



Review

Defining occupational and consumer exposure limits for enzyme protein respiratory allergens under REACH

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ABSTRACT

A wide range of substances have been recognized as sensitizing, either to the skin and/or to the respiratory tract. Many of these are useful materials, so to ensure that they can be used safely it is necessary to characterize the hazards and establish appropriate exposure limits. Under new EU legislation (REACH), there is a requirement to define a derived no effect level (DNEL). Where a DNEL cannot be established, e.g. for sensitizing substances, then a derived minimal effect level (DMEL) is recommended. For the bacterial and fungal enzymes which are well recognized respiratory sensitizers and have widespread use industrially as well as in a range of consumer products, a DMEL can be established by thorough retrospective review of occupational and consumer experience. In particular, setting the validated employee medical surveillance data against exposure records generated over an extended period of time is vital in informing the occupational DMEL. This experience shows that a long established limit of 60 ng/m³ for pure enzyme protein has been a successful starting point for the definition of occupational health limits for sensitization in the detergent industry. Application to this of adjustment factors has limited sensitization induction, avoided any meaningful risk of the elicitation of symptoms with known enzymes and provided an appropriate level of security for new enzymes whose potency has not been fully characterized. For example, in the detergent industry, this has led to general use of occupational exposure limits 3–10 times lower than the 60 ng/m³ starting point. In contrast, consumer exposure limits vary because the types of exposure themselves cover a wide range. The highest levels shown to be safe in use, 15 ng/m³, are associated with laundry trigger sprays, but very much lower levels (e.g. 0.01 ng/m³) are commonly associated with other types of safe exposure. Consumer limits typically will lie between these values and depend on the actual exposure associated with product use.

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1. Introduction

The potential for enzyme proteins to give rise to respiratory allergy has been recognized for several decades, since the time of the introduction of these materials into fabric washing products. The subject and its history has been extensively reviewed elsewhere, such that details do not need to be extensively repeated here (Flindt, 1969; Pepys et al., 1969; Zachariae et al., 1981; Juniper et al., 1977; Schweigert et al., 2000). The salient points are that initially, the risk of the generation of respiratory allergy was not fully appreciated when bacterial proteolytic enzyme was first introduced in the 1960s, such that after period of about a year, an occupational problem began to appear. It transpired that a substantial proportion of the exposed workforce had developed specific immunoglobulin E (IgE) antibodies against the enzyme, i.e. sensitization had been induced. Furthermore, of this group a fair proportion also displayed symptoms of respiratory allergy, including asthma, i.e. elicitation had occurred. These aspects, exposure, the lag phase, induction and then elicitation, are key characteristics of allergy. Once the problem had been identified, then substantial steps were taken over the next few years to reduce the level of occupational exposure until evidence of respiratory allergy could be shown to be absent (Schweigert et al., 2000; Sarlo and Kirchner, 2002; Sarlo, 2003). In essence, this is the situation that still pertains to this day.

Whilst the occupational situation was the most acute and widely reported, and since the risk was not fully appreciated initially, consumer exposure to the proteolytic enzyme being incorporated into the fabric washing product was not sufficiently well controlled. As would be expected, the consumer exposure was much lower than that experienced occupationally, but nevertheless, a number of reports of adverse effects were published in the early 1970s (Belin et al., 1970; Bernstein, 1972; Zetterstrom and Wide, 1974). The efforts to limit occupational exposure were also relevant to consumer exposure insofar as they involved encapsulation of the enzyme which dramatically limited the level of dustiness of the raw material. Consequently, since that time, as far as we are aware, there have been no further reports of adverse effects in consumers, whereas there has been some clear demonstration of the absence of adverse effects (US SDA, 2005; Basketter et al., 2008).

In the present review, we have examined this historical experience from the perspective of the establishment of safe limits for occupational and consumer exposure in order to make recommendations for generically applicable levels which can be used for both existing and new bacterial and fungal enzyme proteins. Furthermore, it is suggested that this knowledge and the limits recommended should also be suitable for application to other enzymes (including engineered enzyme proteins) unless there is additional information which would suggest that a different limit would be appropriate. However, it is also important to appreciate that the DMEL values proposed represent a starting point for the definition of a safe exposure level, since these will always depend on the characteristics of occupational and/or consumer exposure associated with a particular use scenario.

