Toxicology 271 (2010) 87–93

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Assessing the risk of type 1 allergy to enzymes present in laundry and cleaning products: Evidence from the clinical data

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ARTICLE INFO

Article history:
Received 25 January 2010
Received in revised form 22 February 2010
Accepted 3 March 2010
Available online 17 March 2010

Keywords:
Enzymes
Allergy
Asthma
IgE antibody
Risk
Consumer products
Sensitization
Exposure

ABSTRACT

Microbial enzymes have been used in laundry detergent products for several decades. These enzymes have also long been known to have the potential to give rise to occupational type 1 allergic responses. A few cases of allergy among consumers using dusty enzyme detergents were reported in the early 1970s. Encapsulation of the enzymes along with other formula changes were made to ensure that consumer exposure levels were sufficiently low that the likelihood of either the induction of IgE antibody (sensitization) or the elicitation of clinical symptoms be highly improbable. Understanding the consumer exposure to enzymes which are used in laundry and cleaning products is a key step to the risk management process. Validation of the risk assessment conclusions and the risk management process only comes with practical experience and evidence from the marketplace. In the present work, clinical data from a range of sources collected over the past 40 years have been analysed. These include data from peer reviewed literature and enzyme specific IgE antibody test results in detergent manufacturers' employees and from clinical study subjects. In total, enzyme specific IgE antibody data were available on 15,765 individuals. There were 37 individuals with IgE antibody. The majority of these cases were from the 1970s where 23 of 4687 subjects (0.49%) were IgE positive and 15 of the 23 were reported to have symptoms of allergy. The remaining 14 cases were identified post-1977 for a prevalence of 0.126% (14/11,078). No symptoms were reported and no relationship to exposure to laundry and cleaning products was found. There was a significant difference between the pre- and post-1977 cohorts in that the higher rates of sensitization with symptoms were associated with higher exposure to enzyme. The clinical testing revealed that the prevalence of enzyme specific IgE in the population is very rare (0.126% since 1977). This demonstrates that exposure to these strong respiratory allergens via use of laundry and cleaning products does not lead to the development of sensitization and disease. These data confirm that the risk to consumers has been properly assessed and managed and support the concept that thresholds of exposure exist for respiratory allergy. Expansion of enzyme use into new consumer product categories should follow completion of robust risk assessments in order to continue ensuring the safe use of enzymes among consumers.
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1. Introduction

In toxicological safety assessment, it is critical to differentiate between the hazards presented by substances and the risk they may present to human health. Whereas the hazard is an intrinsic property of the substance, the risk to human health also depends very heavily on exposure. While the bacterial/fungal enzymes used in laundry detergent products represent a substantial respiratory allergy hazard, the risk of this being expressed in consumers is practically eliminated by appropriate control of the exposure (SDA, 2005). At the beginning of the use of such enzymes nearly 40 years ago, the hazard was not adequately characterized and the exposure was not properly controlled. The evidence then accumulated relatively quickly that the hazard translated not only into a significant occupational allergy problem (reviewed in Sarlo, 2003), but also into a risk for consumers, albeit at a low level (Belin et al., 1970; Bernstein, 1972; Zetterstrom and Wide, 1974). Rapidly, the industry worked to reduce the occupational and the consumer risks, the latter particularly by the encapsulation of enzymes and other formulation changes which dramatically reduced the exposure level. This resulted in a high degree of confidence that respiratory allergy to enzymes in consumers should not occur (SDA, 2005). Occupationally, enzyme exposure is controlled using the strictest airborne limits applied to any respiratory allergen, but while this ensures

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doi:10.1016/j.tox.2010.03.007

Table 1
Summary of sensitization data on consumer populations using enzyme-containing laundry and cleaning products: English language peer reviewed literature.

