# Guidance for the Risk Assessment of Enzyme-Containing Consumer Products

american cleaning institute®

A·I·S·E since 1952

# Guidance for the Risk Assessment of Enzyme-Containing Consumer Products

Sponsored by
The American Cleaning Institute (ACI) and
The European cleaning & hygiene products industry association (A.I.S.E.)


# Authors

## 2025 Edition:

**ACI participating members:**
Beth Concoby (IFF)
James Kim (ACI)
Francis Kruszewski (ACI)
Nathan Pechacek (3M; formerly Ecolab)

**A.I.S.E. participating members:**
Phillip Davison (A.I.S.E.)
Mark Fieldsend (formerly Ecolab)
Anthony Panepinto (P&G)
Merete Simonsen (Novonesis)

## 2019 Edition:

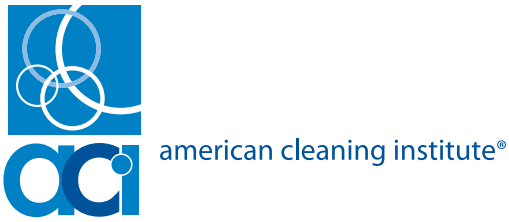
**ACI participating members:**
Beth Concoby (DuPont)
Mekine Baccam (P&G)
Robert Bookstaff (formerly P&G)
Francis Kruszewski (ACI)
Gregory Ladics (DuPont)
Jessica Paschal (formerly Novozymes)
Richard Sedlak (formerly ACI)

**A.I.S.E. participating members:**
Mark Fieldsend (formerly Ecolab)
Giulia Sebastio (A.I.S.E.)
Merete Simonsen (Novozymes)

## 2005 Edition:

**ACI participating members:**
Beth Concoby (formerly Genencor)
Leigh Henderson (formerly Unilever)
Francis Kruszewski (ACI)
Mark LaFranconi (formerly P&G)
Veronique Lauriault (formerly Genencor)
Richard Sedlak (formerly ACI)
Kathy Sarlo (formerly P&G)
Lawrence Smith (formerly P&G)
Robert Stachlewitz (formerly P&G)
John Weeks (formerly SCJ)

**A.I.S.E. participating members:**
David Basketter (formerly Unilever)
Wendy Laing (formerly Novozymes))
Margaret Richold (formerly Unilever)
Carlos Rodriguez (formerly P&G)
Merete Simonsen (Novozymes)
Hans Erik Schiff (formerly Novozymes)





**American Cleaning Institute (ACI®)**
1401 H Street NW, Suite 700
Washington, DC 20005

**TEL:** 202-347-2900

**WEB:**
www.cleaninginstitute.org
www.cleaninginstitute.org/understanding-products/about-cleaning-product-ingredients#enzymes

**The European cleaning & hygiene products industry association (A.I.S.E.)**
Boulevard du Souverain 165, 4th Floor
1160 Brussels

**TEL:** +32 (0)2 679 62 60

**WEB:**
www.aise.eu
www.aise.eu/enzymes

The American Cleaning Institute® is the Home of the U.S. Cleaning Products Industry® and represents the $60 billion U.S. cleaning product supply chain. ACI members include the manufacturers and formulators of soaps, detergents, and general cleaning products used in household, commercial, industrial and institutional settings; companies that supply ingredients and finished packaging for these products; and chemical distributors. ACI serves the growth and innovation of the U.S. cleaning products industry by advancing the health and quality of life of people and protecting our planet. ACI achieves this through a continuous commitment to sound science and being a credible voice for the cleaning products industry.

A.I.S.E. represents the detergents & maintenance products industry in Europe. Based in Brussels, A.I.S.E. has been the voice of the industry to EU regulators since 1952. Membership consists of 29 national associations across Europe, 19 corporate members and 23 value chain partners. Through this extensive network, A.I.S.E. represents over 900 companies supplying household and professional cleaning products and services across Europe. The industry is a substantial contributor to the European economy with an annual market value of €45,5 billion, directly employing 95 000 and 360 000 throughout the value chain. A.I.S.E. has a long history in leading voluntary industry initiatives that focus on sustainable design, manufacturing and consumption, product safety and safe use of products by consumers and professional customers.

# Table of Contents



**NOTE:** The above chapter descriptors/page numbers and *Table of Contents* at the bottom of each page are hot-linked.

# Preface

The laundry product industry has implemented a successful product stewardship program to promote the safe use of enzymes in the workplace and by users of their products, using both appropriate risk assessment and risk management practices. Much of the information about enzymes for laundry applications can be applied to other finished products including those in the cleaning and personal care markets.

This document* provides guidance on risk characterization, that is, hazard identification, dose-response assessment and exposure assessment, in the development of new products containing enzymes. This information is used to develop an appropriate risk management strategy that avoids unacceptable risks to the user of enzyme-containing products. It is not intended as a requirement nor a standard of care for manufacturers or the industry.

The intended audiences for this document are toxicologists, risk assessors and product safety professionals in industries formulating products containing enzymes.

The information presented here may not be entirely applicable to all situations where enzymes are used. Furthermore, certain conclusions are of limited certainty as detailed in the document. Product manufacturers should consult individuals with appropriate expertise in order to judge the applicability of this information, as well as information from other sources.

For additional information on risk assessment and risk practices for enzymes, contact your enzyme supplier, or

American Cleaning Institute
1401 H Street NW, Suite 700
Washington, DC 20005
Tel: 202-347-2900
Web: www.cleaninginstitute.org

The European cleaning & hygiene products industry association (A.I.S.E.)
Boulevard du Souverain 165, 4th Floor
1160 Brussels
Tel: +32 (0)2 679 62 60
Web: www.aise.eu

* Note – The content of this 2025 issued document is the same as that in the 2019 second edition (ACI 2019). The original version was prepared by the Soap and Detergent Association (SDA) in 2005 (SDA 2005). The SDA formally changed its name to the American Cleaning Institute (ACI)® in 2010. The contents of the 2019 second edition and the 2005 first edition were jointly prepared by ACI and A.I.S.E., and this 2025 issue fully acknowledges this collaboration by listing all of the co-authors.

# Executive Summary

Enzymes are proteins that speed (catalyze) reactions. They have the potential to improve efficiencies and provide previously unavailable product benefits. In the last few years, the use of enzyme-containing products has increased significantly and the number of applications in which enzymes are being incorporated is continuing to expand. Enzymes generally have good safety profiles. However enzymes, like many other proteins, can act as allergens and induce the production of enzyme-specific IgE antibodies upon repeated exposure, primarily via inhalation or exposure to mucous membranes that may lead to allergy symptoms, including asthma.

The purpose of this document is to describe the potential health hazards of enzymes present in consumer products and provide a framework for manufacturers of these products to conduct risk assessments to help ensure the safety of new products containing enzymes. The primary challenge associated with enzyme use is preventing the generation of enzyme-specific IgE antibodies and the development of symptoms of respiratory Type 1 hypersensitivity. This hazard is the primary focus for the risk assessment for enzymes and must be managed carefully. Another hazard that also should be addressed includes primary irritation of the eye and skin which can be caused by enzymes belonging to the class of proteases. Only enzymes of the class of protease will have this irritating effect.

Experience in the cleaning products industry demonstrates that the potential risk of adverse effects can be successfully managed by identifying the hazards to be managed, carefully assessing exposure, characterizing the risk and then applying appropriate risk management. If the risks are not managed appropriately, the consequences may spread beyond a single product or company. This could lead to unwarranted limitations on the use of enzyme technology in other consumer applications. Therefore, it is recommended that companies using enzymes consider how they are managing enzyme safety, including the conduct of appropriate risk assessments and risk management programs. This will demonstrate that they are using enzymes responsibly.

The preferred approach as presented here is for product manufacturers to develop comprehensive programs to assess and manage the risks of using enzymes in consumer products. The program design should be developed on a case-by-case basis to address parameters specific to the type of product and its applications. Key elements of this program include hazard identification, exposure assessment and risk characterization. Good understanding of these areas will lead to informed decisions about the potential risks and the development of sound approaches to manage these risks.

The cleaning product manufacturing industry's recent experience shows how successful implementation of a product stewardship program, which includes appropriate risk assessment and risk management practices, helps to promote the continued safe use of enzymes. Such practices can minimize the risk of acquiring enzyme allergies by workers and consumers. This document outlines strategies and methods that have been used successfully by the industry.

## 1. What Are Enzymes and Why Do We Use Them?

Enzymes are proteins produced by all living organisms. They act as catalysts to increase the rate of chemical reactions. They are generally named after the reactions they catalyze. Amylases catalyze reactions with carbohydrates; cellulases react with cellulose; lipases with lipids (e.g. fats and oils); and proteases with proteins in general.

Enzymes used in cleaning products usually "break down" soils and stains on surfaces into their basic components to allow the detergent ingredients to remove them. For instance, protease in laundry detergents breaks peptide bonds in proteins that make up general food stains. The detergent ingredients in the product are then able to remove the breakdown products formed from the action of enzymes much more easily and at lower temperatures than if the enzymes were not in the product.

## 2. How Do Enzymes Work?

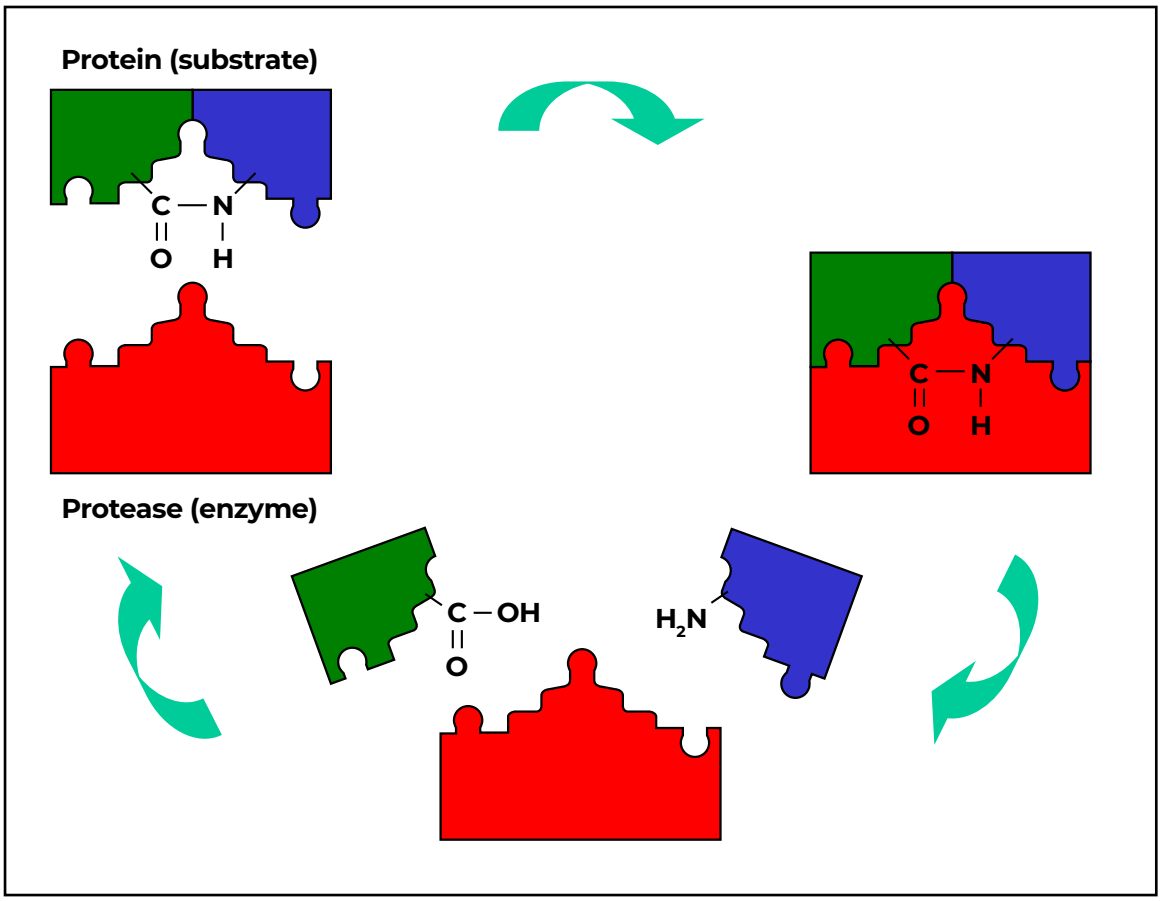
As discussed above, enzymes accelerate reactions that usually occur at a much slower rate or allow us to clean at lower temperatures. A remarkable property of enzymes is that they complete reactions without being destroyed, allowing a single enzyme molecule to catalyze many individual reactions. Therefore, they can be used at very low levels in cleaning products and still contribute to product performance. The action of a model enzyme that breaks down substrate (the name used for a substance upon which the enzyme is acting) is shown in Figure 1.

The first step in an enzymatic reaction is the binding of an enzyme to a substrate. The enzyme binds via an active site in its molecular structure that is specific to certain types of substrates. In this example, a protein binds to the reactive site of a protease. This portion of the enzyme is shaped specifically to allow entrance of only certain substrates with the corresponding shape, much like a key fits into a lock. If the substrate does not fit the shape of the active site, it cannot attach itself to the enzyme and no reaction occurs. In the figure below, the enzyme breaks down the substrate bound to the active site into smaller parts while the enzyme itself is unchanged. Once the enzyme breaks the substrate, the resulting products are released from the enzyme. Thus the enzyme reaction process can take place over and over as the enzyme repeatedly binds to more of the same substrate molecules and the process repeats. Some types of enzymes can bind two substrates at a time and catalyze a reaction to link the substrates together.

## 3. Historical Perspective of Enzyme Use in Laundry Products

In the 1960s little was known about the hazards of enzymes, and they were considered to be a natural and safe ingredient. The early enzymes used in detergent manufacturing plants were in a finely powdered form, which led to high airborne levels, believed to be > 1mg/m$^3$. Inhalation of this enzyme dust led to the production of allergen-specific immunoglobulin E (IgE) antibodies and induced respiratory allergies among workers (Flindt, 1969; Pepys et al., 1969). Furthermore, a few sporadic cases of allergies were observed in consumers and also in some women handling the clothing contaminated with enzyme dust that was brought home for laundering by industrial workers (Zetterstrom, 1974; Belin et al., 1970; and Bernstein, 1972).

**Figure 1 - How Enzymes Work: the enzyme binds to a substrate and cleaves bonds, breaking the substrate into small components**




Since that time, solid enzymes have been encapsulated within granules to reduce greatly their dustiness. With the introduction of encapsulated enzymes and the implementation of improved handling procedures and manufacturing controls, the incidence of respiratory allergy symptoms has disappeared in consumers and has been greatly diminished in the workplace (Basketter et al., 2015; Zachariae et al., 1981; Schweigert, 2000; Pepys et al., 1973; Pepys et al., 1985). These observations provide compelling evidence that enzymes are safely managed for use in consumer products. However, due to their performance enhancement of cleaning formulations, the use of enzymes in both consumer and industrial products is increasing in the numbers of enzymes included, the type of product, concentration and frequency of use.

Good stewardship of enzymes involves accurate hazard characterization of enzyme-containing products and thorough risk assessment for both existing and new uses to prevent the development of allergy in workers and consumers. To those ends the American Cleaning Institute (ACI) and the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) continue to develop and publish best practice control measures for handling and use of enzymes by the detergent industry as well as recommending routine airborne monitoring and ongoing specific

employee health surveillance protocols as part of a comprehensive risk management program. Enzyme suppliers continue to operate stewardship programs to ensure that enzymes are only used in appropriate product types and that users are aware of the industry best practices regardless of their affiliation with the detergent associations.