2. Induction versus elicitation

In toxicology, the expression of any adverse effect requires that there is exposure. However, for allergy, the situation is a little more complex and occurs in two distinct phases. Allergy requires that

the immune system is first exposed in a manner that enables it to recognize the allergen (in this case enzyme protein) so that it can proceed to develop a specific response (in this case, the production of enzyme specific IgE). This is termed induction. The exposure characteristics necessary for this to occur are not fully appreciated (Thorne et al., 1986; Hillebrand et al., 1987; Jones, 2008). Once the induction process is complete, an individual has become “sensitized” and further exposure to a sufficient dose can give rise to the second phase, the elicitation of clinical allergy symptoms.

There is no doubt that there exists a (complex) relationship between exposure level, exposure duration, exposure interval, (i.e. frequency) and of course individual susceptibility for induction and for elicitation. Questions arise also about the relative importance of peak exposures versus more chronic low level exposure. None of these aspects have been well characterized, either by in vivo experimentation or by interrogation of occupational health data, not least since these would represent very substantial challenges in their own right. The limited information that is available has been reviewed very recently (Jones, 2008; Basketter et al., submitted for publication). Despite the limitations, what is quite certain though is that ultimately, it has been the reduction in airborne exposure which resolved the occupational and consumer problems of approximately 35 years ago.

The induction of the sensitized state can be detected in a number of ways. Most commonly, the presence of (enzyme specific) IgE antibody is assessed either by a skin prick test or by radioallergen sorbent test applied to a blood sample (Wide et al., 1967; Pepys, 1972). It is not appropriate to review the details of these and other diagnostic tests here. What is important is that these tests, with a considerable degree of accuracy, demonstrate the presence or absence of IgE sensitization. What they do not do is to indicate anything about whether the elicitation of allergy has occurred. The existence of the clinical symptoms of allergy requires that a sensitized individual has a sufficient degree of exposure to produce the classic signs of respiratory allergy, these being rhinitis, conjunctivitis, bronchoconstriction and asthma (Bernstein, 2007; Chan-Yeung and Malo, 1999). Note that the sensitized state is required for elicitation, but does not mean that clinical symptoms are inevitable.

3. Thresholds

Given the above, it is evident that for allergy there are two general thresholds that can be derived, one related to the induction of the sensitized state and another for the elicitation of clinical symptoms. This of course raises a number of questions, not least which of these thresholds is the most important, relevant, practical and so forth. Before that though, it is worthwhile to consider some background information on our current understanding of the science in this area. In allergy, it is commonly stated that once sensitized, an individual will react to much lower levels of exposure (Chan-Yeung and Malo, 1999). Teleologically, this seems self evident in that the induction process involves a dramatic expansion of the number of cells producing IgE antibody to allergen. Experimentally, such an apparent increase in sensitivity is what has been seen when guinea pigs have been sensitized experimentally (Thorne et al., 1986; Hillebrand et al., 1987; Magnusson and Kligman, 1970; Buehler, 1985) or when humans have been deliberately sensitized (Friedmann, 2007), accepting of course that some of these studies were with a different form of allergy. However, when it comes to

the practical experience with enzyme allergy, this classic situation does not seem to pertain and this will have an important impact on the conclusions within this review. For enzyme allergy, when occupational health problems were apparent in the 1960s and early 1970s, there was a preponderance of sensitized individuals over those with clinical symptoms of allergy (Flindt, 1969; Pepys et al., 1969; Zachariae et al., 1981; Juniper et al., 1977; Schweigert et al., 2000; Sarlo and Kirchner, 2002; Sarlo, 2003). Clearly, if it was the case that those with detectable IgE (i.e. the individuals who had become sensitized) were now able to react to much lower levels of enzyme exposure, then at least the numbers of sensitized individuals should have equalled those with symptoms. Furthermore, as the reduction in factory exposure to airborne enzyme led to a sharp fall in sensitization, symptomatic individuals also became rarer, but, surprisingly, their number fell to zero even though a significant percentage of the workforce had detectable IgE. Over the following decades, the situation has remained very much the same, such that even with further reductions in enzyme exposure, sensitization induction can still occur, although all of the individuals are free of symptoms. Currently, one large multinational company has published its view that, using the best industry controls (SDA, 1995; IASD, 2002) then up to 3% of new sensitizations annually amongst its workforce represents a pragmatically acceptable upper limit, and one which is not associated with the generation of clinical symptoms, either in newly sensitized workers or in those that have been sensitized for some time (Peters et al., 2001).

