Review

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

D.A. Basketter [a,*], C. Broekhuizen [b], M. Fieldsend [c], S. Kirkwood [d], R. Mascarenhas [e], K. Maurer [f], C. Pedersen [g], C. Rodriguez [h], H.-E. Schiff [i]

[a] DABMEB Consultancy Ltd., Sharnbrook, UK
[b] Genencor International BV, Leiden, The Netherlands
[c] Unilever, Sharnbrook, UK
[d] Robert McBride Ltd., Manchester, UK
[e] Reckitt-Benckiser, Hull, UK
[f] Henkel, Dusseldorf, Germany
[g] AISE, Brussels, Belgium
[h] Procter & Gamble Eurocor, Strombeek-Bever, Belgium
[i] NovozymesA/S, Bagsvaerd, Denmark

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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 $60\,\mathrm{ng/m^3}$ 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 $60\,\mathrm{ng/m^3}$ 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, $15\,\mathrm{ng/m^3}$, are associated with laundry trigger sprays, but very much lower levels (e.g. $0.01\,\mathrm{ng/m^3}$) 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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Contents

* Corresponding author at: 2 Normans Road, Sharnbrook, MK44 1PR, UK.
Tel.: +44 1234782944.
E-mail address: david.basketter@ukonline.co.uk (D.A. Basketter).

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 radioallergosorbent 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/m^3 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/m^3 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 lead 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/m^3. For example, a level of 15 ng/m^3, 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/m^3 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/m^3 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/m^3 (proteases), 5–20 ng/m^3 (lipases), 5–15 ng/m^3 (amylases) and 8–20 ng/m^3 (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/m^3 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/m^3 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/m^3 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/m^3 (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/m^3 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/m^3 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/m^3 was not associated with any induction or elicitation effects, then it is suggested that a consumer DMEL of 15 ng/m^3 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 fol-
lows: "*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 accor-
dance 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 experi-
ence 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 pro-
gression 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 sensitiza-
tion (and thus, by definition, will eliminate any possibility of clinical
symptoms).

7. Conclusion

The enzymes of bacterial and fungal origin widely used in indus-
try and in consumer products represent an important hazard – they
are respiratory sensitizers. On the other hand, decades of experi-
ence demonstrates that enzymes can be used safely by ensuring
that the exposure is strictly limited. Occupationally, a DMEL of
60 ng/m^3 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 work-
ing 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/m^3 is proposed, but with the recogni-
tion 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 lead 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

David Basketter was paid by the International Association for
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