan-Yeung, M. 1999. Non-specific bronchial hyper-responsiveness. In: *Asthma in the Workplace* (Bernstein, I. L., Chan-Yeung, M., Malo, J. L. and Bernsein, D. I., Eds.). New York: Marcel Dekker, pp. 173-192.
- Johnson, G. Q., Kruszewski, F. H. and Panepinto, A. S. 2012. Detergent enzyme exposure control methodologies. *J Occup Eng Hyg* (submitted).
- Jones, M. G. 2008. Exposure-response in occupational allergy. *Curr Opin Allergy Clin Immunol* 8:110-114.
- Juniper, C. P., How, M. J., Goodwin, B. F. and Kinshott, A. K. 1977. *Bacillus subtilis* enzymes: A 7-year clinical, epidemiological and immunological study of an industrial allergen. *J Soc Occup Med* 27:3-12.

- Kirchner, D. B., Kruszewski, F. H. and Sarlo, K. 2012. Medical surveillance in detergent production facilities. *J Occup Eng Hyg* (submitted).
- Larsen, A. I., Johnsen, C. R., Frickmann, J. and Mikkelsen, S. 2007. Incidence of respiratory sensitisation and allergy to enzymes among employees in an enzyme producing plant and the relation to exposure and host factors. *Occup Environ Med* 64:763–768.
- Linnett, P. J. and Hughes, E. G. 1999. 20 years of medical surveillance on exposure to allergenic and non-allergenic platinum compounds: The importance of chemical speciation. *Occup Environ Med* 56:191–196.
- Mergert, R., Kulzer, R., Dierkes-Globisch, A., Breitstadt, R., Gebler, A., Kniffka, A., Artelt, S., Koenig, H. P., Alt, F., Vormberg, R., Baur, X. and Schultze-Werninghaus, G. 2000. Exposure-effect relationship of platinum salt allergy in a catalyst production plant: Conclusions from a 5-year prospective cohort study. *J Allergy Clin Immunol* 105:364–370.
- Nicholson, P. J., Newman Taylor, A. J., Oliver, P. and Cathcart, M. 2001. Current best practice for the health surveillance of enzyme workers in the soap and detergent industry. *Occup Med (Lond)* 51:81–92.
- Pepys, J. 1972. Laboratory methods in clinical allergy: Skin tests for immediate Type I allergic reactions. *Proc Royal Soc Med* 65:271–272.
- Pepys, J., Longbottom, J. L., Hargreave, F. E. and Faux, J. 1969. Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet* 1:1181–1184.
- Platts-Mills, T. A. and Woodfolk, J. A. 2011. Allergens and their role in the allergic immune response. *Immunol Rev* 242:51–68.
- Rietschel, R. L. and Fowler, J. F. (Eds.). 2008. Predictive testing for human contact dermatitis. In: *Fisher's Contact Dermatitis, 6th Edition*. Hamilton, ON: BC Decker, pp. 30–34.
- Rodriguez, C., Calvin, G., Lally, C. and LaChapelle, J. M. 1994. Skin effects associated with wearing fabrics washed with commercial laundry detergents. *J Toxicol Cut Ocular Toxicol* 13:39–45.
- Sarlo, K. 2003. Control of occupational asthma and allergy in the detergent industry. *Ann Allergy Asthma Immunol* 90:32–34.
- Sarlo, K. and Kirchner, D. B. 2002. Occupational asthma and allergy in the detergent industry: New developments. *Curr Opin Allergy Clin Immunol* 2:97–101.
- Sarlo, K., Cormier, E., Scott, L. and MacKenzie, D. 1996. Lack of Type I sensitization to laundry enzymes among consumer in the Philippines. *J Allergy Clin Immunol* 97:749a.
- Schweigert, M. K., Mackenzie, D. P. and Sarlo, K. 2000. Occupational asthma and allergy associated with the use of enzymes in the detergent industry—a review of the epidemiology, toxicology and methods of prevention. *Clin Exp Allergy* 30:1511–1518.
- Sekkat, A., Boghossian, J. and Cookman, G. R. 1995. Risk assessment: A clinical study demonstrating the safety of a laundry detergent under paste use conditions. Proceedings of the 7th Pan-African Conference of Dermatology, 1–5 December 1995. Cairo, Egypt.
- Thorne, P. S., Hillebrand, J., Magreni, C., Riley, E. J. and Karol, M. H. 1986. Experimental sensitization to subtilisin I. Production of immediate- and late-onset pulmonary reactions. *Toxicol Appl Pharmacol* 86:112–123.
- UK Health and Safety Executive. 2001. *OELs and the Effective Control of Exposure to Substances Hazardous to Health in the UK* (Version 3). October 2001. London.
- US SDA. 2005. *Risk Assessment Guidance for Enzyme-containing Products*. Soap and Detergent Association, Washington, DC.
- Vanhnen, M., Tuomi, T., Tiikkainen, U., Tupasela, O., Voutilainen, R. and Nordman, H. 2000. Risk of enzyme allergy in the detergent industry. *Occup Environ Med* 57:121–125.
- Vaz, E. M., Vaz, N. M. and Levine, B. B. 1971. Persistent formation of reagins in mice injected with low doses of ovalbumin. *Immunology* 21:11–15.
- Wide, L., Bennich, H. and Johansson, S. G. 1967. Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet* 2:1105–1107.
- Zachariae, H., Høech-Thomsen, J., Witmeur, O. and Wide, L. 1981. Detergent enzymes and occupational safety. Observations on sensitization during Esperase production. *Allergy* 36:513–516.
- Zetterström, O. and Wide, L. 1974. IgE-antibodies and skin test reactions to a detergent-enzyme in Swedish consumers. *Clin Allergy* 4:273–280.