Lastly in this section, let us just briefly consider what constitutes a threshold. A practical working definition would probably refer to the level of exposure which just failed to cause an effect, and which, by such definition, would necessarily be close to the level which would just cause an effect. Thus in establishing a safe limit for exposure, where there are uncertainties, the threshold itself might not be the best choice for a generic safe limit. What does seem clear however is that for allergy, there are safe limits. These can be hard to define in relation to induction, but are clear for the elicitation of allergic reactions. Having said that, then it is also true that this is easier to demonstrate in cell mediated allergy, notably allergic contact dermatitis (Friedmann et al., 1983; Kimber et al., 1999), but has also been done pragmatically in respiratory allergy, where an induction threshold for the halogenated platinum salts was indicated by retrospective occupational survey (Merget et al., 2000) and for the type of enzymes under consideration in the present paper (vide infra).

4. Uncertainties

As just mentioned, where there are uncertainties, an appreciation of these needs to be developed to permit a safe exposure limit to be derived. In the particular case of respiratory allergy to enzymes, uncertainty surrounds the measurement of exposure, to a great extent because the exposure is assessed largely as a time weighted average airborne concentration, such that it is not possible to determine individual exposure. This situation will not change in the short term due to the technical difficulties in measurement of personal exposure and the time required to develop a sufficient body of data/experience. Other uncertainty arises from the impact of interpersonal variation, but in the case of enzyme allergy, as with other forms of occupational allergy, it is recognized that the most significant risk factor that can be defined is smoking (Merget et al., 2000; Barker et al., 1998; Cathcart et al., 1997).

Last, but not least in terms of the consideration of uncertainties, there must be the question of the relative allergenicity of enzymes. Considerable effort has been expended, notably with animal models, to try to find ways to measure this. They may have met with rather limited success, particularly in terms of their more general

adoption, but they have served to show that while enzymes do vary in their relative allergenic potency, that variation seems to be within a fairly restricted window. The original enzyme allergens used and on which the current occupational exposure limits are set have turned out to be amongst the more potent substances tested to date, giving some confidence that in reality it is unlikely that enzymes of dramatically increased allergenic potency will arise. Indeed, it seems quite possible in the opinion of these authors that newer materials, either by chance or more likely by design, will tend to be no more, or even less potent as respiratory allergens.

5. DMEL definition

Under REACH, a defined no effect level (DNEL) is the preferred option for thresholded mechanisms such as sensitization. However, it is also recognized that where there is insufficient data to reliably determine a no effect level, then the DMEL route should be adopted (REACH Technical Guidance Document ref here). It is worth reminding ourselves at this point that a DMEL identifies a level at which exposure may result in a limited degree of response; it does not define a no adverse effect level.

5.1. Occupational

Based on all of the above considerations, the point of departure for the definition of an occupational derived minimal effect level (DMEL) has been the knowledge of the exposure levels associated with respiratory allergy problems (approximately 200 ng/m³ and above) set against a careful consideration of the extensive industrial experience of safe use of enzymes for more than three decades. The details of this approach are summarised in important industry guidance documents (SDA, 1995; IASD, 2002) as well as in formally documented risk assessments, which also contain the most detailed overview of historical material relating to protease mediated occupational respiratory allergy (Human and Environmental Risk Assessment, 2007, 2005). In these documents, the exposure limit of 60 ng/m³ for pure enzyme protein suggested by an independent body, the American Conference of Governmental and Industrial Hygienists (ACGIH) (ACGIH, 2004) is endorsed as a sensible, pragmatic value, not least since its generic application has led to decades of successful occupational control (Schweigert et al., 2000; Sarlo and Kirchner, 2002; Sarlo, 2003; Cathcart et al., 1997). The quality of this success is further established by the rare occasions when an absence of adequate control has led to the occurrence of occupational enzyme asthma (Cullinan et al., 2000; Vanhanen et al., 2000; Brant et al., 2004; Van Rooy et al., 2009).