This document is part of that program, to help users and formulators of products to ensure the safety of the end users of consumer products containing enzymes.

1 European Detergents Industry Association

Risk assessment is the process of identifying the hazard profile of a given material and gauging the likelihood of adverse effects occurring during handling or use. Risk assessment is important for enzyme-containing consumer products since it helps ensure the continued safety of currently marketed products and is a basis for determining the safety of new applications under commercial development.

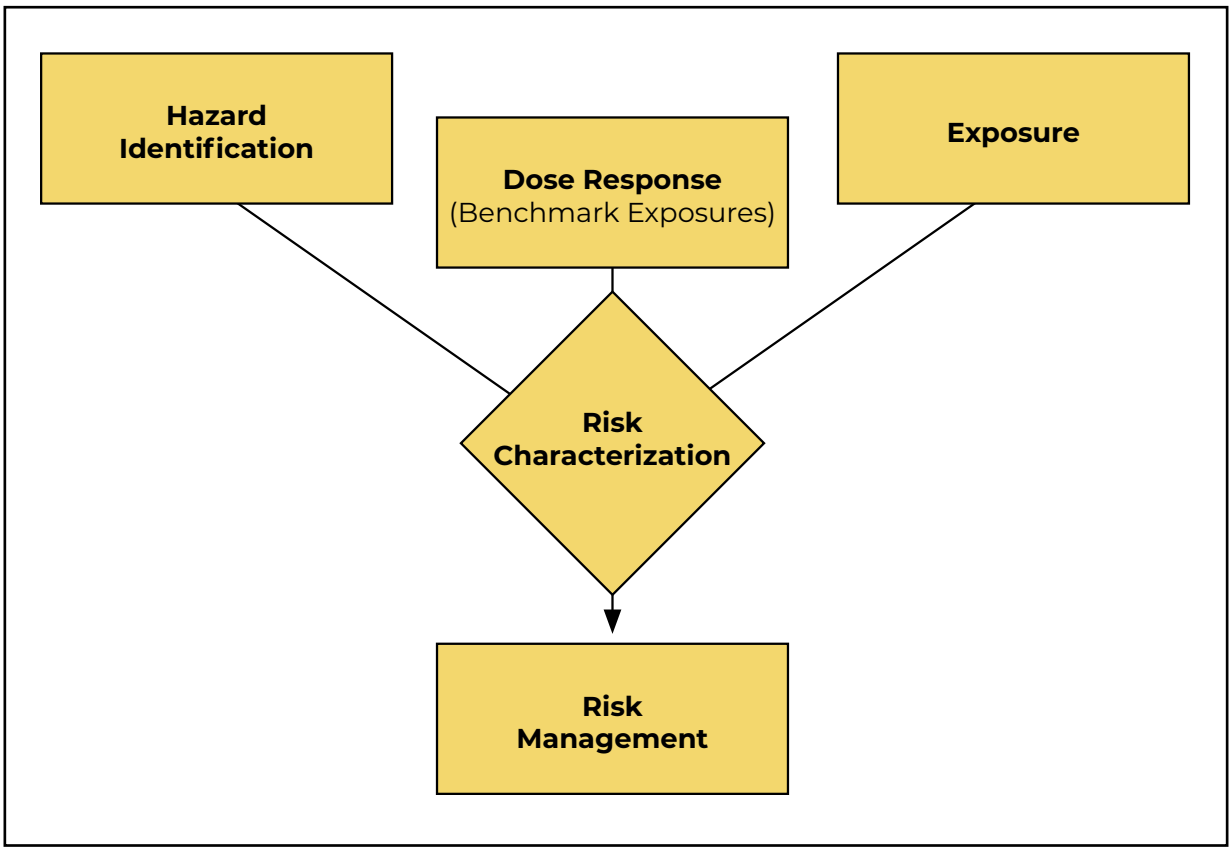
## 1. Steps of Risk Assessment

Risk assessment can be divided into four areas: hazard identification, dose-response assessment, exposure assessment and risk characterization (NAS NRC 1994).

The risk assessment process for enzymes follows this general approach, but benchmark doses, to define effect and no-effect thresholds, are used instead of classic dose-response curves. Figure 2 outlines the risk assessment process used for enzyme-containing products.

In hazard identification, the generation and collection of data on the inherent toxicity of a substance is needed to assess and define the hazard. Hazard information can be generated from clinical studies, animal tests, in vitro tests, structure-activity models, etc. In dose-response assessment, the

**Figure 2 - Risk Assessment Paradigm**



relationship between the magnitude of exposure (i.e., the dose) and the toxic response is investigated. In exposure assessment, routes and magnitude of the exposure under various product use and foreseeable misuse by the consumer are characterized. High levels of uncertainty in the assessment may require measuring exposure under simulated use conditions or during actual consumer use to learn about potential enzyme exposure in a new product. The data gathered in the first three areas (hazard identification, dose-response assessment, exposure assessment) can then be used in risk characterization to make a determination of the likelihood of humans experiencing adverse effects from using a product. This should also describe the uncertainties related to the risk estimates.

## 2. Risk Management

Management of risk to the consumer from enzyme-containing products is an essential part of good stewardship for any company. Risk management is the process whereby the results of the risk assessment are considered and a strategy is developed to manage, control or eliminate the exposures likely to cause health effects. This process could involve introducing appropriate risk-reduction procedures that control or eliminate sources of exposure. Although risk management is often thought of as a process that occurs after the risk assessment is completed, risk management decisions can be made throughout the course of acquiring the risk assessment data. Proper risk management should provide a product to the consumer that is safe for use under intended and reasonably foreseeable misuse conditions.

The following chapters describe the risk assessment steps in greater detail and their application to enzymes being considered as ingredients in finished consumer products.

Hazard identification is the characterization of the physical, chemical and biological effects of a material on humans, other biological systems and the environment. It is generally conducted without regard for dose-response, i.e. it is meant to define the probable consequences of exposure to a material and identify relevant target organs and systems.

The health hazards of commercially available enzymes used in detergent products have been well characterized through toxicological, epidemiological and case studies. The toxicology of these enzymes is generally unremarkable. Acute and sub-chronic toxicity is not of concern; they are effectively nontoxic. To some extent, acute toxicology data generated on one enzyme may be applied to the evaluation of other enzymes in the same class with similar activity.

Proteases, may produce skin and eye irritation. However the most significant hazard of enzymes identified to date is an adverse immune response known as respiratory Type 1 hypersensitivity. Type 1 hypersensitivity is a T helper cell-dependent, IgE-mediated immune response to common environmental proteins that results in tissue injury and/or disease. The terms antigen or allergen are sometimes used to describe these proteins. Type 1 hypersensitivity is also known as immediate type hypersensitivity due to symptoms occurring within 30 minutes of allergen exposure; symptoms can also occur up to several hours after exposure. The symptoms of this type of response are typically seen in the general population as allergic reactions to pets, dust mites, pollens, etc. Almost all enzymes used in consumer products are proteins which are foreign to the human immune system and can act as allergens through a Type 1 hypersensitivity mechanism following exposure, typically by inhalation.

Data on the health hazards associated with enzymes can be obtained from a variety of reports (e.g. toxicology, epidemiology and case reports) and a variety of sources, including enzyme suppliers, published reports (e.g. journal articles, textbooks) and unpublished studies. For enzymes used in industrial applications, the health hazards have been well characterized by a variety of authors (e.g. Griffith et al., 1969; How et al., 1989; Kondo et al., 1994; Briatico-Vangosa, 1994; Pariza and Johnson, 2001; Greenough et al., 1991 and 1996; Hjortkjaer et al., 1986 and 1993; Coenen et al., 1995; Basketter et al., 2012b and 2012c).

Potential routes of exposure via normal use, foreseeable misuse or accidental contact with end use products are through: 1) inhalation, 2) skin contact, 3) eye contact and 4) ingestion. The potential hazards from these exposures are discussed in the following subsections.

## 1. Effects from Inhalation Exposure

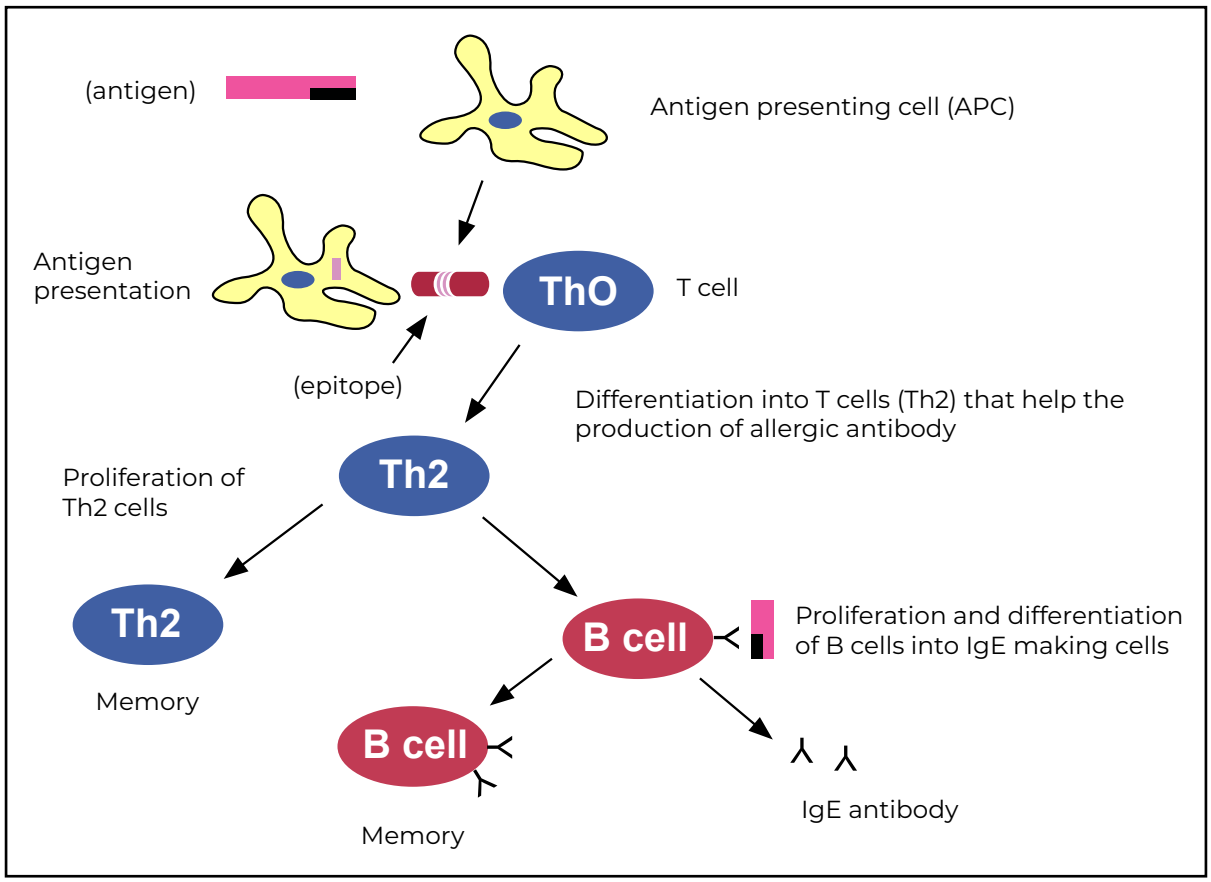
### A) Type 1 Hypersensitivity

Inhalation of enzymes and other high molecular weight proteins has been shown to cause the development of allergen-specific IgE antibodies and elicit symptoms of Type 1 hypersensitivity in certain individuals (Mak and Saunders, 2006). It is important to note that the ability of an enzyme to cause this sort of reaction is not dependent on enzymatic activity but particularly on its protein structure.

There are two main phases in the development of Type 1 hypersensitivity, as shown in Figures 3-A and -B. The first phase is called the induction or sensitisation phase and occurs during the initial exposures of an individual to an allergen, in this case an enzyme (LaDou, 1997; Timbrell, 1982; Glaister, 1986; Shearer, 1998). The induction phase is defined as the development of allergen-specific IgE antibodies that specifically bind to the allergen when it is present. Development of allergen-specific IgE antibodies may result from a single high exposure or from lower level repeated exposures, by inhalation (for respiratory Type 1 hypersensitivity), over a period of weeks, months or even years. Further, exposure levels which cause IgE development in one individual may not cause it in another due to the great variability in the immune response that exists within the human population. As with other sensitisers this makes establishing a dose-response relationship for 100% of the population practically impossible (Delves et al., 2017).

To generate allergen-specific IgE antibodies, airborne enzyme must either be inhaled into the lungs and/or enter the body through other mucous membranes, notably in the upper respiratory tract. The immune system recognizes the enzyme as a foreign protein or antigen and triggers a series of events that lead to the initial production of antigen-specific antibodies. Antigen-presenting cells (APCs), such as macrophages, in the airways engulf the protein and process it into smaller peptide

**Figure 3 – A: Induction of Allergic Antibody to Protein Antigen**

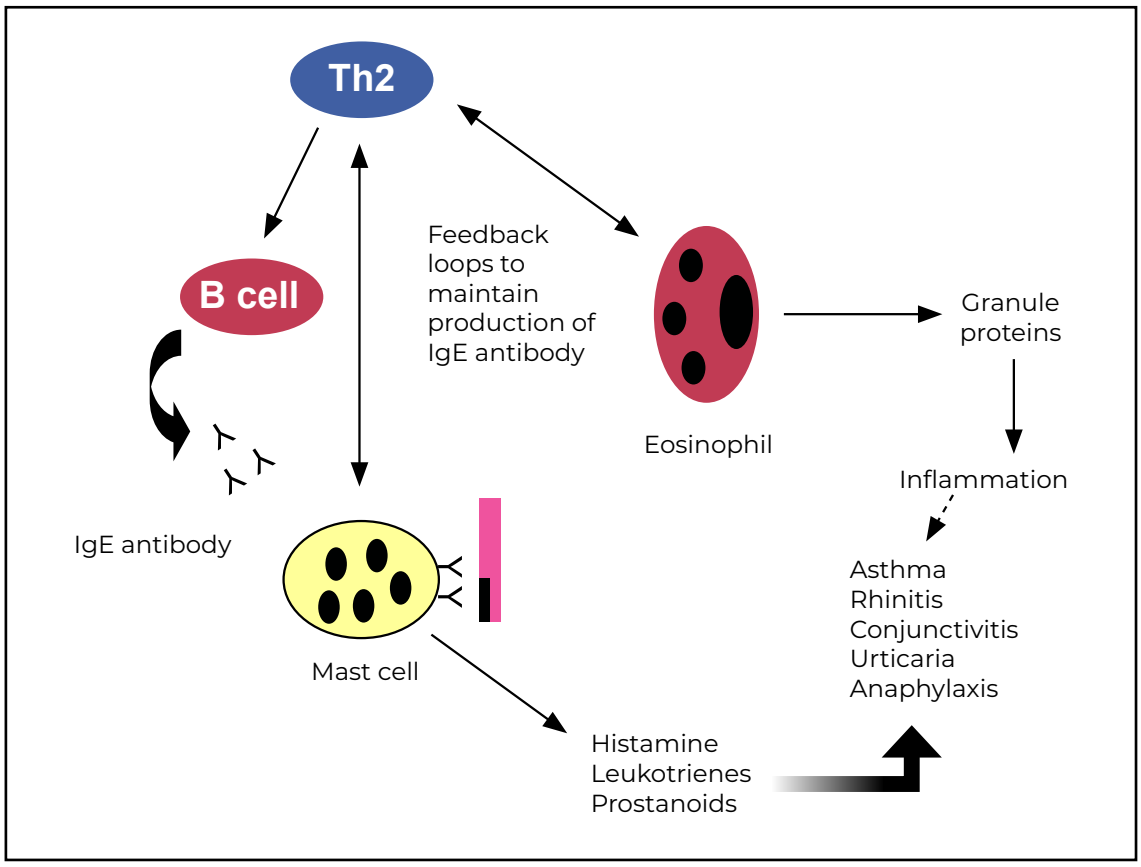


fragments that are associated with peptide-presenting molecules called major histocompatibility complex (MHC) class II molecules. The MHC class II/peptide complexes make their way to the surface of the APCs where the peptides are presented to naïve T cells or ThO cells resulting in activation and differentiation into Th2 cells. The activated Th2 cells are capable of stimulating antigen-specific B cells to differentiate into antibody producing plasma cells and secrete antigen-specific IgE antibodies. Peptides that are recognized by T cells and B cells are called T and B cell epitopes, respectively. IgE antibodies will bind to cell surface receptors of cells involved in the inflammatory process, such as mast cells found in tissue. At this point a person is considered to be sensitized; they have developed IgE antibodies to a specific enzyme. In addition, a subset of antigen-specific T cells and B cells remain in the secondary lymphoid tissues as memory cells. Re-exposure to the antigen leads to stimulation of memory cells with an accelerated immune response. During the induction phase (Fig. 3-A), the exposed person shows no clinical symptoms. Thus, the induction of allergen-specific IgE antibodies is not of itself a clinical outcome or disease, it has no symptoms, and it is only an indication of exposure, one which can be measured by immunological techniques.