Size	IgE[a]	Enzyme	Symptoms[b]	Reference
$N = 3$	+3/3[c]	*B. licheniformis* protease	+($N = 3$)	Belin et al. (1970)
$N = 1645$	+15/1645	*B. licheniformis* protease	+($N = 8$)	Zetterstrom and Wide (1974)
$N = 539$	+4/539[c]	*B. licheniformis* protease	+($N = 4$)	Bernstein (1972)
$N = 2500$	+1/2500[c]	*B. subtilis* Koch-Light protease; *B. subtilis* amylase; *B. licheniformis* protease	–	Pepys et al. (1973)
$N = 136$	−0/136	*B. licheniformis* protease	–	Pepys et al. (1985)
$N = 655$	−0/655	*B. lentus* protease; *B. licheniformis* amylase	–	Cormier et al. (2004)
Total pre-1977 $N = 4687$	Total IgE (+) = 23			
Total post-1977 $N = 791$	Total IgE (+) = 0			

[a] IgE antibody detected by SPT and/or by serology and/or by passive serum transfer.
[b] Symptoms were self-reported or confirmed in the clinic in subjects with IgE antibody.
[c] SPT antigen concentration equal to or greater than 1 mg/ml.

exposure is below the threshold for the elicitation of allergy, nevertheless there is a limited degree of the induction of enzyme specific IgE that is typically observed (Nicholson et al., 2001; Sarlo, 2003). In the present paper, we have surveyed and critically reviewed all the evidence available to us from directed clinical studies and other related and relevant pieces of information which contributes to our understanding of the status of the induction of enzyme specific IgE amongst populations exposed to enzyme-containing cleaning products. This information includes a review of published data plus presentation of previously unpublished information from clinical studies and the occupational medical surveillance program at Procter & Gamble. The picture that emerges is that the absence of respiratory allergy to laundry detergent enzymes in consumers is complemented by an absence of the induction of IgE. These data show that exposure to these strong respiratory allergens occurring under the conditions of use in laundry and cleaning products does not lead to the development of IgE or disease, and support the concept that thresholds of exposure exist for respiratory allergy.

2. Materials and methods

2.1. Analysis of information from literature

A search of the English language peer review literature using combinations of MESH terms that included enzyme, allergy, allergic antibody, sensitization, detergent, skin prick test, clinical trial along with key author searches identified 603 papers of which 10 were selected for inclusion in this study. Adequate information on the population studied, how the population was assessed, the enzymes used in the testing, and the concentration of enzymes used in diagnostic testing was available in these papers (see Tables 1 and 2). Also, these studies met the criteria that any indication of enzyme IgE antibody was confirmed by repeat skin tests, serology or passive transfer tests. In addition, three abstracts that described consumer exposure to enzymes in cleaning products were found in Soap and Detergent Association industry trade association guidance documents (Weeks et al., 2001b; Troyano et al., 2003; Sekkat et al., 1995). Enzyme specific allergic antibody data were available for 10,178 individuals.

2.2. Skin prick test (SPT) for enzyme allergic antibody

Assessment of enzyme specific allergic antibody in populations not included in the published literature was done using the skin prick test. These populations included clinic study subjects and detergent manufacturing employees in the medical surveillance program. The enzyme SPT reagents were prepared by the Procter & Gamble Co. from purified versions of the commercially used enzymes obtained from Genencor International (Palo Alto, CA, USA) or Novozymes, A/S (Denmark). These included the alkaline serine proteases from *Bacillus amyloliquifaciens*, *Bacillus licheniformis* (Alcalase®) and *Bacillus claussi*, along with the alpha-amylases from *B. amyloliquifaciens* and *B. licheniformis*. Fungal (*Humicola lanuginosa*) cellulase and lipase made in *Aspergillus oryzae* and alpha-amylase from *A. oryzae* were also tested in the occupational medical surveillance program. Solutions of 500 μg protein/ml and 50 μg protein/ml were prepared using a 50% glycerin/saline solution. The negative control was the glycerin/saline vehicle and the positive control was 2.75 mg/ml histamine phosphate in the glycerin/saline vehicle. Tests were performed using the method described (Pepys, 1972). A drop of the test or control material was placed on the volar surface of the forearm and a slight prick of the surface epidermis was made using a sterile, 26–27 gauge needle. The test site was observed after 15 min for the presence of a wheal and flare reaction. Resulting reactions were measured,

Table 2
Summary of sensitization data in test populations enrolled in clinical studies designed to assess safety of enzyme-containing products: English language peer review literature.