However, this successful history of occupational control should not be adopted with any air of complacency. In reality, the working limit in many factory locations is likely to be substantially below 60 ng/m³. For example, a level of 15 ng/m³, adopted to take account of additional factors, such as the extent to which co-exposure with surfactants may enhance the allergenic effect of the enzyme (Schweigert et al., 2000; Sarlo, 2003). In the UK, an occupational exposure limit of 40 ng/m³ is required for proteases (UK Health and Safety Executive, 2009). We also know that even where there appears to be thorough control, very occasional problems can and do arise (Vanhanen et al., 2000; Brant et al., 2004). Of course, this can be argued to be consistent with the definition of a DMEL – see above.

There have also been recent suggestions that long-term exposure to proteolytic enzymes may result in a modest predisposition to upper and lower airway disease (Brant et al., 2009). However, as the authors themselves acknowledge, there are significant difficulties regarding exposure estimation and many assumptions in their work and several other possible explanations of their data and thus

it would be inappropriate to use this perspective in establishing DMEL value.

Guidance documentation within the detergent industry is worth noting here. Although some manufacturers have adopted the limit of 60 ng/m³ set by the ACGIH, others have established internal occupational exposure guidelines (OEGs) for each enzyme. The ranges for internally defined OEGs (8 h time weighted average) used by some of the major detergent manufacturers in Europe are 8–20 ng/m³ (proteases), 5–20 ng/m³ (lipases), 5–15 ng/m³ (amylases) and 8–20 ng/m³ (cellulases) (IASD, 2002). What this demonstrates is that this wide range of enzymes is not particularly varied in their relative allergenic potency but also, and significantly, that these companies have taken the ACGIH value as an appropriate starting point, not a conclusion.

Given the above considerations, it is suggested that an occupational DMEL of 60 ng/m³ is adopted and remains as the starting point for new and existing enzymes which do not have a limit and/or for which there is no other data to indicate that a different value may be more appropriate. Where uncertainties exist, this value may be reduced appropriately.

5.2. Consumer

Whereas employees might reasonably be expected to have exposure up to the occupational limit for approximately 5 days per week, 8 or 12 h per day, consumer exposure will generally be of a very much lower order than this. In such a situation, it might be argued that the occupational limit will be even more protective for the consumer and that the occupational DMEL should be sufficient. Set against that however, is that whereas occupational exposure is controlled and monitored, and the workforce actively monitored by occupational health, this is not true for the general consumer. Here, and notwithstanding the use of enzyme encapsulation and general formulation with low levels of enzyme, consumer exposure is not subject to control, nor does specific health monitoring occur. Furthermore, it has to be recognized that the control point for occupational exposure is the induction of sensitization, which although not an adverse health effect is evidence of immune activation. As such, it provides opportunity for early intervention, whereas with the consumer, only clinical disease symptoms would trigger intervention. As a consequence, it would seem appropriate to adopt a more cautious limit.

The levels of exposure which led to consumer problems in the mid-twentieth century were not comprehensively characterized, but as a consequence of the use of unencapsulated enzymes, clearly were too high, being in the order of 200 ng/m³ or more. When consumer enzyme exposure subsequently was controlled by ensuring that the enzyme was not dusty, then the exposure to enzymes from laundry detergents fell to 1 ng/m³ or even lower. In this situation, clinical symptoms of allergy were entirely absent as was any detectable production of enzyme specific IgE. This latter aspect is critical: occupationally, health monitoring of the workforce at the level of IgE has proven to be a highly successful tool, but is of course one which cannot be actively applied to consumers. For that group, the most appropriate endpoint is an absence of IgE induction, which demands exposure is effectively reduced to a very low level.