Subsequent exposures to the enzyme by inhalation may lead to clinical symptoms in some sensitized people (Figure 3-B). However, experience has shown that not all sensitized people will develop symptoms of allergy when re-exposed to the enzyme. This symptomatic stage is called the elicitation phase, which is the disease state of Type 1 hypersensitivity. The IgE antibodies specific for the enzyme (produced during the induction phase) are localized in tissue on special cells called "mast cells" throughout the body, including in the lung. Mast cells in the tissues have granules that contain chemical compounds that mediate the inflammatory events that lead to the symptoms of allergy. Once the enzyme allergen binds to and crosslinks the specific IgE molecules on the surface of the mast cell, the cell is activated to release pro-inflammatory chemical mediators (including histamine) that cause the allergic symptoms. These mediators can have a direct effect on tissue as well as attract other inflammatory cells (e.g. eosinophils) to the area. Where a sensitized individual is exposed to a dose level above their elicitation threshold, symptoms of allergy range from rhinitis (watery eyes, a runny nose and a scratchy throat) to urticaria (hives), to asthma, or in extreme cases, to anaphylaxis (LaDou, 1997; Timbrell, 1982; Glaister, 1986; Freye, 1988; Flindt, 1978).

For a population of people exposed to allergens, certain predisposing factors, such as atopy, may make some people more susceptible to developing IgE antibodies (becoming sensitised) and possibly developing clinical symptoms. Smoking is another (important) risk factor for making people more susceptible to making IgE antibodies to a protein (Chan-Yeung, 1990; Nordman, 1984; Romano, 1995). However, it is very difficult to predict whether any particular individual will develop IgE antibodies or clinical symptoms. Indeed, individuals who are found to have allergen-specific antibodies using diagnostic methods, such as the skin prick test, may never develop allergic symptoms. This is true for enzymes as well as for all other allergens. It may simply be that for some individuals, they have a relatively high elicitation threshold, and if this is never exceeded, symptoms do not appear.

**Figure 3 – B: Elicitation of an Allergic Response upon Re-exposure to the Protein Antigen**




### B) Irritation

Inhalation of high concentrations of some enzymes (e.g. proteases) may cause irritation of the respiratory tract due to the proteolytic activity of the enzyme (Kilburn et al., 1971; Gibson et al., 1976; HERA, 2007).

## 2. Effects from Skin Exposure

### A) Irritation

Proteases can be irritating to the skin of animals and humans when applied at high concentrations, mainly due to the proteolytic action on the skin (Griffith et al., 1969; How et al., 1989). Other enzymes have been found generally to be nonirritating to the skin of humans or animals (Kondo et al., 1994; Greenough et al., 1991 and 1996; Hjortkjaer et al., 1986 and 1993; Coenen et al., 1995; Briatico-Vangosa, 1994; Basketter et al., 2012b). Irritation potential of proteases is important when considering the risk of enzyme products containing protease coming into contact with the skin. Furthermore, the encapsulated or formulated enzyme preparation may also contain other ingredients that may cause skin irritation. These should be included as part of the overall assessment of the product.

### B) Immunologic Contact Urticaria (A Form of Type 1 Hypersensitivity)

There are two types of urticaria, immunological and non-immunological. The majority of contact urticaria is not mediated by the immune system. In very rare cases, enzymes may elicit symptoms of Type 1 allergy on the skin, called immunologic contact urticaria. The major symptom is the classic "hives" reaction of redness, swelling and itching. This rare condition can be elicited in people who had already made allergen-specific antibodies to an enzyme, most likely in response to inhalation exposure. However, to reiterate, this is extremely rare for enzymes. The common causes of contact urticaria are reviewed in Basketter and Lahti (2011).

### C) Allergic Contact Dermatitis (Type 4 Hypersensitivity)

Allergic contact dermatitis is caused by specific T cell responses to a material that comes in contact with the skin. As with Type 1 hypersensitivity, induction of the sensitised state is required before the disease can be elicited. Typically, dermal symptoms start to show 24 to 48 hours after skin contact in a sensitized individual. The inability of enzymes to induce allergic contact dermatitis (ACD) has been confirmed in numerous studies in which volunteers were tested by a variety of standard tests used to assess skin sensitization (e.g. Human Maximization Test, Modified Draize Test, Human Repeat Insult Patch Test). Most of these tests were conducted with proteases, but other enzymes (including amylases, cellulases and lipases) have also been investigated. In addition, many similar tests conducted on detergents containing enzymes have shown that the presence of enzymes in detergents does not result in skin contact sensitization (A.I.S.E., 2018; Andersen et al., 1998; Bannan et al., 1992; Basketter et al., 2008; Griffith et al., 1969; Rodriguez et al., 1994).

## 3. Effects from Eye Exposure

### A) Irritation

Proteases have been shown to be irritating to the eye when applied in high concentrations for much the same reason that these enzymes are irritating to the skin. Other classes of enzymes are either less or not irritating to the eye compared to proteases (Griffith et al., 1969; Greenough et al., 1991 and 1996; Coenen et al., 1995; Briatico-Vangosa, 1994). Information on the eye irritation potential of ingredients used in enzyme preparations and product matrices should be evaluated.

### B) Type 1 Hypersensitivity

Although development of allergen-specific antibodies through contact with the eyes is believed to be rare, technically the potential still exists, as illustrated by reports of allergy to enzymes used in contact lens cleaning solutions (Bernstein, 1984; Fisher, 1985). Papain-specific IgE antibodies were found in people with symptoms of rhinoconjunctivitis.

## 4. Effects from Oral Exposure

The oral toxicity and allergic potential of the ingestion of various enzymes have been evaluated and reviewed (Pariza and Johnson, 2001; Basketter et al., 2012c). Enzymes, at the very low levels that have been traditionally found in food, are inherently nontoxic via the oral route and development of allergen-specific antibodies by ingestion of enzymes has not been documented (AMFEP, 1998). Guidelines and principles for assessing the safety of traditional food enzymes and food enzymes derived by modern biotechnology have been developed and should be referred to when evaluating an enzyme product where exposure via the oral route might be anticipated, e.g. use associated with food preparation areas or equipment (Bindslev-Jensen et al., 2006; Pariza and Johnson, 2001; SCF, 1992; OECD, 1993).

## Summary

The hazard of any substance is defined as the intrinsic potential of that substance to cause harm. The hazards of enzymes used in detergents and cleaning products have been well characterized over many years for inclusion in consumer products as well as for safe handling in a manufacturing setting. Hazard identification is important because it helps determine the endpoints to focus on during risk characterization. For all enzymes, the primary hazard is Type 1 hypersensitivity. For proteases, skin and eye irritation is also a hazard. As described in the next chapter, it is important to understand the dose at which the hazard is expressed.

There are many hazardous materials in all types of products that we use in the home, including personal products, but the hazard only becomes a concern if the exposure (or dose) achieved in use is significant enough to cause an acute or chronic effect under normal conditions of use, and foreseeable misuse. As we will see in the next chapter, the potential exposure a user might receive to any hazardous ingredient is based on the level of inclusion, the product format, the mode of use and frequency and duration of use. These potential exposures are then compared against established safe benchmarks to allow the risk of exposure to be characterized.

In this step of the risk assessment process, the relationship between the level of exposure and the specific biological effect is characterized. The dose-response assessment consists of determining the amount of exposure to relevant tissues (i.e. the delivered dose) and the corresponding biological effect. The delivered dose will be a function of the level, duration, pattern and route of exposure. This process is not trivial, since the dose-response relationship for enzymes is not clearly defined for Type I hypersensitivity. For enzymes, there remain limitations in our knowledge on safe levels of exposure and for example, the role of peak exposures in the development of enzyme-specific antibodies and elicitation of symptoms. Furthermore, for a variety of reasons, there is little prospect of these gaps being filled. Therefore, benchmark values rather than more traditional dose-response measures generally are used to support decisions in enzyme risk assessments. Such benchmark values are based on studies in which measured or estimated exposure levels are associated with a demonstrated effect or the lack of an effect in the people exposed.

One very important benchmark is the Derived Minimal Effect Level (DMEL), which has been established to comply with the European legislation for chemicals: REACH. (Basketter et al., 2010). A DMEL of 15 ng/m$^3$ has been adopted by industry (Basketter et al., 2010) and recognized by the EU for consumer exposure to enzymes as a starting point for risk assessment (Basketter et al., 2012b). However, as will be pointed out later in this chapter, there may be consumer uses that take place under special conditions, which will call for a specific assessment of which benchmark would be appropriate for the specific use.

Also, it should be noted that neither the DMEL nor any other benchmark will provide an assurance of absolute safety in all conceivable exposure situations. As described below it is difficult to link airborne levels of enzymes to actual individual exposure and induction of sensitization. Additionally, the complex picture of the role of duration, peaks vs. troughs, frequency, etc. associated with exposure and the presence or absence of individual's concomitant respiratory irritation or personal susceptibility factors is not fully understood (Basketter et al., 2012a). For Type I hypersensitivity caused by exposure to enzymes, also it appears that the elicitation of clinical symptoms occurs at similar or higher exposure levels than those that trigger the induction of sensitization (Basketter et al., 2012a). Thus, when exposure is sufficiently low to prevent sensitization, the likelihood that clinical symptoms will appear among sensitized but yet non-symptomatic individuals is very low.

Other end points, including eye and skin irritation and oral toxicity, do have dose-response relationships defined (HERA, 2007). However, in this chapter, only the dose-response relationship for Type I hypersensitivity is discussed.

As explained in Chapter 3, enzymes used in consumer applications are essentially nontoxic when ingested (Ladics and Sewalt, 2018).

## 1. Dose-Response Estimations and Benchmarks

Dose-response estimation for populations is inherently a statistical process. Ideally, a mathematical model is developed that relates patterns of exposure to the likelihood of biological or adverse effects. In the absence of that level of predictability, exposures are estimated or measured empirically to establish uses that avoid biological effects and adverse effects. These values are known as benchmarks. A benchmark is a value derived from a study or studies in which a specific biological effect (e.g. production, or absence of production, of allergen-specific antibodies) is associated with an exposure level. In the risk assessment process, the exposure level estimated for a use application is compared to benchmark values to judge risk.

For enzymes, there are very little data on dose-response, so a benchmark approach has to be used to assess risk. There are some data on dose-response to enzymes related to Type 1 hypersensitivity, but not enough to develop predictive models. In these models, values of estimated or measured exposures are compared to the highest exposure level previously shown not to induce the generation of allergen-specific antibodies (the "No Observed Effect Concentration," or NOEC), or to the lowest exposure level previously shown to induce the generation of allergen-specific antibodies (the "Lowest Observed Effect Concentration," or LOEC). The threshold for inducing the generation of enzyme-specific antibodies presumably lies between these two levels. Such comparisons require a consideration of the uncertainty in estimated exposures, as well as uncertainty in the NOEC or LOEC. The existence of a threshold for allergen-specific antibody production to enzymes must be considered a reasonable assumption, as similar thresholds are generally assumed for most biological effects, including allergy (Cohrssen and Covello, 1989; Basketter et al., 2002). From occupational data, a decrease in exposure to enzymes led to a sharp decline in the incidence of allergic symptoms among workers to the point where the symptoms were eliminated. In addition, the rate at which workers developed IgE antibodies to enzymes also declined with a decline in exposure (for a review, see Schweigert, 2000; Sarlo and Kirchner, 2002). These studies demonstrated a dose-response relationship for antibody production and elicitation of symptoms and support the existence of thresholds for both events. The occupational data also point to peak exposure levels as playing an important role in antibody production and symptoms. It is reasonable to assume that such thresholds and dose-response relationships exist for consumer exposures.

Another issue in building a dose-response model for enzyme allergy is the limitations in air monitoring technology. When air monitoring is used to determine delivered dose, the time of exposure is a direct controlling factor. Ideally, the entire exposure duration should be recorded. However, limitations in air monitoring technology preclude minute-to-minute monitoring of air concentrations of proteins. Instead, time-weighted averages along with activity patterns of exposed individuals are measured. Currently, there are no universally accepted models applicable to humans that permit the determination of the dose-response relationship of enzymes for causing production of allergen-specific antibodies or symptoms of allergy via the inhalation route (Basketter et al., 2012c). Although the generation of IgE antibodies to enzyme is often used as a component of the benchmark value, it is not of itself a disease state, but it is an essential precursor. Therefore, the generation of IgE antibodies is a conservative endpoint on which to establish a benchmark. In order to make a risk assessment of a new enzyme use, the exposure data

from exaggerated use condition of the new enzyme use should be compared to benchmarks obtained from occupational and consumer studies. Ideally, the benchmark used should have well-characterized exposure and clinical endpoints as well as a duration and frequency of exposure that are comparable to the new enzyme use, in order to be relevant to the new exposure value being evaluated. The majority of data on consumer experience with enzyme-containing consumer products are from the use of laundry detergents. These studies are discussed later in this chapter.

## 2. Clinical Studies for Obtaining Benchmark Data

The three main types of clinical studies used to obtain benchmark data are the prospective test, retrospective test and provocative tests. Study designs can be mixed to combine any or all of the test types. All clinical testing should be conducted in accordance with the principles of the Helsinki Agreement (World Medical Association, 2000). The parameter usually measured in clinical studies of Type 1 hypersensitivity is the development of allergen-specific IgE antibodies. Detection of specific IgE antibodies is an indication of exposure and sensitization, not disease. IgE antibodies can be detected by serological tests such as the radioallergosorbent test (RAST) or by the skin prick test (SPT). Methods for these particular tests are found in the general allergy literature (Bernstein et al., 2008). In addition, exposure assessment should be made under conditions of exaggerated use and misuse of the product. These exposures can be calculated or measured (see Chapter 5 on Exposure Assessment).