Size	IgE[a]	Enzyme	Reference
$N = 65$	0/65	Alcalase	White et al. (1985)
$N = 400$	3/400[b]	*B. amyloliquifaciens* protease *B. licheniformis* amylase	Bindslev-Jensen et al. (2006)
$N = 108$	0/108	*B. amyloliquifaciens* protease	Kelling et al. (1998)
$N = 1325$[c]	0/1325	*B. claussi* protease *B. licheniformis* amylase	Cormier et al. (2004)
$N = 2500$	4/2500	*B. amyloliquifaciens* protease *B. claussi* protease, *B. licheniformis* protease and *B. licheniformis* amylase	Sarlo et al. (2004)
$N = 519$ (subset of 2500)	0/519		
Total $N = 4398$	Total IgE (+) = 7		

[a] IgE detected by skin prick test and/or by serology; antigen concentration <1 mg/ml.
[b] Subsequent tests to confirm IgE were negative.
[c] Population does not include the 655 test subjects reported in Table 1.

and the sizes of the wheal and flare at the largest points were recorded. A test was considered positive if the site exhibited a wheal of at least 3 mm larger than the negative control, together with a visible flare and a reaction to the positive control. Any positive reactions to the enzymes were confirmed by repeat testing on the opposite arm. Positive responses among test subjects in the clinical studies were verified by repeat SPT at different skin sites on a separate day.

2.3. Procter & Gamble medical surveillance program

Male and female employees of the Procter & Gamble Company were enrolled in a medical surveillance program designed to detect the development of IgE antibody to enzymes used in the occupational environment (Schweigert et al., 2000). Participation in the program was voluntary although enrolment was near 100%. The SPT database from the P&G medical surveillance program was reviewed to identify previously unreported baseline (pre-occupational exposure) responses to 8 different enzymes among 5156 employees in North America from 1972 to 2008.

2.4. Clinical study populations

Data from clinical study populations were evaluated. Population #1 consisted of 500 atopic, female subjects from North America, France and Germany. The subjects were between the ages of 18 and 60 and in good health, participated in controlled clinical trials of new enzyme-containing products and were part of a larger group described in 1 of the 10 publications (Sarlo et al., 2004). These 500 test subjects were tested with selected enzymes 6 months after their first evaluation (not reported in Sarlo et al., 2004). Any subject who displayed a positive SPT response was asked to return to the test laboratory for a repeat SPT and their serum was collected and tested for circulating specific IgE antibody to the enzymes. These subjects were also interviewed about potential enzyme exposure. Population #2 consisted of 208 test subjects in the United Kingdom (UK), between the ages of 18 and 65 (Troyano et al., 2003). Fifty percent were atopic (based on positive skin prick test response to one common aeroallergen) and all were in good health. These test subjects were asked to use a dishwashing liquid containing proteolytic enzyme along with their regular laundry and cleaning products for 1 year. Subjects were assessed for IgE antibody to the enzyme by SPT at the start and end of the study. Population #3 consisted of 406 test subjects in the UK and in North America enrolled in a human repeat insult patch test of a protease enzyme in lotion (unpublished data). These test subjects were assessed by SPT before and after the patch test study. One hundred and eighty one (44.5%) of these test subjects were atopic (based on positive skin prick test response to one common aeroallergen). Population #4 consisted of 76 Egyptian male mechanics that used enzyme-containing granule detergent to wash their bodies (Sekkat et al., 1995). The wash was done with sponges and buckets of water (no shower). Individuals were identified by interviews. In total, 1190 clinic test subjects were evaluated. The Procter & Gamble Company Institutional Review Board as well as the Ethics Committees of the different testing laboratories reviewed and approved all clinical protocols. All subjects signed an informed consent. The study was conducted in accordance with Good Clinical Practices (GCP) for Trial of Medicinal Products and the ICH Guideline for GCP (CPMP/ICH/135/95).