With modern detergent products, the reality of typical airborne exposure to enzymes in association with fabric washing is of the order of 0.01 ng/m³ (Human and Environmental Risk Assessment, 2007, 2005; UK Health and Safety Executive, 2009). This exposure is not associated globally, over decades, with any evidence of the induction or elicitation of respiratory allergy. Complementing this absence of evidence, there are a variety of types of testing which have been carried out in a number of geographic locations (North America, Europe, Asia, Africa) which ensured that a broad range

of consumer habits had been taken into account. This work has shown that allergic reactions do not occur in groups with widely varying use habits and that there is no evidence of the production of enzyme specific IgE (US SDA, 2005; Bannan et al., 1992; Rodriguez et al., 1994; Sekkat et al., 1995; Sarlo et al., 1996; Cormier et al., 1997). This provides evidence of the absence of an adverse effect. In this context, it is very important to note that other published work investigating different uses of detergent enzyme has found evidence of potential problems and in each case this has led to a cessation of the project prior to marketing (Johnson et al., 1999; Kelling et al., 1998; Sarlo et al., 2004). Arguably, some of these tend to indicate that longer exposure may be just as important as high “spot” exposures, but as already mentioned, detailed understanding of these variables is lacking.

A very recent survey including the information above together with additional previously unreported data has been prepared for publication (Sarlo et al., submitted for publication). A significant observation within this work is that examination of several thousand individuals demonstrates that there is an absence of evidence of the induction of sensitization in consumers. These included new employees being screened prior to starting work in detergent factories around the world as well as volunteers for various consumer studies involving enzymes and who were tested at the outset of the study to ensure they were not already positive.

So, past levels of airborne exposure to enzymes which caused consumer problems of an allergic nature were over 4 orders of magnitude higher than typical exposure from laundry products today. In terms of trying to establish a DMEL, this leads to a key question: where, between these levels, is exposure still safe for the consumer? The highest reported safe consumer exposure level has been in association with spot cleaning with a trigger spray device, as reported in the documentation of the US Soap and Detergent Association and elsewhere (UK Health and Safety Executive, 2009; Weeks et al., 2001a; Weeks et al., 2001b). This value of approximately 15 ng/m³ was associated with a 30 sprays per day, daily use of the product for a six month period, equivalent to the use of about 2 product packs per month, so representing very heavy use. The study demonstrated that this use gave rise to no adverse effects over a 6 month period in a carefully monitored atopic population, the 96 subjects who completed the study all being negative to skin prick test at study termination. The exposure discussed here for a trigger spray represents frequent, but nevertheless short duration exposure. Where exposure is prolonged and/or more intimate in nature, then experience shows that a careful risk assessment must be undertaken and that such a high level of exposure could not be tolerated (UK Health and Safety Executive, 2009; Johnson et al., 1999; Kelling et al., 1998; Sarlo et al., 2004; Blaikie et al., 1999).

In drawing together the pieces of information which can contribute to the establishment of a general consumer DMEL, it has to be recognized that the available data is relatively limited and that the types of exposure involved are varied. The occupational evidence that a OEL (and thus a DMEL) of 60 ng/m³ is acceptable for daily 8 h workplace exposure during detergent production provides a useful backdrop, whereas the experience of decades of safe exposure at very much lower levels is reassuring but not especially helpful in determining a limit. So, taken together, and in particular with the evidence from a single but comprehensive study that daily consumer exposure at 15 ng/m³ was not associated with any induction or elicitation effects, then it is suggested that a consumer DMEL of 15 ng/m³ can be adopted as the starting point for new and existing enzymes which do not have a limit and/or for which there is no other data to indicate that a different value may be more appropriate. However, it is important that, just as for the occupational limit, this value is not taken as assurance of absolute safety in all conceivable exposure situations (Johnson et al., 1999; Kelling et al., 1998; Sarlo et al., 2004).