A prospective study is conducted with individuals who have never been previously exposed to the enzyme or who have never developed allergen-specific antibodies to the enzyme. The prospective test can be one of the best tests to conduct since it evaluates a selected population over time. Important considerations when designing such a study include its length; number of test subjects; type and frequency of measurements undertaken; and target population (e.g. inclusion of atopics). The size of the study is dependent on the level at which one needs to predict the risk of allergen-specific antibody production occurring in the population. For example, to predict the risk of development of enzyme-specific IgE antibodies occurring at 0.1% rate at the 95% confidence level, a study would need to include 3,000 test subjects (Hanley, 1983; Eypasch, 1995). It is appropriate to consult various experts, including clinicians, statisticians and epidemiologists before conducting clinical tests.

A retrospective study examines effects, or lack thereof, in a population exposed to enzymes. A retrospective study can be used to help position a new use versus an existing use of an enzyme. These studies are limited since they evaluate responses at a single point in time. In addition, retrospective tests tend to involve “healthy” self-selected individuals. Individuals who have selected themselves out of a population due to adverse effects from exposure will be difficult to recruit into retrospective studies. In any retrospective study there is the potential for biases that may arise if the test subjects are not representative of the general population.

A provocative test is conducted among individuals confirmed to have allergen-specific antibodies to the enzyme being considered for use in the product. For enzymes, this population is found mainly among occupationally exposed individuals. Provocative tests should only be done under very special circumstances (Zetterstrom, 1977). Clinical testing in a population with allergen-specific antibodies to the enzyme must be done with great care since these individuals are at high risk of developing symptoms. Positive responses in the clinical test can have an impact on the ability of an individual to work and use products. The study size becomes limited due to the small, finite pool of eligible individuals. Therefore, the statistical power of a provocative test is likely to be limited.

Although data from occupational exposure scenarios in facilities producing enzymes or enzyme-containing products may be useful, caution should be exercised when applying them to finished product safety evaluations because of differences in frequency, duration, route of exposure and possible variations in the exposure to specific enzymes in the workplace.

## 3. Case Studies

### A) Studies in Which Biological Effects Were Observed

**Type I Hypersensitization and Respiratory Allergy**

There are some documented cases of consumers who used dusty laundry products in Sweden in the late 1960s and early 1970s and became allergic to enzymes (Belin, 1970; Zetterstrom, 1974). An analysis of 1,645 individual serum samples showed that 15 individuals had enzyme-specific IgE antibodies (0.91%). These 15 were also skin prick test (SPT) positive to the enzyme. Exposure data have been generated retrospectively to simulate the exposure that occurred to these materials from filling a sink with water and adding laundry detergent for hand laundering. The results estimated average peak levels to be 212 ng/m$^3$ for this use (see Appendix 1, Estimation of Exposure to Enzymes from Early Detergent Formulations, for more details). This was estimated by reconstructing a similar type of product many years later and measuring exposure under simulated use conditions. This example demonstrates the effects resulting from high exposure over a short duration that occurred on a regular basis. Some of these 15 individuals reported symptoms of allergy when they used the dusty enzyme-containing laundry powder. A provocative test of some of these consumers showed that 8 out of 12 patients who had IgE antibodies to enzymes had symptoms after challenge with an enzyme-containing product (laundry powder mixed with enzyme). None of the 12 patients had symptoms from exposure to garments and bed linen laundered with an enzyme-containing granule laundry product (Zetterstrom, 1977). This is an example of a benchmark where the generation of enzyme-specific IgE antibodies and the elicitation of symptoms were associated with an exposure to enzyme-containing product.

In addition to historical laundry practices, another use of enzymes has resulted in the development of allergen-specific antibodies in a study population. Additional data on exposure levels that have produced these antibodies were obtained from a prospective study on a prototype personal cleansing beauty bar that contained a protease enzyme. This product was not commercialized. Average airborne exposure during the use of this prototype product during showering was measured and determined

to be approximately 5.7 to 11.8 ng/m$^3$ with a total range of 3 to 29 ng/m$^3$. These exposures occurred daily for several minutes within the confines of a shower or bath enclosure. These individuals not only had measurable inhalation exposure to enzyme, but also were intentionally applying the enzyme product to hydrated skin and mucosal surfaces. As revealed in pre-market clinical testing, this use situation led to 4 of 61 (6.5%) individuals developing enzyme-specific, allergen-specific antibodies between four and six months of product use as determined by skin prick testing at 50 µg/ml (Kelling et al., 1998). None of these four individuals developed symptoms of allergy during the course of the study. This is an example of antibody production associated with lower exposure levels on a daily basis but for longer duration and multiple routes of exposure (inhalation, hydrated skin, mucosal membranes). Therefore, the lowest benchmark concentration that is associated with a biological effect (IgE antibody) is in the area of 5.7 to 11.8 ng/m$^3$, noting the unique conditions involved with this non-commercialized product. This type of exposure may not have direct applicability to other uses of enzymes (e.g. uses other than personal cleansing) because of the pattern of exposure, but it does demonstrate that antibody production to enzymes can occur at low airborne levels.

### B) Studies in Which Biological or Adverse Health Effects Were Not Observed

**Type 1 Hypersensitivity**

There is a long history of safe use of enzymes in laundry products, even though such use can result in low level exposures during dryer venting, cleaning the dryer lint screen, machine filling and hand washing.

For indoor pouring of laundry product, exposures from 0.01 to 1 ng/m$^3$ are considered safe, see Table 1 below and in the paragraphs that follow, which links the exposure to the biological or clinical endpoint (Sarlo, 2010). From experience with powdered laundry detergents and current understanding of habits and practices, it is recognized that these types of exposures are safe, in the context of their use. These exposures are of very short duration (seconds), may not occur on a daily basis, and do not involve intimate contact of the enzyme-containing product with the body.

A retrospective evaluation of nearly 2,500 patients who attended an allergy clinic in the early 1970s showed that at least 80% had used coated enzyme laundry detergents for nearly 2 years and none had developed IgE antibodies to enzymes (Pepys, 1973). Continued skin testing of consumers of granulated and encapsulated laundry products over the years confirmed these original findings that exposure to enzymes via laundry use does not result in IgE antibody production (Pepys, 1985). In addition, baseline skin prick testing of (several tens of thousands of) prospective employees in the detergent industries has shown no reaction to detergent enzymes among this population. This observation supports Pepys's work that exposure to enzymes via laundry use will not lead to allergen-specific antibody production among consumers (Sarlo, 2010). Table 2 shows the effect of changes in formulation on clinical outcome among consumers using enzyme-containing products.

A retrospective study analyzed clinical data from a range of sources collected over a period of 40 years. In total, the sensitization towards common laundry enzyme from more than 15,000 individuals were included in the study. No individuals having allergy symptoms related to the enzyme exposure from laundry or cleaning products were found (Sarlo, 2010). This study also measured exposure data for some of the most abundant situations related to the use of enzyme-containing laundry or cleaning products are given:

**Table 1*: Exposure measurements upon use of enzyme-containing laundry detergents**

| Task | Magnitude (ng protein/m³) | Duration of Task | Frequency of Task |
|---|---|---|---|
| Pour liquid detergent into top-loader wash machine | 0.012 | <30 s | 4 - 7 x/week |
| Pour granular detergent into top-loader wash machine | 0.00022 | <30 s | 4 - 7 x/week |
| Addition of water to liquid or granule detergent in top-loader wash machine | 0.7 - 2.9 | <30 s | 4 - 7 x/week |
| Addition of detergent to front-loader wash machine | 0 | <30 s | 3 - 10 x/week |
| Detergent refill (pour granule from 6 kg sack) | 0.5 | <1 min | Once/month |
| Dryer vent (indoors) | <0.5 | <30 s to 1 hr [a] | <4 - 7 x/week |
| Clean dryer lint trap | 0.04 - 1.2 | <30 s | <4 -7 x/week |
| Spray pre-treat laundry items [b] | 14.5 | <1 min | 4 - 7 x/week |
| Hand wash dishes using liquid dish soap [c] | 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] Weeks et al., 2011.

[c] Measurement includes initial peak exposure as water and soap first mix followed by the exposure during the hand wash task.

* Adapted from Sarlo et al., 2010. Assessing the risk of Type 1 allergy to enzymes in laundry and cleaning products: Evidence from the clinical data, Toxicology 271: 87-93.

All exposures were associated with tasks of short duration (seconds to minutes) and occurred several times over a period of one week.

The highest exposure, which is seen for the spray pre-treatment of laundry items, is according to the clinical data from that study not associated with any sensitization response among the study population. This study is described in more details in Appendix 3 (Weeks, 2011). Similarly, no sensitizations were observed in a clinical study covering hand dishwashing exposure (Troyano et al., 2003).

A retrospective study of 655 atopic women in the Philippines showed no skin prick test positive responses to protease and amylase enzyme at a test reagent concentration of 50 µg/ml in those individuals who used enzyme-containing granule laundry detergents for hand laundering for at least one year. Another 1,300 women who had sporadic to no exposure to these enzymes via laundry product were also skin prick test

negative to these enzymes. Many of these women had compromised skin due to mechanical abrasion associated with hand laundry habits used in this region. The enzyme exposure from hand laundry with granule products ranged from 0.06 to 0.18 ng/m$^3$. These were daily exposures lasting minutes to hours per day (Sarlo et al., 1996).

A separate two-year prospective study among 581 atopic women in the Philippines showed no IgE antibody production to enzymes after use of enzyme-containing granule detergent for hand laundry supplemented with an enzyme-containing synthetic laundry bar (exposures from bar use for hand laundry ranged from 0.004 to 0.026 ng/m$^3$). These women also used the bar for personal cleansing with measured exposures less than 0.01 ng/m$^3$ (Cormier, 2004). The personal cleansing habit was daily use, lasting several minutes per day, and it involved intimate contact with the body. The bathing did not occur in a shower enclosure but rather in an open-air environment with washtubs, buckets, etc.

A retrospective study of 76 mechanics in Egypt who (mis-)used enzyme-containing laundry granules for personal cleansing showed that none of the 76 had allergen-specific antibodies to enzymes in the detergent product (Sarlo et al., 2010). The great majority of these individuals had performed this practice for more than one year. Washing was performed using a ladle and bucket, and the exposures were calculated to be less than 0.01 ng/m$^3$. Only seven of these individuals performed this habit for more than one year using shower conditions. The exposures were measured to be less than 0.5 ng/m$^3$ (Procter & Gamble, unpublished data). These were daily exposures, lasting several minutes per day, and they involved intimate body contact with enzyme. Consequently, the absence of induction of specific IgE antibodies nevertheless provides a useful negative benchmark.

## 4. Caution in the Use of Benchmarks

Caution should be used in the application of benchmarks. There is likely to be a complex relationship among frequency, magnitude and duration of exposure to the generation of enzyme-specific IgE antibodies. Exposure data needs to be relevant to a particular use or misuse for comparison of a new derived value to the existing benchmark value. Furthermore, the limitations in measurement at the point of exposure may or may not relate to the actual internal body dose. As discussed in previous chapters, the actual dose is nearly impossible to obtain with current methodology. Care should also be taken when extrapolating from one product type to another (e.g. rinse-off to leave-on conditioners; formulations' dustiness, delivery systems, adjuvancy) since the exposure conditions may be too different to be comparable. Finally, one very important parameter when assessing the potential enzyme exposure formation during use is the dosage of enzyme used. Often benchmark studies do not clearly reveal the enzyme protein dosage. Instead % w/w is often given without specifying how much enzyme protein is contained in this percentage. As the amount of enzyme used is directly related to the potential for enzyme exposure, benchmarks can be difficult to use directly without the exact information on enzyme dosage. Hence, it is essential when using benchmarks for making risk assessment of new enzyme uses that specific exposure data for the new use are generated and compared to the exposure data given by the benchmark.

**Table 2: Exposure and Clinical Outcome Among Consumers Using Enzyme Products: Decreasing Exposure Resulting from Transition to Granular Formulations**

| Product Type | Exposure | Duration | Frequency | Clinical Outcome |
|---|---|---|---|---|
| Dusty laundry detergent (1960s) | 212 ng/m$^3$ (measured) | Seconds - minute | 3-5/week | (+) SPT, RAST, Clinical symptoms (Belin, 1970; Zetterstrom,1974) |
| Laundry hand wash in Philippines (granular detergent) | 0.015-0.18 ng/m$^3$ (protease) 0.086-0.22 ng/m$^3$ (amylase) | Hours | 5-7/week; 28 min/task; twice daily | 0/1,980 subjects SPT (+), no development of IgE to enzyme (Cormier, 2004) |
| Prilled laundry product (1970s) | 1 ng/m$^3$ (calculated) | Seconds - minute | 3-5/week | (-) IgE, no symptoms (Pepys, 1973) |
| Granulated (encapsulated) laundry product (1980s-current) | 0.0057 ng/m$^3$ (calculated) | Seconds - minute | 3-5/week | (-) IgE, no symptoms (personal communication, P&G) |

Exposure assessment evaluates the amount of enzyme the user may be exposed to during intended use and foreseeable misuse. This value is then compared to the benchmark exposure to make risk decisions. Measuring or even estimating exposure to enzymes is a complex process. The determination of these consumer exposure values is essential for thorough risk assessment. In the absence of good quality exposure data, conservative worst-case assumptions and uncertainty factors are employed, which may lead to an overestimation of exposure levels and thereby unnecessarily limit the amount and type of enzyme that can be used in a consumer product. Therefore, it is important that the exposure assessment be conducted thoroughly to enable the optimum use of enzymes in consumer products.

This chapter describes methods and approaches used to estimate exposure to enzymes from the use of enzyme-containing products. Since the primary hazard of enzymes is Type 1 allergy from inhalation, this chapter will focus on those exposures that can lead to inhalation of airborne enzyme.

## 1. Factors Influencing Exposure

Many factors related to product use or applications are important determinants of overall exposure. Comprehensive answers to the following questions are needed to conduct optimal exposure assessment and risk assessment.

- What is the formulation and delivery mechanism of the product being assessed?
- How is the product going to be used under normal conditions and what may be the conditions of foreseeable misuse?
- Where will the product be used?
- What is the potential for user exposure to the product (direct or indirect)?

### A) What Is the Formulation and Delivery Mechanism of the Product Being Assessed?

**i) Product Formulation**

The physical and chemical properties of a formulation influence the exposure. The potential for aerosolization of liquids (sprays) and powders, leading to inhalation and contact with mucosal membranes, should be evaluated during product development. This can be affected by delivery mechanism and viscosity of the product. Aerosols should be characterized in terms of their droplet or particle size distribution. The size of droplets or particles, along with their density, determines their rate of settling and thus, the concentration of enzymes in the air during and after use. Large droplets or particles have the advantage of settling out of the air quickly. However, droplet and particle size can change during application. For example, liquid droplet size can decrease after impact on a surface during spray application. This has been demonstrated in spray application studies for fabric pre-treatment. This leads to a higher percentage of particles that are of inspirable size (Appendix 3; Weeks et al., 2011; A.I.S.E., 2013).

The potential for aerosolization can also be affected by enzyme form. Non-encapsulated enzyme powders are more easily aerosolized than encapsulated enzyme granules or enzymes in liquid and slurry formulations. In fact, enzymes used in granular detergents are encapsulated in order to greatly reduce the potential for dust generation. For these reasons, commercial enzyme granulate preparations do not result in any significant level of inhalable particles during normal handling (SDA, 1995; Sarlo et al., 2010; A.I.S.E., 2018 ).

Unit dose formulations are either encased in a water-soluble film or in a tablet that greatly minimizes exposure to the ingredients including enzymes.

Regional differences in product formulation may also influence the amount of enzyme released as dust and aerosols. For example, regions with higher concentration of enzymes in formulations may be a factor in exposure.