2.5. Measurement of exposure to enzymes upon use of products

Laundry and cleaning products containing 1× to 100× the enzyme levels used in marketed products were used to assess exposure upon simulation of consumer usage. Air samples were collected using General Metal Works P-2000, Handi-Vol high volume air sampler equipped with air flow meters and 4″ round filter pad holders containing Whatman GFC grade glass microfibre filters. The sampler was placed near the machine or sink so that it could capture aerosol in the breathing zone. Air samples were collected during different phases of washing and drying tasks. These tasks included pouring granule or liquid detergent into the washing machine, pouring liquid dish soap into the sink, running water into the machine or sink, spraying fabric with laundry pre-treat product, cleaning dryer vents, and washing and rinsing dishes. The total air volume sampled during a trial was determined by measuring air flow at frequent time intervals, and summing intermediate volumes [time elapsed for interval × airflow (m^3/min) for that interval]. The amount of specific enzyme protein on the filters was measured by enzyme linked immunosorbent assay (ELISA), as previously described (Miller et al., 1994). All samples were run in triplicate and were not diluted unless they were expected to be above the detection range of the assay. The detection range of the assay was 1.9–190 + 0.5 ng/m^3 at an air sampling flow rate of 0.67 m^3/min.

2.6. Statistical analysis

The Fisher's exact test was used to identify differences between cohorts of populations identified in this review. All statistical analyses of the data were conducted in StatXact 4 for Windows. Cohorts were treated as independent populations and evaluated for statistical significance by binary response methods. Confidence intervals were calculated for the individual cohorts. Significance was defined at $p < 0.05$.

3. Results

3.1. Peer reviewed literature: allergy and enzymes in consumer populations

Table 1 summarizes the enzyme sensitization data described in 6 published studies of consumer populations using laundry detergent products. The presence of anti-enzyme IgE antibody and symptoms upon exposure to enzyme was apparent in some populations during the very early days of use of enzyme-containing laundry products. These early products were dusty and were used in machines as well as for hand washing of clothes. In Sweden, 3 patients with symptoms of type 1 allergy associated with use of enzyme-containing powder (dusty) detergent were described (Belin et al., 1970). The description of the 3 patients showed that symptoms arose after 6 months to 2 years of use of product. Symptoms were noted with both hand and machine washes of laundry items. Zetterstrom used serology and skin prick test to evaluate clinic patients for IgE antibody to Alcalase (Zetterstrom and Wide, 1974). He evaluated 1523 serum samples in a radioallergosorbent test (RAST) and tested 122 subjects by skin prick test finding that 15, or 0.91% (15/1645) were confirmed as consistently positive for IgE antibody to Alcalase. Laboratory-generated enzyme aerosols from these dusty products showed enzyme levels greater than 100 ng/m^3 (SDA, 2005). Bernstein (1972) studied a total of 539 individuals in the US and found 4 individuals that were sensitized and symptomatic due to enzymes, confirmed by positive results from skin prick tests, passive transfer of serum plus challenge, bronchoprovocation challenge and clinical symptoms after product exposure. This experience, along with the manufacturing experience led the industry to granulate the enzyme and detergent products to reduce dust.

Pepys (1972) tested 2500 allergy clinic patients with *Bacillus* protease enzyme using the SPT and concluded that 1 had allergic IgE antibody that recognized the protease. A subset of 506 patients was also tested to different protease and amylase enzymes; all were negative. Approximately 2000 of the allergy clinic patients had used enzyme-containing granulated detergent products for at least 1 year with measured airborne exposure levels of 1 ng/m^3 or less. It was determined that the individual with the IgE antibody to enzyme had been exposed to a very dusty detergent product similar to the those associated with allergy and asthma among Swedish consumers, where airborne enzyme exposure was believed to exceed 100 ng/m^3 (Belin et al., 1970; Zetterstrom and Wide, 1974). Several years later, Pepys et al. (1985) tested 136 clinic patients; 88 of these patients were from the original pool of 2500. None were SPT or RAST positive to protease enzyme.