6. Enzymes, REACH and the DMEL

Within new European regulations commonly referred to as REACH (Registration, Evaluation, Assessment and restriction of Chemicals; (Commission of European communities, 2006), there is the requirement to define acceptable exposure limits. For allergens, including respiratory allergens such as the enzyme proteins which form the topic of this paper, it has been proposed that a threshold cannot be determined and hence a minimum effect level should be established (ECHA, 2008). Key wording in this document is as follows: “assuming that there are data allowing it, the registrant should develop a **DMEL** (derived minimal effect level), a reference risk level which is considered to be of very low concern. DMEL derived in accordance with the guidance should be seen as a tolerable level of effects and it should be noted that it is not a level where no potential effects can be foreseen.” The DMEL values proposed above in this document have been developed on the basis of decades of practical experience with enzymes in occupational and consumer settings. This experience is strongly indicative of thresholds below which (the adverse) events associated with allergy no longer occur, or will do so only at a very low level and not be associated with clinical allergy symptoms. For the factory situation, this is based on the elicitation of allergy and the highly effective minimization of clinical symptoms as there is no significant data indicating where a threshold for the induction of sensitization may be and it is abundantly evident that at such exposure levels the presence of sensitization does not lead to a progression to clinical disease. In contrast, for the consumer, where the patterns of exposure are such that the exposure burden is typically far lower, then experience indicates that it is possible to derive an exposure limit which should avoid even the induction of sensitization (and thus, by definition, will eliminate any possibility of clinical symptoms).

7. Conclusion

The enzymes of bacterial and fungal origin widely used in industry and in consumer products represent an important hazard – they are respiratory sensitizers. On the other hand, decades of experience demonstrates that enzymes can be used safely by ensuring that the exposure is strictly limited. Occupationally, a DMEL of 60 ng/m³ provides an excellent starting point for safety assessment, with experience showing that downward adjustment of this value to take account of particular circumstances ensures safe working practice, a view supported by the observation where systems which go out of control lead to problems (Cullinan et al., 2000). For consumers, a DMEL of 15 ng/m³ is proposed, but with the recognition that this is the highest tolerable value and is associated with the situation where there is only short term, perhaps frequent, exposure. Where there are circumstances which would lead for example to prolonged intimate exposure, then a lower level would need to be adopted (Human and Environmental Risk Assessment, 2007; Johnson et al., 1999; Kelling et al., 1998; Sarlo et al., 2004).

Application of the above limits has led to safe occupational and consumer use of these substances and thus adoption of these values as DMELs in the context of REACH would seem to be appropriate.