**ii) Delivery Systems**

When formulating a product, consideration should be given to how the design of a delivery system can affect user exposure. The product delivery system should minimize the amount of product that can be inhaled or exposed to mucous membranes. Packaging can have a significant impact on the extent and route of exposure to the product. Unit dose delivery systems provide an inherent reduction of exposure by design. A spray delivery system has the highest potential for inhalation exposure and should be designed carefully to minimize the production of inhalable mists. The delivery system should minimize available enzyme by limiting the production of particles small enough to be captured in the inhaled air stream. An additional consideration should be the effect of “bounce back” (i.e. the product bounces off a surface being sprayed), which may generate smaller droplets or particles than those produced by the sprayer originally. Therefore, assessment of exposure should not only include the product as it is delivered from the bottle, but should also include an evaluation of secondary exposures such as aerosols generated during bounce back.

### B) How Is the Product Going to Be Used Under Normal Conditions and What May Be the Conditions of Foreseeable Misuse?

For product use under normal conditions, the amount of product used per application, the duration of usage and the frequency of use are factors that affect the exposure to the product. Knowledge of the habits and practices of product users is important for a thorough understanding of enzyme exposure during a product’s use. These data can be obtained by conducting market surveys and consumer tests (discussed below) to determine how the product will be used.

In addition, there are special cases that should be considered in exposure assessment. For example, product exposure should be assessed for misuses. Misuses may result in higher exposures than can be anticipated during recommended product use. To illustrate this principle, consider a case of laundry detergent misuse. Non-recommended uses of laundry detergents can lead to worst-case exposures to the product by inhalation, skin, eye, mucosal and oral exposure through bathing, hair washing, pet washing, car washing, hard surface cleaning or using the product in a pump spray

bottle. Non-recommended uses of a product may be more common in some parts of the world than others and in certain socioeconomic segments of the population. For example, people in many developing regions use laundry detergent or water from washing clothes for bathing since it is not common or economically feasible to buy different detergents for different tasks (Cormier et al, 2004; A.I.S.E., 2017). Such factors should be included in exposure assessments for enzyme-containing laundry detergents. These differences should be considered before extrapolating the results of any exposure assessment from one geography to another. These differences should be investigated carefully to ensure proper characterization of exposures in all parts of the world where the product will be marketed.

### C) Where Will the Product Be Used?

The physical environment in which the product is used also influences the extent of exposure. Several factors should be considered with respect to the physical environment. For example, factors such as room size and ventilation will affect exposure. Use of a product outdoors, where there are air currents, can lead to a different exposure in the breathing zone of the user as compared to the use of a product in a small room with poor ventilation. The orientation of the consumer relative to the product during use, i.e. breathing zone relative to the source of enzyme aerosols, will influence exposure.

### D) What Is the Potential for User Exposure to the Product?

The possible exposure routes for the product under evaluation should be considered during evaluation of exposure. The most common routes of exposure are listed below. Again, consideration of potential misuse of the product should be assessed for these endpoints.

#### i) Inhalation

The major route of exposure to be considered is inhalation of dusts or aerosolized products. This may arise from intentional pouring of powdered or liquid products; stirring or agitating product solutions (e.g. hand laundering); spray applications; or blowing or vacuuming powder products or liquid products that have dried (e.g. carpet cleaning).

#### ii) Mucous Membranes

Exposure to enzymes may occur following application of product to the eyelid, lips, mouth and genitalia (e.g. from an enzyme-containing personal cleansing product such as a shampoo, skin cream or soap).

#### iii) Eyes

Exposure to enzymes in the eyes is possible from the use of contact lens cleansing solutions, or by incidental exposure (e.g. splashing, hand to eye).

#### iv) Skin

Skin exposure may occur during product use (e.g. during pre-treatment, hand laundering and hand dishwashing) or from incidental exposure during pouring of machine laundry detergents.

## 2. Assessments of Consumer Exposure

There are several approaches that can be taken to collect the information on product use (How et al., 1978 and 1989). Some approaches are described below:

### A) Consumer Tests

The habits and practices of the product user can be evaluated by conducting tests in a setting where the product will be used under circumstances of normal use. It may be useful to obtain product-specific exposure measurements during user tests and laboratory studies simulating in-use exposures. In addition, indirect exposures (e.g. deposition on fabric, glassware, utensils, solid surfaces) should be assessed, when appropriate.

### B) Market Surveys and Questionnaires

Market surveys and questionnaires are used to evaluate parameters of product use. Generally, they are used to assess product efficacy and safe use of the product. In the event a consumer questionnaire or survey is present, the consumer can be asked questions on the use of the product (e.g. How much product was used for the task? How long did the task take? Do they follow the use instructions and precautionary labeling?). Feedback from consumers can be used to guide further product development as well as validate and refine existing safety risk assessments. In addition, market surveys help identify potential non-recommended uses of the product. These non-recommended uses may increase exposure to levels that were not considered in the initial exposure assessment for foreseeable misuse, thereby warranting a reassessment of exposure.

### C) Poison Control Centers

Poison control centers can provide valuable information regarding misuse or accidental exposures to products. In the United States, summarized poison control center data are published in the "Toxic Exposure Surveillance System." These data can be obtained by contacting the American Association of Poison Control Centers via email at aapcc@poison.org, or through their website: aapcc.org.

### D) Government Agencies

Exposure information for products can be obtained from the government. For example, the U.S. Environmental Protection Agency sponsors telephone and diary surveys to determine the extent of consumer exposure to various household products (EPA, 1989).

### E) Manufacturer's Telephone Help-Line

Consumer comments received via telephone help-lines operated by product manufacturers provide additional useful information concerning use and misuse of products by consumers. This information is typically not found in the public domain.

### F) Publications

Information on habits and practices of consumers are described in various publications (EPA, 1989; Weegels, 1997; ECETOC, 2001; A.I.S.E., 2017; HERA, 2007).

## 3. Estimating Exposure

Exposure can be estimated initially from available data. The assumptions used in the estimation should be based on consumer habits and practices and the other factors referred to in the preceding paragraphs.

The first step is usually a conservative theoretical calculation using reasonable worst-case assumptions (e.g. using all of the product at one time) and uncertainty factors. If there are insufficient data to allow a reliable estimate of exposure to be developed, then actual exposure measurements should be obtained before making a final risk estimation. An example is provided in Appendix 1 of a study to evaluate exposure from filling a washing machine with an early detergent formulation. For granular laundry detergents used in machines, there is limited potential from inhaling materials that become airborne during routine laundry tasks, as well as some potential for enzyme deposition on skin during hand-wash tasks.

## 4. Measurements of Exposure

In cases where estimated exposures exceed safe benchmark levels or are unable to be calculated, it is necessary to refine the exposure estimate by conducting actual measurements under simulated use. Measurements provide a more accurate assessment of the exposure and thus produce a more reliable basis for estimating the risk of using that product. Table 1 lists exposure measurements from a variety of consumer activities associated with cleaning products (Sarlo et al., 2010).

The measurement process can be divided into simulation of the exposure, sample collection, measurement of enzyme concentration in the samples, background assessment and carryover prevention.

### A) Simulation of the Exposure

The exposure simulation procedure used should be developed based on habits and practices data, including visual observation of product use habits. This is important for the development of a procedure that provides an accurate representation of the consumer habits and, thus, is representative of consumer exposure.

### B) Sample Collection

Sample collection can vary depending on the type of exposure being assessed. Kelling et al. (1998) have described air-sampling equipment and set-up approaches that can be used for air collections (A.I.S.E., 2018; Appendix 2). It should be noted that the measurement procedure developed may need to be specific for the product, the enzyme type and the enzyme level used. Validation of the procedure and equipment should be conducted prior to making the exposure assessment. Such validation is necessary to ensure that new data can be compared to values obtained previously.

### C) Measurement of Enzyme Concentration in Samples

There are two main approaches that can be utilized for measurement of enzyme concentrations in air samples. The historical approach has been through the use of activity measurement to detect the enzyme and then converting the value to enzyme protein based on specific activity (Bruce et al., 1978; Rothgeb et al., 1988). This is an indirect measurement of the enzyme protein as it is based on the assumption or knowledge that the activity-to-protein ratio of the standards used in calibration is maintained during the manufacturing or consumer use process. A more direct measurement of the enzyme in the product is through the use of immunological methods, such as the enzyme-linked immunosorbent assay (ELISA). The ELISA method directly measures the enzyme protein concentration and is capable of doing so in the presence of other proteins, even with the same catalytic activity as long as they are immunochemically distinct. The accuracy of the data is dependent on the availability of the appropriate standards. For instance, the antibody used in the assay should have been produced in response to an enzyme that is immunologically equivalent to the one being tested. Examples of these types of assays, along with set-up and experimental procedures applied specifically to the detection of enzymes in detergents, are given by Kelling et al. (1998) and Miller et al. (1994).

Characterization of the standard used in the ELISA (or any immunoassay) is important. The level of enzyme assigned to each should not be based on the level of active enzyme but rather the total enzyme protein (A.I.S.E., 2018). This would include both active and some inactive forms of the enzyme. For that reason, polyclonal antibodies developed against the ingredient enzyme should be used. Antibodies to all forms of the enzyme will be produced in this manner and will contribute to detection of the total enzyme protein present in the product. Information such as the level of active enzyme, the total protein and the trichloroacetic acid (TCA) precipitable protein (Bradstrut, 1965; Lowry, 1951) should be considered collectively before making the assignment. Details of analytical methodology are described elsewhere (A.I.S.E., 2018). The suppliers of enzyme ingredients are a good source for information on protein contents and the level of active enzyme in their preparations.

The detection limit for the analytical method should be chosen based on the needs of the exposure assessment. If this limit is too high and does not meet the risk assessment need, either modification of the method to increase sensitivity or spiking with an increased level of enzyme without changing the product's physical characteristics should be done. The latter option is a reasonable approach, as demonstrated in the example below.

### D) Background Assessment and Carryover Prevention

Before conducting any exposure measurements, assessment of the test area for the presence of contaminating enzymes should be performed. Moreover, the ability to remove any enzyme left from one exposure simulation trial before conducting the next trial should be demonstrated. This can often be done by conducting a short number of trials at exaggerated levels of enzyme exposure and looking at the impact of cleanup procedures on trials that follow without enzyme present. Good cleanup should show no enzyme to be present in the no-enzyme-containing trials that follow.

## 5. Examples of Airborne Exposure Measurement

Data from exposure measurements are discussed in the Sarlo et al. (2010). A table summarizing exposure measurement from this journal article is listed above in Chapter 4.

Studies have been conducted to measure airborne enzyme concentrations that may be present during use of enzyme-containing laundry granule and bar products under Philippine hand-laundering conditions. Full details of the studies are included in Appendix 2, Enzyme Risk Assessments of Hand-Laundering Practices. The procedures used in simulating product use habits, air sampling and measurements are good examples of how exposure can be determined for a product under consumer use conditions. Typical Philippine hand-laundering practices include use of both a granular detergent and a laundry bar, either separately or in combination. In the study, either the powder and bar products were used at the same time or the granular product was used alone. For each laundering trial, three air samples were collected. The first was collected during fabric washing with granular detergent only. The second sample collection followed the first as fabric washing changed to a laundry bar but still using the previous granular wash solution. The third collection was taken after fabric washing and during the fabric rinsing task. Two different enzymes were used in this study to allow evaluation of granular and laundry bar contributions to aerosolized enzyme. It was necessary to spike the powder and bars with enzyme in order to reach detectable aerosolized enzyme concentrations (detection limit of 0.1 ng/m$^3$).

The granular product was spiked to give a level of 2,933 µg enzyme/gram granular product (which was 36-fold greater than the level in the product intended to be marketed). The measured airborne enzyme levels were 1.67 to 6.54 ng/m$^3$ for granular-only hand wash; 1.81 to 3.05 ng/m$^3$ in the combined granular-plus-bar wash; and 0.18 to 2.34 ng/m$^3$ in the rinse portion of the hand-laundering trial. Adjusting these levels downward by a factor of 36 to predict exposure from non-spiked product yields a maximum airborne enzyme level of 0.18 ng/m$^3$ in the case of the granular-only hand wash.

The laundry bar was spiked at 100-fold enzyme level (to give a concentration of 2,248 µg enzyme/gram laundry bar) compared to current marketed product. The derived airborne enzyme levels ranged from 0.41 to 2.62 ng/m$^3$ in the combined granular-plus-bar wash and from <0.1 to 2.29 ng/m$^3$ in the rinse portion of the hand-laundering trial. Adjusting these levels downward by a factor of 100 to predict consumer exposure from non-spiked product yields airborne enzyme levels of 0.004 to

0.026 ng/m$^3$ for granular-plus-bar wash. These levels can be compared to benchmark values as part of the risk characterization process (see Chapter 6).

Other examples of exposure assessment using application include a protocol for assessing enzyme exposure via trigger spray laundry pre-treatment prototype products described in Appendix 3, Spray Pre-treater Case Study (Batelle, 2000) and a prospective investigation in humans (Weeks et al., 2011).

The results of the exposure assessment along with the benchmark data are utilized in risk characterization discussed in Chapter 6.

Risk characterization is the examination of the relationship between human exposure (calculated or measured) and the inherent toxicity of a substance to assess the likely incidence and severity of any effect. This step is important because it integrates information regarding the hazard identification and exposure assessment associated with use and foreseeable misuse of a product. In traditional risk assessment, dose-response analysis is typically used to estimate risk to humans from a material based on its hazard profile and the exposure level at which effects are expected. However, adequate dose-response relationships are not available for Type 1 hypersensitivity to enzymes. Instead, the risk characterization process for enzymes relies on comparing potential exposure to benchmark values causing irritation or development of allergen-specific antibodies.

Although the information presented in this section is generally representative of current risk assessment practices, it should be recognized that this is an ever-evolving discipline. In the future, the methods and procedures used by practitioners should be modified, as necessary, to reflect the most current (and best) scientific practices.

## 1. Risk Characterization Process

The components necessary for risk characterization of enzymes include hazard identification, a dose-response relationship, an estimate of potential exposure, comparison of exposure to benchmarks and an application of general knowledge regarding IgE antibody production and allergy. Chapter 3 describes the hazards associated with enzymes, with respiratory Type 1 hypersensitivity being the predominant hazard for most classes of enzymes and irritation also requiring consideration for proteases. Chapter 4 describes the benchmarks that exist for enzymes.

The estimate of potential consumer exposure requires information on the pattern (frequency and duration), magnitude and route of exposure; product handling; use habits and practices; and demographics of the use conditions. Where possible, when considering the application of an enzyme that is used under conditions different from those used to develop a benchmark, other sources of exposure should be taken into account. Also, the potential for exposure from accidents and foreseeable misuses in addition to recommended uses should be considered in the risk characterization process.

If the value generated for the new exposure is at or below an applicable no-effect benchmark, then the risk may be judged acceptable.

If the exposure value for the new use is above the acceptable benchmark range but below the applicable effect benchmark, then the evaluator should make a decision as to the use of the product. A better understanding of potential exposure may be needed to refine the comparison to the benchmarks. Alternately, the product may have to be reformulated to reduce the exposure. It is also possible that the product will need to be evaluated in an appropriate test to establish a new benchmark. These decisions then become part of the risk management process.

General information on the relationship among exposure, development of IgE antibodies and development of allergic symptoms can be applied to the risk characterization process. For example,

it is well recognized from the occupational literature from enzyme processing or product formulation facilities that the intensity of exposure (e.g. magnitude, duration, frequency) is associated with the development of occupational allergy and asthma (Cullinen, 1994a; Cullinen, 1994b). As the intensity of exposure drops, the likelihood of having symptoms also drops. In the detergent enzyme industry, more intense exposures have been associated with symptoms, while less intense exposures have been associated with production of allergen-specific antibodies (Sarlo and Kirchner, 2002). The occupational data indicate that there are thresholds for the induction of antibodies and for the elicitation of symptoms.