A retrospective examination of 655 atopic women in the Philippines showed no SPT positive response to *Bacillus* protease or amylase enzymes in individuals who used enzyme-containing granule laundry detergents for hand laundering for at least 1 year. A further 1300 atopic women who had sporadic exposure to these enzymes via laundry product were also SPT negative to these enzymes. Many of these women had compromised skin due to mechanical abrasion associated with hand laundry habits used in the region. It is notable that enzyme exposures were up to 0.18 ng/m^3 and occurred for minutes to hours, daily (Cormier et al., 2004). A 2-year clinic study of 581 of these women showed that no IgE antibody to enzymes was found after use of enzyme-containing granule detergent for hand laundry supplemented with an enzyme-containing synthetic laundry bar. The women also used the bar for daily personal cleansing. The highest exposure level from the bar soap was 0.026 ng/m^3 (Cormier et al., 2004).

Assessments of the development of IgE antibody to enzymes were also done in populations that were not necessarily being studied for safety of laundry and cleaning products. Table 2 provides a

Table 3
Summary of sensitization data from studies described in abstract; also unpublished studies.

Size	IgE[a]	Enzyme	Reference
$N = 100$	–	*B. claussi* protease	Weeks et al. (2001a, 2001b)
$N = 151$	–	*B. amyloliquifaciens* protease	Troyano et al. (2003)
$N = 406$	–	*B. amyloliquifaciens* protease	P&G HRIPT studies (unpublished, post-1977)
$N = 76$	–	*B. claussi* protease	Sekkat et al. (1995)
Total $N = 733$	Total IgE (+) = 0		

[a] IgE detected by skin prick test; antigen concentration <1 mg/ml.

summary of these data from the remaining publications found in the peer review literature. In total, 4398 subjects were variously assessed by SPT. Of these, only 7 (0.16%) were positive and 3 of the 7 could not be confirmed as positive by additional testing. Sarlo et al. (2004) reported SPT results for 2500 atopic female test subjects screened for participation in clinical trials. All subjects were tested with the protease from *B. amyloliquifaciens*. A total of 4 women were found to be SPT (+) to this enzyme but SPT (−) to the other *Bacillus* enzymes. Only 1 of the 4 had detectable, low levels of IgE antibody in serum. The results of a product use questionnaire plus interview did not reveal an obvious source of exposure to the *B. amyloliquifaciens* protease enzyme. Furthermore, these women did not report any symptoms of allergy upon use of household cleaning products, including detergents. A subset of this group ($N = 519$) was tested with 4 other *Bacillus* enzymes; all were SPT negative and remained SPT negative to these enzymes 6 months after their initial test. Bindslev-Jensen et al. (2006) tested 400 clinic patients with a variety of enzymes used in the food industry. The serine protease enzymes and alpha-amylase enzymes from *B. licheniformis* and *B. amyloliquifaciens* that are also used in laundry and cleaning were included in the SPT antigen battery. Three individuals gave an initial positive SPT response but subsequent tests to confirm the IgE antibody were negative. It is possible that these 3 were false positive responses. The other studies all generated negative skin prick test data among 2498 test subjects (see references, Table 2).

Table 3 lists data from published abstracts or from unpublished clinical studies that also describe the lack of IgE antibody to *Bacillus* enzymes, albeit in small cohorts. The first study showed negative SPT to *B. claussi* protease in 100 atopic, female test subjects were asked to use a protease containing laundry spray pre-treater product for 6 months on a daily basis (Weeks et al., 2001a, 2001b). These test subjects were SPT negative at the beginning and end of the study. The work described by Troyano showed negative SPT results to *B. amyloliquifaciens* protease in 208 subjects recruited to use protease containing dishwashing liquid or control product for 1 year. The product was used at least once per day. All of the 151 subjects that completed the study were SPT negative (Troyano et al., 2003). The remaining 49 subjects dropped out of the study due to non-health related reasons. Data on 406 test subjects recruited to participate in human repeat insult patch tests (HRIPT) on a prototype body lotion containing *B. amyloliquifaciens* protease showed that all were SPT negative before and after the patch test (unpublished data). One hundred and nine of the 406 were atopic. Lastly, a retrospective study of 76 male mechanics in Egypt who used enzyme-containing laundry granules for daily personal cleansing (the great majority for more than a year) showed that none of them had enzyme specific IgE antibody (Sekkat et al., 1995). These exposures occurred primarily via use of detergent products with buckets of water to cleanse the body.