Conflicts of interest statement

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References

- American Conference of Governmental and Industrial Hygienists 2004 Enzymes in the Workplace: Strategies for Minimising Health Effect. September.
- Bannan, E.A., Griffith, J.F., Nusair, T.L., Sauer, 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.
- Barker, R.D., van Tongeren, M.J., Harris, J.M., Gardiner, K., Venables, K.M., Newman Taylor, A.J., 1998. Risk factors for sensitization and respiratory symptoms among workers exposed to acid anhydrides: a cohort study. *Occup. Environ. Med.* 55, 684–691.
- Basketter, D.A., English, J., Wakelin, S., White, I.R., 2008. Enzymes, detergents and skin: facts and fantasies. *Br. J. Dermatol.* 158, 1177–1181.
- Basketter, D.A., Berg, N., Kruszewski, F., Sarlo, K. and Concohy, B. Relevance of sensitization to occupational asthma and allergy in the detergent industry. MS for submission to *J. Occup. Environ. Hyg.*
- Belin, L., Hoborn, J., Falsen, E., Andre, J., 1970. Enzyme sensitization in consumers of enzyme-containing washing powder. *Lancet* 2, 1153–1157.
- Bernstein, I.L., 1972. Enzyme allergy in populations exposed to long-term low level concentration of household laundry products. *J. Allergy Clin. Immunol.* 49, 219–237.
- Bernstein, J.A., 2007. Occupational asthma. In: Mahmoudi, M. (Ed.), *Allergy and Asthma – Practical Diagnosis and Management*. McGraw-Hill, pp.145–155.
- Blaikie, L., Richold, M., Whittle, E., Lawrence, R.S., Keech, S., Basketter, D.A., 1999. Airborne exposure from topically applied protein (proteolytic enzyme). *Hum. Exp. Toxicol.* 18, 528.
- Brant, A., Hole, A., Cannon, J., Helm, J., Swales, C., Welch, J., Taylor, A.N., Cullinan, P., 2004. Occupational asthma caused by cellulase and lipase in the detergent industry. *Occup. Environ. Med.* 61 (9), 793–795.
- Brant, A., Upchurch, S., van tongeren, M., Zekveld, C., Helm, J., Barnes, F., Newman-Taylor, A.J., Cullinan, P., 2009. Detergent protease exposure and respiratory disease: case-referent analysis of a retrospective cohort. *Occup. Environ. Med.* online.
- Buehler, E.V., 1985. A rationale for the selection of occlusion to induce and elicit delayed contact hypersensitivity in the guinea pig. *Curr. Probl. Dermatol.* 14, 39–58.
- Cathcart, M., Nicholson, P.J., Roberts, D., et al., 1997. Enzyme exposure, smoking and lung function in employees in the detergent industry over 20 years. *Occup. Med. (London)* 47, 473–478.
- Chan-Yeung, M., Malo, J.-L., 1999. Natural history of occupational asthma. In: Bernstein, I.L., Chan-Yeung, M., Malo, J.-L., Bernsein, D.I. (Eds.), *Asthma in the Workplace*. Marcel Dekker, New York, pp. 129–144.
- Commission of the European Communities 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directive 91/155/EEC, 93/67/EEC, 93/105/EEC and 2000/21/EC. *Off. J. Eur. Union*, L 396/1 of 30.12.2006. <http://europa.eu.int/comm/enterprise/reach/index-en.htm>.
- Cormier, E., Sarlo, K., Scott, L., Vasunia, K., Smith, M., Payne, N., MacKenzie, D., 1997. Lack of sensitization to laundry enzymes among consumers in the Philippines. Results of an 18 month clinical study. *J. All. Clin. Immunol* 99, 579.
- Cullinan, P., Harris, J.M., Newman Taylor, A.J., et al., 2000. An outbreak of asthma in a modern detergent factory. *Lancet* 356, 1899–1900.
- ECHA, 2008. Guidance on information requirements and chemical safety assessment. Chapter Volume 8: Dose (concentration) – Response characterization. Helsinki, May 2008.
- Flindt, M.L., 1969. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1, 1177–1181.
- Friedmann, P.S., 2007. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br. J. Dermatol.* 157, 1093–1102.
- Friedmann, P.S., Moss, C., Shuster, S., Simpson, J.M., 1983. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin. Exp. Immunol.* 53, 709–715.
- Human and Environmental Risk Assessment 2005. (Amylases, Cellulases and Lipases. 1st edition, dated November 2005. Last accessed 09. 06. 09. <http://www.heraproject.com/files/38-F-Hera.Bridging.document.28.10.05.pdf>.
- Human and Environmental Risk Assessment. Subtilisins 2007. 2nd edition, dated February 2007. Last accessed 09. 06. 09. [http://www.heraproject.com/files/22-F-07.PROTEASE.HERA.Final%20Edition%20\(unsecured%20-%20PDF-A-1b\).pdf](http://www.heraproject.com/files/22-F-07.PROTEASE.HERA.Final%20Edition%20(unsecured%20-%20PDF-A-1b).pdf).
- Hillebrand, J.A., Thorne, P.S., 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.
- The International Association for Soaps, Detergents and Maintenance Products. 2002; Guidelines for the safe handling of enzymes in detergent manufacture. Brussels: www.aise-net.org.
- Johnson, G., Innis, J.D., Mills, K.J., Bielen, F., Date, R.F., Weisgerber, D., Sarlo, K., 1999. Safety assessment for a leave-on personal care product containing a protease enzyme. *Hum. Exp. Toxicol.* 18, 527.
- Jones, M., 2008. Exposure response in occupational allergy. *Curr. Opin. Allergy Clin. Immunol.* 8, 110–114.
- Juniper, C.P., How, M.J., Goodwin, B.F.J., Kinshott, A.K., 1977. *Bacillus subtilis*: a 7 year clinical, epidemiological and immunological study of an industrial allergen. *J. Soc. Occup. Med.* 27, 3–12.