## 2. Generation of Additional Data

Since risk characterization involves evaluation of substantial amounts of information from a variety of sources, there may be some uncertainty in the final assessment. Sometimes, the overall confidence in the database is low and consideration has to be given to obtaining additional data on likely human exposure or toxicity. Judgment is required to decide whether or not refinement of the risk characterization is warranted and, if so, whether or not development of additional data is practical.

It is recommended that care be taken when considering new applications for an existing enzyme that may already have proved acceptable for another use. Data may need to be generated to support the safety of enzyme use in these new applications. For some new applications, a benchmark may not exist. For example, the introduction of a subtilisin enzyme with a history of safe use in laundry applications into prototype beauty bar soap led to production of allergen-specific antibodies among test subjects in a pilot clinical study resulting in non-commercialization of the product (Kelling, 1998).

If clinical data need to be generated, one should weigh the ethical issues of intentional exposure of human test subjects with the risk of inducing allergen-specific antibodies and possibly eliciting symptoms in this population. The design of these studies must be developed from a thorough understanding of product use habits and in accordance with the Helsinki Agreement (The World Medical Association, 2000). A description of the study types and considerations is given in Chapter 4.

## 3. Examples of the Risk Characterization Process

The following are four examples of how hazard information, exposure data and available benchmark data have been used to assess the safety of enzyme-containing consumer products.

### A) Addition of a New Protease Enzyme to Granular Detergent Used in Machine and Hand Laundering

As shown in Appendix 1, replacement of one enzyme for another on a one-to-one basis (based on total enzyme protein level) results in the same measured air exposure level.

### B) The Introduction of Protease Enzyme into a Bathing Bar for Personal Cleansing

A protease enzyme with a long history of safe use in laundry applications was introduced into a prototype bathing bar for use in personal cleansing. Enzyme exposure data from use of this bar in a shower was 5.7 to 11.8 ng/m$^3$ with a total range of exposure of 3 to 29 ng/m$^3$ (Kelling et al., 1998). These

values were higher than those shown to be safe for machine use of enzyme-containing detergents (1 ng/m$^3$). However, the duration of exposure in the shower was much shorter (minutes) than typical occupational exposure (~8 hours). Also, the routes of exposure (inhalation, mucosal contact, hydrated skin) were different from exposure to laundry product used in machines. Since the duration of exposure to the enzyme in the shower was much shorter than the exposures typically encountered in the detergent manufacturing sites, it was thought that the exposure would carry a very low risk for induction of IgE antibodies. As part of the safety program designed to support the market introduction of the bath bar, a pilot clinical study was initiated to confirm that users would not be sensitized when the product was introduced to the marketplace. Surprisingly, the investigators found that 6.5% (4/61) of study participants developed allergen-specific antibodies to enzymes between 4 to 6 months of use of the bath bar in the shower. These data showed that low-level exposure of short duration but of high frequency (daily) and with multiple routes of exposure (inhalation, mucosal tissue, hydrated skin) could lead to the development of allergen-specific antibodies in a significant portion of a population on a rapid timeline. Therefore, this study generated a biological effect benchmark for the risk characterization process for this type of exposure.

### C) The Introduction of Enzyme-Containing Granular Detergents and Enzyme-Containing Laundry Bars for Hand Laundering

The enzymes used in products marketed in regions where laundering is done predominately by hand are the same enzymes used in regions where laundry is done predominately by machine. Also, the product matrix is not very different in hand vs. machine laundering geographies. Therefore, the benchmark exposures generated in either type of usage are interchangeable. Surfactants in the product matrix are known to have adjuvant effects (Robinson et al. 1996; Sarlo et al., 1997)

Extensive habits and practices data in regions where laundering by hand predominates revealed that 1) Philippine consumers had extreme hand laundry exposures since mechanical friction during this task led to compromised hand skin, and 2) these consumers also used laundry bars for personal cleansing. Exposure data for the hand-laundering task were generated and compared to exposure data associated with machine laundering. Exposure data for the personal cleansing habit were also generated. Benchmark data for exposure to compromised skin as well as for personal cleansing did not exist. Therefore, additional clinical data were generated.

Exposure to enzymes during hand laundering with granular detergent was 0.05 to 0.18 ng/m$^3$ and with a hand-laundry bar product was 0.004 to 0.026 ng/m$^3$ (total protein). Both of these ranges of values are significantly lower than exposures measured or estimated for laundry products with a history of safe use, as described in Table 2. Skin prick testing of consumers with compromised hand skin who used these products for one to three years showed no allergen-specific antibodies to enzymes used in either granular detergent or laundry bars (Sarlo et al., 1996; Cormier et al., 2004). Therefore, these data can be used as a low-risk benchmark for hand laundering when using similar dosing and laundering practices, etc. Exposure to enzymes during personal cleansing with the enzyme-containing laundry bar was extrapolated to be 0.007 ng/m$^3$, about 1,000 times lower than exposures to enzymes in the bath bar/shower application that generated IgE antibodies in test subjects. Since

the exposure levels were low, the development of allergen-specific antibodies was not expected to occur. However, as personal cleansing is a common use of laundry bars in some countries, a clinical study was conducted. The study showed no allergen-specific antibodies among nearly 500 atopic Philippine women who used enzyme-containing laundry bars for personal cleansing for one to two years (Cormier et al., 2004). These data therefore could be used as a low risk benchmark for inhalation exposure in a personal cleansing scenario.

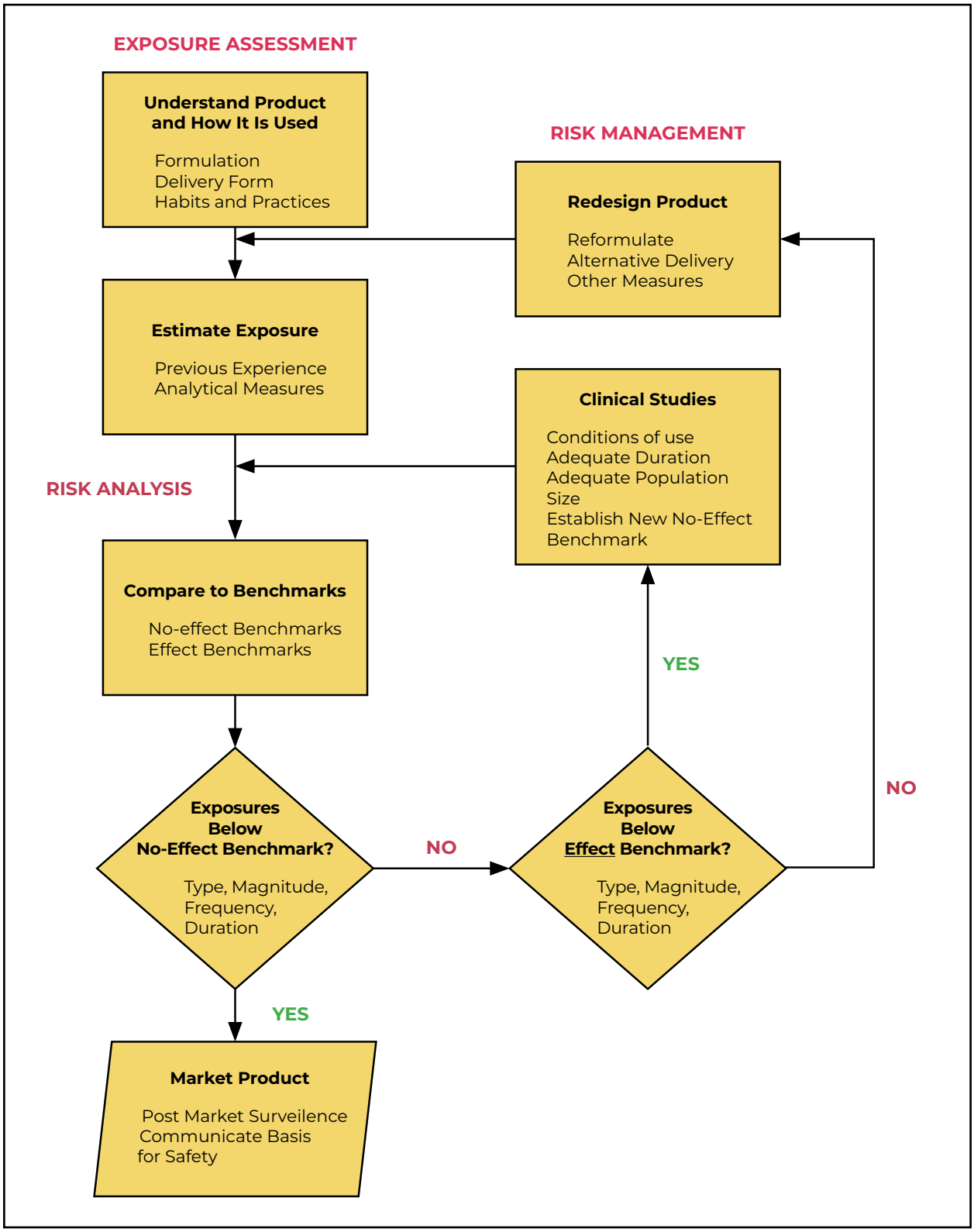
### D) Use of Protease in a Skin Cream/Body Lotion Application

Subtilisin proteases were considered for use in skin lotion/body lotion products (Blaikie et al., 1999; Johnston et al., 1999). Again, these were proteases with a long history of safe use in laundry applications. The main concern with the use of enzymes in skin care applications was the potential for inhalation of the enzyme which could occur through aerosolization of residual enzyme on skin during showering or skin flaking into bedding, clothing, etc.

A very small pilot study of 12 weeks duration was conducted using only five individuals per test group who applied the skin lotion on arms or arms and legs before retiring to bed (Blaikie et al., 1999; Pocalyko et al., 2002). Enzyme exposure was measured from airborne skin squames aerosolized into the air during change of bed linen. Exposures were found to range from non-detectable, < 3 ng/m$^3$, to 29 ng/m$^3$. The exposures occurred about once per week for a few minutes duration. None of the participants showed evidence of allergen-specific antibody. In a separate study, the amount of enzyme aerosolized during showering after application of an enzyme-containing skin lotion was measured (Johnston et al., 1999). These exposures were within one order of magnitude of the range of exposures associated with the development of allergen-specific antibody in test subjects due to use of the enzyme-containing bath bar in a shower (5 to 11 ng/m$^3$). They were also two orders of magnitude higher than exposures associated with safe exposure to personal cleansing with an enzyme-containing laundry bar (Example C, Philippines exposure). Lowering the enzyme level in the lotion helped to reduce the exposure. Prospective clinical testing of this enzyme-containing lotion used about five days per month for 18 months led to the development of enzyme-specific allergic antibodies in a small number of test subjects (Sarlo, et al., 2004).

The results of these two exposure studies showed that skin can be a reservoir of enzymes available for aerosolization. The Blaikie study generated exposure levels near occupational exposure levels but of shorter duration and frequency. The Johnston study generated exposure data that exceeded those not associated with sensitisation through use of a laundry bar for personal cleansing. Both studies showed that there could be daily exposure via showering along with occasional high exposures during changing of bedding. Changes made to the enzyme level in the lotion and use of the lotion did not abrogate the risk of induction of enzyme-specific allergic antibodies as assessed by clinical testing. The above examples illustrate the care and thought necessary before embarking on a new application for enzyme-containing products.

**Figure 4: Risk Characterization Assessment**



## 4. Outcome of Risk Characterization

Prior to commercializing a product, it is often helpful to present the data gathered from the risk evaluation stage to peers internally, if sufficient expertise exists in-house, or to experts external to the company. Conducting a peer review of the decisions made in risk evaluation can provide different perspectives regarding the assumptions, methodology and subjective interpretation inherent in the process. Once the questions identified in risk evaluation have been answered and exposures have been compared with the level of acceptable risk, the company may conclude whether the product is suitable for consumer use.

If the risks associated with product use are acceptable, then surveillance of the marketplace experience can be used to assure that exposure to the enzyme is indeed safe. If the risks associated with the product uses are unacceptable, product modification and re-evaluation of the risk characterization using information based on the modified product is recommended. The overall process is depicted graphically in Figure 4.

The objectives of the risk management process are to determine the significance of risks to human health, to ensure that the product use is and remains within the acceptable risk levels and to effectively communicate risks, or lack thereof, to appropriate audiences.

## 1. Determining the Relevance of a Risk Assessment

Risk assessment provides useful information to weigh alternatives and analyze tradeoffs in addition to providing a means of organizing relevant information in order to estimate the potential impact on human health. In doing so, assessments may convey a level of precision that fails to reflect the shortfalls of the underlying assumptions and the uncertainties that may characterize the risk assessment. The quality and reliability of the risk assessment is dependent on, and is only as good as, the data used to conduct the assessment. Uncertainties may exist in dose-response relationships, defined benchmarks, exposure data and estimates from exposure models. Assumptions and estimations need to be stated clearly as they can affect the reliability and quality of the risk assessment. It is important to consider these points when evaluating information from the risk assessment in determining whether or not the risk is considered acceptable.

## 2. Acceptable Level of Risk

The risk assessment process does not define an acceptable level of risk. No numerical level of risk will receive universal acceptance. Further, it is impossible to eliminate all risks associated with a particular activity, and this is also true for the use of enzymes in finished products.

Risk management approaches should be based on critical evaluation of the risks associated with the use of the product and the data generated from the quantitative risk assessment process. If screening level assessments based on estimates of exposure and available hazard information are not sufficient to support the safe use of the product, then the collection or generation of additional data as discussed in the risk characterization section could be considered. If the completion of the risk assessment results in the determination that the risk is unacceptable, then appropriate risk control measures should be carried out to reduce the exposure to within acceptable risk levels.

Risk assessments for a given product and usage may not be applicable for another product or application. It is important to understand these differences as well as the effect of other exposure factors, such as frequency and duration of exposure, on the development of allergen-specific antibodies. Inter-individual variability and susceptible subpopulations that are predisposed to allergy development are important factors to consider in the risk management process and can further complicate the establishment of an acceptable level of risk.

## 3. Risk Control

In general terms, the risk control step of the risk management process should strive to reduce the risk by limiting exposure to enzymes from the product. Risk reduction options may include product modification, product use restrictions or a decision not to market the enzyme-containing product.

Modification options may include changing the matrix or delivery of the enzyme product, reducing the enzyme concentration in the product, substituting other ingredients that may be affecting the potency of the enzyme, or a combination of these approaches. In the detergent industry, great steps have been taken to minimize risk through product modification. For example, enzymes are encapsulated to limit consumer and worker exposure. This risk control method was relatively easy to achieve for consumer laundry products and, in turn, provided a reduction of risk in the work environment. Alternatively, appropriate labeling to restrict certain end uses may be considered and weighed against the likelihood that consumers will read the label.

As stated previously, the goal of risk control is to decide, based on an acceptable risk level, whether product modification or restrictions on its use is necessary. If product modification or restriction on use are not alternatives, and there is a likelihood of an adverse experience or event, not selling the product is also an option to be considered.

## 4. Risk Communication

An integral part of the risk management process is to effectively communicate the potential risks to appropriate audiences. There are two important audiences to target in designing a risk communication program: 1) users of the company's products and 2) other stakeholders, such as the general public and public interest groups.

### A) Product Users

Product labels and digital communications are the primary means of informing consumers. For enzyme-containing products, as with all consumer products, many countries require that the label include appropriate warning statements. In the U.S., the regulations of the Consumer Product Safety Commission apply; in Canada, those of the Consumer Chemicals and Containers Regulations apply; and in the European Union, those of CLP Regulation and Detergent Regulation apply. In addition, there may be requirements to place handling instructions and information or first aid information on the label. Product manufacturers can also supplement this safety-related information, as needed.