3.2. Unpublished SPT results from employees prior to enzyme exposure

All new employees entering the detergent manufacturing workforce are tested to obtain a baseline value for continued occupational health monitoring. In addition, all employees are tested prior to the introduction of new enzymes into the manufacturing site. Table 4 shows the SPT results of 5156 employees (North America) tested from 1972 to 2008 prior to occupational exposure to enzyme. The concentration of SPT antigen used from 1972 to 1992 was 500 μg protein/ml. During this time there was a prevalence of 0.137% baseline SPT positive results to the serine protease Alcalase among this cohort. The concentration of SPT antigen used from 1993 to 2008 was 50 μg/ml. The antigen concentration was dropped to improve specificity without a loss in sensitivity (Bernstein et al., 1994). During this time there was a prevalence of 0.135% SPT (+) to any enzyme. There was one SPT (+) individual each to the serine protease and alpha-amylase from *B. licheniformis*. There were 2 SPT (+) individuals to the fungal cellulase. All 4 individuals were identified prior to 2002. Since 2002, 1341 new employees were tested and none showed baseline SPT (+) responses. The results of the product use questionnaire given to 100 employees in North America showed that >80% of these individuals were consumers of several types of enzyme-containing detergents and used them regularly for their laundry needs.

3.3. Summary of population data

A total of 15,765 individuals were tested for IgE antibody to enzymes. Of these, 37 were confirmed as IgE positive to enzyme for a prevalence of 0.23%. In order to assess whether changes to detergent plus enzyme formulations had an impact on this prevalence, the sensitization data were split into 2 pools representing IgE positive subjects identified before 1977 and those identified after 1977. This year was chosen since there was a transition in exposure to enzymes from high (>100 ng/m^3) to low (<1 ng/m^3) in the early 1970s and the papers published at this time included many individuals who were exposed during this transition period. There were a total of 23 of 4687 individuals with enzyme specific IgE antibody pre-1977 (see Table 1, total pre-1977). There were a total of 14 of 11,078 individuals with enzyme specific IgE antibody post-1977 (see Table 1, total post-1977, Tables 2–4). This difference is statistically different at $p = 0.0001$.

3.4. Exposure assessment: air sampling

Consumer use of detergent products was assessed by extensive habits and practices surveys of consumer populations in North America and Europe. Enzyme exposure associated with product use was estimated in the laboratory by reproducing the tasks identified in these surveys, using commercially available washing machines and dryers and measuring the resulting concentrations of airborne enzymes (SDA, 2005; HERA, 2007). Table 5 shows the measured airborne enzyme levels during a variety of cleaning tasks. All of the exposures were associated with tasks of short duration (seconds to minutes) and occurred several times over a period of 1 week. The highest exposure level was measured upon use of a trigger spray laundry pre-treat cleaner. This exposure occurred when consumers sprayed fabric held in a vertical position and there was opportunity for the spray to bounce off the fabric and into the breathing zone.

Table 4
SPT results of 5156 employees prior to occupational exposure to enzymes (unpublished data).

Enzyme SPT reagent	1972–1992[a] cohort	1993–2002[b] cohort	2002–2008[b] cohort	Total
B. amyloliquifaciens protease	0/2192	0/1623	0/1341	0/5156
B. licheniformis protease	3/2192	1/1623	0/1341	4/5156
B. claussi protease	0/2192	0/1623	0/1341	0/5156
B. amyloliquifaciens amylase	0/2192	0/1623	0/1341	0/5156
B. licheniformis amylase	0/2192	1/1623	0/1341	1/5156
H. lanuginosa cellulase	NT	2/1623	0/1341	2/5156
H. lanuginose lipase	NT	0/1623	0/1341	0/5156
A. oryzae alpha-amylase	NT	0/1623	NT	0/1623

[a] SPT at 500 μg/ml.
[b] SPT at 50 μg/ml.

Table 5
Exposure measurements upon use of enzyme-containing laundry detergents (unpublished data).