- Kelling, C.K., Bartolo, R.G., Ertel, K.D., Smith, L.A., Watson, D.D., Sarlo, K., 1998. Safety assessment of enzyme-containing personal cleansing care products: exposure characterization and development of IgE antibody to enzyme after a 6-month product use test. *J. Allergy Clin. Immunol.* 101, 179–187.
- Kimber, I., Gerberick, G.F., Basketter, D.A., 1999. Thresholds in contact sensitization: theoretical considerations and practical applications. *Food Chem. Toxicol.* 37, 553–560.
- Magnusson, B., Kligman, A.M., 1970. Allergic Contact Dermatitis in the Guinea Pig: Identification of Contact Allergens. Charles C. Thomas, Springfield, IL, USA, 141 pp.
- Merget, R., Kulzer, R., Dierkes-Globisch, A., et al., 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.
- Pepys, J., 1972. Laboratory methods in clinical allergy – skin tests for immediate, type I, allergic reactions. *Proc. R. Soc. Med.* 65, 271–272.
- Pepys, J., Longbottom, J.L., Hargreave, F.E., Faux, J., 1969. Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet* 1, 1181–1184.
- Peters, G., Johnson, G.Q., Golembiewski, 2001. Safe use of detergent enzymes in the workplace. *Appl. Occup. Environ. Hyg.* 16, 389–396.
- Rodriguez, C., Calvin, G., Lally, C., 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., 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., MacKenzie, D., 1996. Lack of type I sensitization to laundry enzymes among consumer in the Philippines. *J. Allergy Clin. Immunol.* 97, 749a.
- Sarlo, K., Adamson, G.M., Hollis, V.L., Innis, J.D., Babcock, L.S., Kirchner, D.B., 2004. Development of allergic antibody to an enzyme in a body lotion: results of an 18-month clinical study. *J. Immunotoxicol.* 1, 71–77.
- Sarlo, K., Kirchner, D.B., Troyano, M.E., Smith, L.A., Carr, G. and Rodriguez, C. Assessing the risk of type I allergy to enzymes present in detergent products: a review of clinical data. MS for submission to Toxicology.
- Schweigert, M.K., Mackenzie, D.P., 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.
- The Soap and Detergent Association (SDA). 1995. Work practices for handling enzymes in the detergent industry. New York: The Soap and Detergent Association.
- Sekkat, A., Boghossian, J., Cookman, G.R., 1995. Risk assessment: A clinical study demonstrating the safety of a laundry detergent under paste use conditions. In: of 7th Pan African Conference of Dermatology, 1–5 December 1995, Cairo, Egypt.
- Thorne, P.S., Hillebrand, J., Magreni, C., Riley, E.J., 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. Last accessed 09. 06. 09. <http://www.hse.gov.uk/consult/condocs/cd187result2.htm>.
- US SDA. Risk assessment guidance for enzyme-containing products. Soap and Detergent Association, Washington, (2005).
- Van Rooy, F.G.B.G.J., Houba, R., Palmén, N., Zengeni, M.M., Sander, I., Spithoven, J., Rooyackers, J.M., Heederik, D.J.J., 2009. A cross-sectional study among detergent workers exposed to liquid detergent enzymes. *Occup. Env. Med.*
- Vanhanen, M., Tuomi, T., Tiikkainen, U., Tupasela, O., Voutilainen, R., Nordman, H., 2000. Risk of enzyme allergy in the detergent industry. *Occup. Environ. Med.* 2, 121–125.
- Weeks, J.A., Burdick, J.D., Rogers, R.E., Prento, A., 2001a. Assessment of sensitization risk to consumers using a laundry prespotter product containing protease. *Toxicol. Sci.* 60, 20.
- Weeks, J.A., Burdick, J.D., Harper, R.A., Smith, K., Simon, R.A., 2001b. A controlled use study of a laundry prespotter containing protease in an atopic population. *J. Allergy Clin. Immunol.* 107, S28.
- Wide, L., Bennich, H., Johansson, S.G.O., 1967. Diagnosis of allergy by and in vitro test for allergen antibodies. *Lancet* ii, 1105–1107.
- Zachariae, H., Hoeh-Thomsen, J., Witmeur, O., Wide, L., 1981. Detergent enzymes and occupational safety. Observations on sensitization during Esperase production. *Allergy* 36, 513–516.
- Zetterstrom, O., Wide, L., 1974. IgE antibodies and skin test reactions to a detergent enzymes in Swedish consumers. *Clin. Allergy* 4, 272–280.