To address other questions, manufacturers should have properly educated customer support personnel to provide answers to customers and effectively communicate issues to the public regarding the safety of enzyme-containing products. Examples of essential information that customer support personnel should be able to communicate are as follows:

- Composition of the product;
- First aid information;
- Use and handling guidelines, with detailed examples of correct use and concrete recommendations to steer consumers from misuses.

Further work is needed in risk communication for enzymes to the general public since understanding the use and function of enzymes in products varies. Thus, an important part of risk communication is to ensure that the audience understands what enzymes are and what they do, so that their benefits and risks are understood.

### B) Other Stakeholders

Other stakeholders may be governmental, non-governmental organizations or industry partners. An important route toward gaining acceptance of stakeholders is through interaction among experts in the field or the industry, government authorities and the interested stakeholders, such as consumer associations, scientific journalists and academia. The goal is to build confidence in the company or industry using the technology.

An attitude of openness and willingness to share information and data is essential, while recognizing the legitimate needs of companies to protect competitively sensitive information. The amount and detail of information that may be needed in dialogues with some stakeholders may be more extensive than what is provided to the general consumer. Position papers and dossiers giving details of the product with particular reference to the enzymes used may be considered. In addition to information relevant to consumers and workers, product manufacturers should anticipate requests from some stakeholders related to the production process and containment procedures employed in the production facility, since levels of exposure in the workplace are generally higher than in the product use setting if exposure management steps are not taken. Finally, any document or dossier provided might be more readily accepted if it has been subjected to a peer-review process.

In short, an effective risk communication program requires the ability to provide useful information in response to worker, consumer and other stakeholders' inquiries. By providing this information, it is possible to promote the safe use of the product and, in turn, reduce the risks associated with exposure to the enzymes contained in the product.

Enzymes can bring significant benefits to consumer products, including improved efficiencies and previously unavailable product benefits. However, prior to introducing an enzyme preparation into a product, a risk assessment should be conducted to continue the safe use of the enzymes by the consumer. The primary challenge associated with enzyme use is preventing the generation of enzyme-specific antibodies and the development of symptoms of respiratory Type 1 hypersensitivity.

Experience in the cleaning products industry demonstrates that potential risk of adverse effects can be successfully managed by identifying the hazards to be managed, carefully assessing exposure, characterizing the risk and then applying appropriate risk management. This document has outlined strategies and methods that have been used successfully by the industry. While it sets forth recommended options for individual consideration, it is not designed to constitute a standard of care for the industry.

Each company that intends to use enzymes in its products can play an active role in understanding and managing the risks associated with enzymes. If the risk is created and not appropriately managed, the consequences may spread beyond a single product or company. This could lead to unwarranted limitations on the use of enzyme technology in other consumer applications. Therefore, it is recommended that companies using enzymes responsibly consider how they are managing enzyme safety and whether appropriate risk assessment and risk management programs have been employed.

# Bibliography

American Cleaning Institute. 2019. Risk Assessment Guidance for Enzyme-containing Products. Washington, D.C.: The American Cleaning Institute. https://www.cleaninginstitute.org/newsroom/releases/2019/new-aci-guidance-provides-pathways-stewardship-enzyme-containing-consumer

A.I.S.E. (International Association for Soaps, Detergents and Maintenance Products), 2013/2020. Exposure measurements of enzymes for risk assessment of household cleaning spray products. https://aise.eu/app/uploads/2024/02/enzyme-spray-protocol-revision-AISE-2020.pdf

A.I.S.E ., 2018: Guidelines for the safe handling of enzymes in detergent manufacturing. https://aise.eu/app/uploads/2024/02/enzymes-safe-handling-aise-2018.pdf

A.I.S.E., 2017/2025. Pan-European Consumer Habits Research, survey. https://aise.eu/priorities/science-research/consumer-research/

Andersen, P.H., Bindslev-Jensen, C., Mosbech, H., Zachariae, H., and Andersen, K.E. 1998. Skin Symptoms in Patients with Atopic Dermatitis Using

Enzyme-containing Detergents. Acta Derm Venereol (Stockh) 78: 60-62.

Anson, M.L. 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. Journal of General Physiology 22: 79-89. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2213732/pdf/79.pdf

Association of Manufacturers of Fermentation Enzymes (AMFEP) 1998. Consumer Allergy Risk for Enzyme Residues in Food. Brussels: AMFEP. https://amfep.org/about-enzymes/safety/allergen-labelling/

Bannan, E.A., Griffith, J.F., and Nusair, T.L. 1992. Skin testing of laundered fabrics in the dermal safety assessment of enzyme-containing detergents. Journal of Toxicology Cutaneous and Ocular Toxicology 11 (4): 327339. https://www.tandfonline.com/doi/pdf/10.3109/15569529209042726?needAccess=true

Basketter, D.A., Broekhuizen, C., Fieldsend, M., Kirkwood, S., Mascarenhas, R., Mauer, K., Pedersen, C., Rodriguez, C., and Shiff, H.-E. 2010. Defining occupational and consumer exposure limits for enzyme protein respiratory allergens under REACH, Toxicology 268, 165-170.

Basketter, D., Berg, N., Kruszewski, F.H., Sarlo, K., and Concoby, B. 2012a . Relevance of sensitization to occupational allergy and asthma in detergent industry, J Immunolotoxicol. 9(3): 314-319. https://www.tandfonline.com/doi/pdf/10.3109/1547691X.2012.656855?needAccess=true

Basketter, D., Berg, N., Broekhuizen, C., Fieldsend, M., Kirkwood, S., Mathieu, S., and Rodriguez, C.2012b . Enzymes in cleaning products: an overview of toxicological properties and risk assessment/ management, Regulatory Toxicology and Pharmacology 64, 117-123.

Basketter, D., Berg, N., Kruszewski, F.H., Sarlo, K., and Concoby, B. 2012c. The toxicology and

immunology of detergent enzymes. J Immunotoxicol., 9(3): 320-326 https://www.tandfonline.com/doi/pdf/10.3109/1547691X.2012.659358?needAccess=true

Basketter, D.A., English, J.S., Wakelin, S.H., and White, I.R. 2008. Enzymes, detergents and skin: facts and fantasies. Br J Dermatol. 158, 1177-1181 https://www.ncbi.nlm.nih.gov/pubmed/18422788

Basketter, D.A., Evans, P.E., Gerberick, G.F., and Kimber, I.A. 2002. Factors affecting thresholds in allergic contact dermatitis: safety and regulatory considerations. Contact Dermatitis 47, 1-6. https://www.ncbi.nlm.nih.gov/pubmed/12225405

Basketter, D.A., Kruszewski, F.H., Mathieu, S., Kirchner, D.B., Penepinto, A., Fieldsend, M., Siegert, V., Barnes, F., Bookstaff., R., Simonsen, M., and Concoby, B. 2015. Managing the Risk of Occupational Allergy in the Enzyme Detergent Industry. J Occup. Environ. Hyg. 12(7): 431-437. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4806342/

Basketter, D.A. and Lahti, A. 2011. Immediate contact reactions. In "Contact Dermatitis", 5th edition, Chapter 7. Springer, Berlin, Eds. Johansen, J.D., Frosch, P.F., and Lepoittevin J-P. 137-154. https://www.springer.com/us/book/9783642038266

Battelle Report for the Soap and Detergent Association. 1999. Measurement and Characterization of Aerosols Generated from a Consumer Spray Product – Pilot Study – Phase I. Richland, WA.

Battelle Report for the Soap and Detergent Association. 2000. Internal Report: Characterization of Aerosols Generated from a Consumer Spray Product – Phase II. Richland, WA.

Belin, L., Hoborn, J., Falsen, E., and 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 concentrations of household laundry products. Journal of Allergy and Clinical Immunology 49 (4): 219-237.

Bernstein, D.I., Gallagher, J.S., Grad, M., and Bernstein, I.L. 1984. Local ocular anaphylaxis to papain enzyme contained in a contact lens cleaning solution. Journal of Allergy and Clinical Immunology 74: 258-260. https://www.jacionline.org/article/0091-6749(84)90255-0/pdf

Bernstein, I.L., Li, J.T., Bernstein, D.I., Hamilton, R., Spector, S.L., Tan, R., Sicherer, S., Golden, D.B.K., Khan, D.A., Nicklas, R.A., Portnoy, J.M., Blessing-Moore, J., Cox, L., Lang, D.M., Oppenheimer., J., Randolph, C.C., Schuller, D.E., Tilles, S.A., Wallace, D.V., Levetin, E., and Weber, R. 2008. Allergy Diagnostic Testing: An Updated Practice Parameter. Annals of Allergy, Asthma & Immunology 100: S1-S148. https://www.aaaai.org/Aaaai/media/MediaLibrary/PDF%20Documents/Practice%20and%20Parameters/allergydiagnostictesting.pdf

Bindslev-Jensen, C., Skov, P.S., Roggen, E.L., Hvass, P., and Brinch, D.S. 2006. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. Food Chem Toxicol. 44: 1909-1915.

Blaikie, L., Richold, M., Whittle, E., Lawrence, R.S., Keech, S., and Basketter, D.A. 1999. Airborne

exposure from topically applied protein (proteolytic enzyme). In: Human and Experimental Toxicology, 18(8): 528.

Bolam, R.M., Hepworth, R., and Bowerman, L.T. 1971. In-Use Evaluation of Safety to Skin of Enzyme-Containing Washing Products. British Medical Journal 2: 499-501. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1796038/pdf/brmedj02262-0031.pdf

Bradstrut, R.B. 1965. The Kjeldahl Method for Organic Nitrogen. New York: Academic Press. https://pubs.acs.org/doi/pdf/10.1021/ac60085a028

Briatico-Vangosa, G., Braun, C.L.J., Cookman, G., Hofmann, T., Kimber, I., Loveless, S.E., Morrow, T., Pauluhn, J., Sorensen, T., and Niessen, H.J. 1994. Respiratory allergy: hazard identification and risk assessment. Fundamental and Applied Toxicology 23(2): 145-58.

Bruce, C.F., Dunn, E., Brotherton, R., Davies, D.R., Hall, F., and Potts, S.C.M. 1978. Methods of measuring biologically active enzyme dust in the environment air of detergent factories. Annals of Occupational Hygiene 21: 1¬20. https://academic.oup.com/annweh/article-abstract/21/1/1/149990

Chan-Yeung, M. Occupational asthma. Chest 1990. 98(suppl): 148S-161S.

Clydesdale, F.M. (Ed.) 1996. Critical Review in Food Science and Nutrition. CRC Press. Vol. 36, Supplement.

Coenen, T.M., Schoenmakers, A.C., and Verhagen, H. 1995. Safety evaluation of beta-glucanase derived from Trichoderma reesei: summary of toxicological data. Food and Chemical Toxicology. 33(10): 859-66.

Cormier, E.M., Sarlo, K., Scott, L.A., MacKenzie, D.P., Payne, N.S., Carr, G.J., Smith, L.A., Cua-Lim, F., Bunag, F.C., and Vasunia, K. 2004. Lack of Type 1 sensitization to laundry detergent enzymes among consumers in the Philippines: Results of a two-year study in atopic subjects. Annals Allergy Asthma Immunology, in press.

Cohrssen, J.J., and Covello, V.T. 1989. Risk Analysis: A Guide to Principles and Methods for Analyzing Health and Environmental Risks. Council on Environmental Quality, Executive Office of the President. NTIS No. PB 89-137772. Washington, D.C. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB89137772.xhtml

Cullinen, P., Lowsen, D., Nieuwenhuijsen, S., Gordon, S., Tee, R.D., Venables, K.M., McDonald, J.C., and Newman-Taylor, A.J. 1994a. Work-related symptoms, sensitization and estimated exposure in workers not previously exposed to laboratory rats. Occupational and Environmental Medicine 51: 589-592. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1128051/pdf/oenvmed00057-0013.pdf

Cullinen, P., Lowsen, D., Nieuwenhuijsen, S., Sandiford, C., Tee, R.D., Venables, K.M., McDonald, J.C., and Newman-Taylor, A.J. 1994b. Work-related symptoms, sensitization and estimated exposure in workers not previously exposed to flour. Occupational and Environmental Medicine 51: 579-583. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1128049/pdf/oenvmed00057-0003.pdf

Delves, P.J., Martin, S.J., Burton, D.R., and Roitt, I.M. 2017. Roitt's Essential Immunology, Chapter 14

Allergy and other Hypersensitivities. 405-434. Wiley Blackwell, Chichester.
https://www.wiley.com/en-us/Roitt%27s+Essential+Immunology%2C+13th+Edition-p-9781118415771

ECETOC Technical Report No. 79. 2001. Exposure factors sourcebook for European populations (with focus on UK data). Brussels. (ISSN-0773-0872-79).
http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-079.pdf

Eisen, H.N. 2001. Specificity and degeneracy in antigen recognition: Yin and yang in the immune system. Annual Reviews in Immunology 19: 1-21.
https://www.annualreviews.org/doi/pdf/10.1146/annurev.immunol.19.1.1

EPA (1989) Exposure factors handbook. EPA/600/8-89/043.

Eypasch, E., Lefering, R., Kum, C.K., and Troidl, H. 1995. Probability of adverse events that have not yet occurred: a statistical reminder. British Medical Journal 311: 201-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2550668/pdf/bmj00608-0045.pdf

Fisher, A.A. 1985. Allergic reactions to contact lens solutions. Cutis 36: 209-11.
https://www.ncbi.nlm.nih.gov/pubmed/3931986

Flindt, M.L. 1969. Pulmonary disease due to inhalation of derivatives of Bacillus subtilis containing proteolytic enzyme. Lancet June 14;1(7607): 1177-81.

Flindt, M.L. 1978. Respiratory Hazards from Papain. Lancet February 25; 1(8061): 430-2.
https://www.ncbi.nlm.nih.gov/pubmed/75454

Freye, H.G.B. 1988. Papain anaphylaxis: A case report. Allergy Proceedings. 9(5): 571¬4.
https://www.ncbi.nlm.nih.gov/pubmed/3234762

Gibson, J.C., Juniper, C.P., Martin, and Weill, R.B.H. 1976. Biological effects of proteolytic enzyme detergents. Thorax, Vol. 13 (6): 621-634.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC470486/pdf/thorax00150-0001.pdf

Glaister, J. 1986. Principles of Toxicological Pathology. London and Philadelphia: Taylor and Francis, Ltd.

Greenough, R.J., Everett, D.J., and Stavnsbjerg, M. 1991. Safety evaluation of alkaline cellulase. Food and Chemical Toxicology 29(11): 781¬5.

Greenough, R.J., Perry, C.J., and Stavnsbjerg, M. 1996. Safety evaluation of a lipase expressed in Aspergillus oryzae. Food and Chemical Toxicology 34(2): 161-6.

Griffith, J.F., Weaver, J.E., Whitehouse, H.S., Poole, R.L., Newmann, E.A., and Nixon, G.A. 1969. Safety evaluation of enzyme detergents. Oral and cutaneous toxicity, irritancy and skin sensitization studies. Food and Cosmetic Toxicology 7: 581-593.