Task	Magnitude (ng protein/m^3)	Duration of task	Frequency of task
Pour liquid detergent into top-loader wash machine	0.012	<30 s	4–7×/week
Pour granule detergent into top-loader wash machine	0.00022	<30 s	4–7×/week
Addition of water to liquid or granule detergent in top-loader wash machine	0.7–2.9	<30 s	4–7×/week
Addition of detergent to front-loader wash machine	0	<30 s	3–10 × week
Detergent refill (pour granule from 6 kg sack)	0.5	<1 min	Once/month
Dryer vent (indoors)	<0.5	<30 s to 1 h[a]	<4–7×/week
Clean dryer lint trap	0.04–1.2	<30 s	<4–7×/week
Spray pre-treat laundry items[c]	14.5	<1 min	4–7×/week
Hand wash dishes using liquid dish soap[b]	1–3 followed by <0.3	<30 s followed by several minutes	Daily

[a] Assume an individual remains in the laundry room for any length of time during the typical dry cycle.
[b] Measurement includes initial peak exposure as water and soap first mix followed by the exposure during the hand wash task.
[c] Weeks et al. (2001b).

This exposure was associated with negative SPT responses in a clinical trial (see Table 3, Weeks study). Other high exposures occurred when hot water mixed with liquid detergent product placed at the bottom of a top-loading washing machine or hot water mixed with liquid soap used to clean dishes. These peak concentrations lasted for less than 1 min and the magnitude dropped as a surfactant layer formed on top of the water. The exposure for the dishwashing liquid was associated with negative SPT responses in a clinical trial (see Table 3, Troyano study). Front-loading washing machines generated no airborne levels of enzyme based on the way product is added to these machines. Enzyme levels on lint delivered into the air upon indoor venting of dryers (estimated <5% of homes in North America) is also low (<0.6 ng/m^3).

4. Discussion

Enzyme-containing detergent products have enjoyed a safe market history for nearly 40 years. While enzymes can cause occupational asthma and allergy via an IgE mediated, type 1 hypersensitivity response, this response is rare among the general population. The SPT data generated from evaluation of several populations show that the prevalence of individuals with anti-enzyme IgE antibody is very low. A total of 37 of 15,765 (0.23%) were identified as IgE positive to an enzyme since the original paper by Belin was published in 1970. By splitting the population into pre- and post-1977 we found a significant difference in the prevalence of sensitization and reported symptoms. The majority of the pre-1977 cases were clinically relevant and were linked to high exposure to enzyme in laundry products. None of the cases identified post-1977 was clinically relevant and none could be linked to use of enzyme-containing laundry and cleaning products. This is despite the fact the enzyme-containing laundry and cleaning products are used daily by many millions of consumers in most regions of the world now and over the past 15–20 years (Houston, 1997). These data tell us that intervention in the form of changes to product formulations and hence lowered exposures eliminated the risk to consumers. This information confirms the risk assessment that typical use of enzyme-containing laundry and cleaning products does not lead to the development of enzyme specific IgE antibody among consumers (SDA, 2005; HERA, 2007).

Since the 14 cases identified post-1977 could not be linked to enzymes in laundry and cleaning products we can speculate that there may have been other environmental exposures that led to IgE antibody that recognized the enzymes. *Bacillus* organisms are commonly found in soil and in decomposing plant products and are considered non-pathogenic in humans (EPA risk assessment) (USEPA, 1997). The subtilisin proteases are a superfamily of proteases that are produced by almost all organisms (Kraut, 1977) and some function as virulence factors (St. Leger et al., 1997). Therefore, it is possible that some cross-reactivity exists among some of these enzymes.

There are currently no quantitative risk assessment methods that can be used to identify no adverse effect levels for type 1 allergy to enzyme. Rather, we have to compare exposure assessment results to existing clinical data which associate the exposure levels with the development (or lack thereof) of IgE antibody to enzyme (benchmarks). These benchmark datasets tell us that there are thresholds of exposure (defined by magnitude, frequency, duration and route) below which allergic antibody and subsequent development of disease are not induced. We believe the exposures listed in Table 5 represent a de facto threshold and can be used to establish derived minimal effect levels (DMELs) for the enzymes (Basketter et al., 2009). These exposures do not necessarily translate to other uses of enzymes since clinical testing of enzymes in personal care products such as a bar soap and a body lotion led to development of IgE antibody in a few of the test subjects (Kelling et al., 1998; Sarlo et al., 2004).

The SPT is a very sensitive test for allergen specific IgE antibody but it can be confounded by poor technique and inappropriate aller-

gen concentrations. A report by Bernstein et al. (1994) showed that increased concentrations of enzyme in the SPT can detect lightly sensitized individuals but greater false positive responses could be attained with enzyme concentrations greater than 500 μg/ml. This is consistent with the experience reported by Pepys et al. (1985). In our hands, 500 μg/ml is the highest non-irritating concentration of enzyme that can be used in the SPT. Our investigation uncovered 37 individuals that were IgE positive to enzymes. Twenty-three of the 37 SPT positive individuals identified in this paper were described by investigators in the early 1970s when exposure to enzymes was not adequately controlled. We have confidence that these individuals had true positive responses since their initial responses were confirmed by repeat skin prick testing and/or with serology and/or with passive transfer testing.

P&G has a well structured medical surveillance program to detect the onset of enzyme specific IgE antibody amongst the detergent manufacture workforce (Schweigert et al., 2000). All employees must be assessed prior to exposure to enzyme at hire and before introduction of new enzymes into the manufacturing site. This has provided a well characterized population to evaluate since we also know that these individuals are typical consumers of laundry and cleaning products. From 1972 to 1992 the SPT enzyme concentration was 500 μg/ml protein. The introduction of enzymes derived from a fungal host organism in the early 1990s led to an increase in false positive SPT responses due to the degree of purity of reagents used in the program. Dropping the concentration to 50 μg/ml helped to reduce the false positive responses with these reagents with a modest sacrifice in sensitivity (Bernstein et al., 1994). Since employees are under active medical surveillance and are tested on an annual basis, then some loss in sensitivity can be accepted to improve the assay specificity, i.e., minimize false positive responses to the less pure reagents. Comparing the baseline IgE responses amongst the 2 cohorts of workers (pre-1992, post-1992) showed no difference in the prevalence of SPT positive responses.

It is fair to say that after the initial adverse reactions to enzyme-containing products which occurred in consumers almost 40 years ago, there is now an absence of evidence of respiratory allergy problems. This is supported by the evidence of absence of any significant induction of IgE antibodies in exposed consumers. Where there is no IgE antibody to enzyme there can be no allergic reactivity. Enzymes can be used safely in a range of consumer laundry and cleaning products. This outcome has been predicted by product safety assessments (SDA, 2005; HERA, 2005, 2007). However, expanded use of enzymes outside of these typical laundry and cleaning applications must be supported by a risk assessment and in some cases clinical evaluation. The Soap and Detergent Association has published guidelines that manufacturers of consumer products containing enzymes should follow when performing the consumer safety assessment (SDA, 2005).

In addition to the question of respiratory sensitization, it is worth mentioning that the issue of skin effects, including sensitization, has been considered from theoretical, experimental and clinical perspectives. This work has shown, over a 40-year period, that there is no evidence of immunologically mediated adverse skin affects associated with enzymes in detergent products (Basketter et al., 2008).

In summary, after learning from the early cases of enzyme induced consumer allergy, changes were made such that enzyme-containing detergent products have enjoyed a safe market history for over 35 years. While enzymes can cause occupational asthma and allergy via an IgE mediated, type 1 hypersensitivity response, this response is limited to detergent manufacturing and is very rare among the general population. The SPT data generated from employees prior to occupational exposure to enzymes and in atopic female populations shows that the prevalence of individuals with anti-enzyme IgE antibody is very low, is not clinically relevant and cannot be linked to product exposures. Furthermore, levels of exposure to enzymes that result in neither induction or elicitation of respiratory allergy in consumers have been determined in the context of laundry and cleaning products. Thus, the risk assessment conclusion, that typical use of enzyme-containing detergent products should not lead to the development of enzyme specific IgE antibody among consumers, is not only supported by an absence of evidence, it is further confirmed by evidence of absence of such an effect.

Acknowledgments

The authors wish to thank David Basketter for his assistance in writing this paper.

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