Hanley, J.A., and Lippman-Hand, A. 1983. If nothing goes wrong, is everything all right? J American

Medical Association 249: 1743-1745.
http://www.medicine.mcgill.ca/epidemiology/hanley/reprints/If_Nothing_Goes_1983.pdf

Hendricks, M.H. 1970. Measurement of Enzyme Laundry Product Dust Levels and Characteristics in Consumer Use. Journal of the American Oil Chemists' Society 47(6): 207-211.
https://link.springer.com/content/pdf/10.1007%2FBF02638872.pdf

HERA 2007: Human and Environmental Risk Assessment,
http://www.heraproject.com/RiskAssessment.cfm?SUBID=22

Hjortkjaer, R.K., Bille-Hansen. V., Hazelden, K.P., McConville, M., McGregor, D.B., Cuthbert, J.A., Greenough, R.J., Chapman, E., Gardner, J.R., and Ashby, R. 1986. Safety evaluation of Celluclast, an acid cellulase derived from Trichoderma reesei. Food and Chemical Toxicology 24(1): 55-63.

Hjortkjaer, R.K., Stavnsbjerg, M., Pedersen, P.B., Heath, J., Wilson, J.A., Marshall, R.R., and Clements, J. 1993. Safety evaluation of esperase. Food and Chemical Toxicology 31(12): 999-1011.

How, M.J., Goodwin, B.F., Juniper, C.P., and Kinshott, A.K. 1978. Comparative serological and clinical findings in subjects exposed to environmental allergens. Clinical Allergy 8(4): 347-360.
https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.1978.tb00470.x

How, M.J., Kunstler, K, Thernagand, J., and Walker, A.P. 1989. The A.I.S. approach to assessment of product safety: The A.I.S approach to assessment of the safety of soap and detergent products to consumers. Tenside Surfactants Detergents 26(3): 231-236.

Johnston, G., Innis, J.D., Mills, K.J., Bielen, F., Date, R.F., Weisgerber, D., and Sarlo, K. 1999. Safety assessment for a leave-on personal care product containing a protease enzyme. Human and Experimental Toxicology 18(8): 527.

Johnsen, C.R., Sorensen, T.B., Ingemann Larsen, A., Bertelsen Secher, A., Kofoed, G.S., Fredslund Nielsen, L., and Gyntelberg, F. 1997. Allergy risk in an enzyme producing plant: a retrospective follow up study. Occupational and Environmental Medicine. 54: 671-675.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1128842/pdf/oenvmed00093-0047.pdf

Kelling, C.K., Bartolo, R.G., Ertel, K.D., Smith, L.A., Watson, D.D. and Sarlo, K. 1998. Safety assessment of enzyme-containing personal cleansing products; exposure characterization and development of IgE antibody to enzyme after a 6 month test. J. Allergy & Clinical Immunology, 101: 179-87.
https://www.ncbi.nlm.nih.gov/pubmed/9500750

Klburn, K.H., Dowell, A.R., Pratt, P.C. 1971. Morphological and biochemical assessment of papain-induced emphysema. Archives of Internal Medicine 127(5): 884-890.
https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/577898?redirect=true

Kondo, M., Ogawa, T., Matsubara, Y., Mizutani, A., Murata, S., and Kitagawa, M. 1994. Safety evaluation of lipase G from Penicillium camembertii. Food and Chemical Toxicology 32(8): 685-96.

Ladics, G.S., and Sewalt, V. 2018. Industrial Microbial Enzyme Safety: What does the weight of

evidence indicate? Regulatory Toxicology and Pharmacology 98:151-154.

LaDou, J. 1997. Occupational and Environmental Medicine. Stamford, Connecticut: Appelton & Lange. https://trove.nla.gov.au/work/16026118?q&versionId=29248935

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin-phenol reagents. Journal of Biological Chemistry 193: 265. http://www.jbc.org/content/193/1/265.full.pdf

Mak, T.W. and Saunders, M.E. 2006. The Immune Response, Basic and Clinical Principles. Elsevier Academic Press. https://journals.lww.com/shockjournal/Fulltext/2007/02000/The_Immune_Response__Basic_and_Clinical_Principles.19.aspx

Markham, R.J.F., and Wilkie, B.N.M. 1976. Influence of detergent on aerosol allergic sensitization with enzymes of Bacillus subtilis. International Archives of Allergy and Applied Immunology 51: 1-154, 529-543. https://www.ncbi.nlm.nih.gov/pubmed/823120

Miller, L.S., Bhullar, B.S., Moore, B.S., Scovell, L.J., Lamm, J., Sawhney, A., and Smith, L.A. 1994. A robotic immunoassay system for detergent enzymes. Laboratory Information Management 26: 79-87.

National Academy of Sciences (NAS), National Research Council (NRC) (1994) Science and Judgment in Risk Assessment, the National Academies Press, Washington, D.C. https://www.nap.edu/catalog/2125/science-and-judgment-in-risk-assessment

Nordman, H. Atopy and work. Scand J Work Environ. Health. 1984. 10: 481-5.

Organization for Economic Cooperation and Development. 1993. Safety Evaluation of Foods Derived by Modern Biotechnology. Paris: OECD. https://pdfs.semanticscholar.org/87a0/50d6d060805832a5c4ba0f400be1cd3a5a85.pdf

Pariza, M.W., and Johnson, E.A. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century. Regulatory Toxicology and Pharmacology 33: 173-186.

Pepys, J., Longbottom, J.L., Hargreave, F.E., and Faux J. 1969. Allergic reactions of the lungs to enzymes of Bacillus subtilis. Lancet June 14. 1(7607): 1181-1184. http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(69)92166-7/abstract

Pepys, J., Mitchell, J., Hawkins, R., and Malo, J.L. 1985. A longitudinal study of possible allergy to enzyme detergents. Clinical Allergy 15: 101-115. https://www.ncbi.nlm.nih.gov/pubmed/3888439

Pepys, J., Wells, I.D., D'Souza, M.F., and Greenberg, M. 1973. Clinical and immunological responses to enzymes of Bacillus subtilis in factory workers and consumers. Clinical Allergy 3: 143-160. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.1973.tb01318.x

Pocalyko, D.J., Chander, P., Harding, C.R., Blaikie, L., Watkinson, A., and Rawlings, A.V. 2002.

The efficacy, stability and safety of topically applied protease in treating xerotic skin. In Skin Moisturization, eds Leyden, J.J. and Rawlings, AV. Marcel Dekker, NY, NY. 365-384.

Ritz, H.L., Evans, B.L.B., Bruce, R.D., Fletcher, E.R., Fisher, G.L., and Sarlo, K. 1993. Respiratory and immunological responses of guinea pigs to enzyme-containing detergents: A comparison of intratracheal and inhalation modes of exposure. Fundamental and Applied Toxicology 21: 31-37.

Robinson, M.K., Babcock, L.S., Horn, P.A., and Kawabata, T.T. 1996. Specific Antibody Responses to Subtilisin Carlsberg (Alcanase) in Mice: Development of an Intranasal Exposure Model. Fundamental and Applied Toxicology 34: 15-24.

Rodriguez, C., Calvin, G., Lally, C., and LaChapelle, J.M. 1994. Skin effects associated with wearing fabrics washed with commercial laundry detergents. Journal of Toxicology. Cutaneous & Ocular Toxicology 13(1): 39-45. https://www.tandfonline.com/doi/abs/10.3109/15569529409037508

Romano, C., Sulotto, F., Piolatto, G., Capellaro, E., Falagiani, P., Constable, D.W., Verga, A., Scansetti, G. Factors related to the development of sensitization to green coffee and castor bean allergens among coffee workers. Clinical and Experimental Allergy. 1995. 25:643-650.
https://www.ncbi.nlm.nih.gov/pubmed/8521183

Rothgeb, T.M., Goodlander, B.D., Garrison, P.A., and Smith, L.A. 1988. The raw material, finished product, and dust pad analysis of detergent proteases using a small synthetic substrate. Journal of the American Oil Chemists' Society 65: 806. https://link.springer.com/article/10.1007/BF02542537

Sarlo, K., Cormier, E., MacKenzie, D., and Scott, L. 1996. Lack of Type 1 sensitization to laundry enzymes among consumers in the Philippines. Journal of Allergy and Clinical Immunology 97: 749, 77.
https://www.ncbi.nlm.nih.gov/pubmed/15191024

Sarlo, K., Fletcher, E.R., Gaines, W.G., and Ritz, H.L. 1997. Respiratory allergenicity of detergent enzymes in the guinea pig intratracheal test: Association with sensitization of occupationally exposed individuals. Fundamental and Applied Toxicology 39: 44-52.
https://www.ncbi.nlm.nih.gov/pubmed/9325026

Sarlo, K., and Kirchner, D.B. 2002. Occupational asthma and allergy in the detergent industry: new developments. Current Opinion in Allergy and Clinical Immunology 2: 97-101.
https://www.ncbi.nlm.nih.gov/pubmed/11964756

Sarlo, K., Adamson, G.M., Hollis, V.L., Innis, J.D., Babcock, L.S., and Kirchner, D.B. 2004. Development of allergic antibody to an enzyme in a body lotion: results of an 18-month clinical study. Journal of Immunotoxicology 1: 71-¬77.
https://www.tandfonline.com/doi/pdf/10.1080/15476910490496249?needAccess=true

Sarlo, K., Kirchner, D.B., Troyano, E., Smith, L.A., Carr, G.L., and Rodriguez, C. 2010. Assessing the risk

of Type 1 allergy to enzymes in laundry and cleaning products: evidence from the clinical data. Toxicology 271: 87-93.
https://pdfs.semanticscholar.org/f705/bc6aa477392b3fcef5fe0fd1ffecf93976ba.pdf

Scientific Committee for Food (SCF). 1992. Guidelines for the presentation of data on food enzymes (Opinion expressed on 11 April 1991). Reports of the Scientific Committee for Food 27th Series (Cat. No. EUR 14181). Office for Official Publications of the EEC, 1992. http://aei.pitt.edu/40836/1/27th_good.pdf

Schweigert, M.K., MacKenzie, D.P., and Sarlo, K. 2000. Occupational asthma and allergy associated with the use of enzymes in the detergent industry: a review of the epidemiology, toxicology and methods of prevention. Clinical and Experimental Allergy 30: 1511-1518.
https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2222.2000.00893.x

Shearer, W.T., and Fleisher, T.A. 1998. The Immune System in Allergy, Principles and Practice. 5th ed. Vol. 1. Ed. Middleton, E., Reed, C.E., Ellis, E.F., Adkinson, N.F., Yunginger, J.W., and Busse, W.W. New York: Mosby.

The Soap and Detergent Association. 1995. Work Practices for Handling Enzymes in the Detergent Industry. New York: The Soap and Detergent Association.

The Soap and Detergent Association. 2005. Risk Assessment Guidance for Enzyme-containing Products. Washington, D.C.: The Soap and Detergent Association.

Timbrell, J.A. 1982. Principles of Biochemical Toxicology. London: Taylor & Francis, Ltd.
https://onlinelibrary.wiley.com/doi/abs/10.1002/jat.2550030412

Troyano, E, Page, D.A., Bielen, F., Sarlo, K., Parker, R. and Rodriguez, C. 2003. Safety profile of a dish-washing liquid containing BPN' protease. Eurotox. https://www.researchgate.net/publication/278135181_Proceedings_of_EUROTOX_2003_-_The_XLI_European_Congress_of_Toxicology_-_September_28-October_1_2003_-_Firenze_Italy_-_Science_for_Safety_-_Preface

Weegels, M.F. 1997. Exposure to chemicals in consumer product use. TU Delft, The Netherlands (under the authority of the Dutch General Inspectorate for Health Protection – Project 96/034910/04).

Weeks, J.A., Burdick, J.D., Harper, R.A., Smith, K., and Simon, R.A. 2001a. A controlled use study of a laundry pre-spotter containing protease in an atopic population. Journal of Allergy and Clinical Immunology 107: S28.
https://www.sciencedirect.com/journal/journal-of-allergy-and-clinical-immunology/vol/107/issue/2/suppl/S

Weeks, J.A., Burdick, J.D., Rogers, R.E., and Prento, A. 2001b. Assessment of sensitization risk to consumers using a laundry pre-spotter product containing protease. Toxicological Sciences 60: 20.

Weeks, J.A., Harper, R.A., Simon, R.A., and Burdick, J.D. 2011. Assessment of sensitization risk of a laundry pre-spotter containing protease. Cutan and Ocul Toxicol, 30(4): 272-279.
https://www.tandfonline.com/doi/abs/10.3109/15569527.2011.565010

The World Medical Association. 1964. Revised 1975, 1983, 1989, 1996, and 2000. Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects. Helsinki, Finland.

Zachariae, H., Hoech-Thomsen, J., Witmeur, O., and Wide, L. 1981. Detergent enzymes and occupational safety. Observations on sensitization during Esperase production. Allergy 36(7): 513-516. https://www.ncbi.nlm.nih.gov/pubmed/6461274

Zetterstrom, O. 1977. Challenge and exposure test reactions to enzyme detergent in subjects sensitized to subtilisin. Clinical Allergy 7: 355-363. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.1977.tb01463.x

Zetterstrom, O., and Wide, L. 1974. IgE antibodies and skin test reactions to a detergent enzyme in Swedish consumers. Clinical Allergy 4: 272-280. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.1974.tb01385.x

**Adjuvant** — any substance that enhances an immune response to an antigen.

**Aerosol** — Small airborne solid or liquid particles suspended in air, i.e. dust or mist.

**Allergy** — An immunological condition acquired through exposure to a substance (allergen) that results in an enhanced, adverse reaction to the substance upon re-exposure. Allergies to enzymes, as with other proteins, are mediated by allergic IgE antibodies. Symptoms of enzyme allergies may include any or a combination of the following: sneezing; nasal or sinus congestion; coughing; watery and itchy eyes or nose; hoarseness or shortness of breath; and anaphylaxis. Symptoms not typically observed with allergy to enzymes include GI upset, urticaria and atopic dermatitis.

**Allergen** — A substance that specifically induces the production of allergic antibodies.

**Amylase** — A class of enzymes that speed up the breakdown of the chemical bonds between the connecting sugar molecules in starch

**Anaphylaxis** — An allergic reaction that involves multiple organ systems and can lead to cardio-pulmonary collapse and death.

**Antibody** — Globular proteins (immunoglobulins or Ig) made by B cells. Antibodies recognize and bind to antigens in a specific manner and mediate immune responses to eliminate the antigen.

**Antigen** — A substance (often a protein) involved in the induction of an immune response and recognized by antibodies and T cells during the progression of an immune response. Not all antigens are capable of inducing antibody production or T cell responses; these are considered incomplete antigens.

**Antiserum** — The antibody-containing fluid component of clotted blood.

**Asthma** — The reversible narrowing of the airways of the lungs in response to exposure to irritants, allergens or other stimuli. Symptoms may include shortness of breath, wheezing, labored breathing and cough.

**Atopic** — A genetic tendency for an individual to develop allergic antibodies to antigens in their environment, thereby developing allergies, such as hay fever, more readily after exposure to an antigen.

**Benchmark Values** — Based on studies in which measured or estimated exposure levels are associated with a demonstrated effect or the lack of an effect in the people exposed.

**Cellulase** — An enzyme that breaks down cellulose.

**Consumer Products** — Products used in household and industrial cleaning or personal care applications.

**Detergent** — A mixture of surfactants, builders, bleach and other chemicals used to facilitate cleaning.

**Elicitation of Allergy** — That phase of the immune response where antigen binds to and cross-links allergic antibodies (usually on the surface of tissue mast cells), leading to the release of pro-inflammatory mediators (e.g. histamine) resulting in symptoms of allergy.

**ELISA (Enzyme-Linked Immunosorbent Assay)** — A sensitive laboratory immunoassay for detection of antibodies or quantitation of antigen, widely used in biology and medicine.

**Encapsulation** — A chemical coating applied to an enzyme granule to reduce the potential for dust generation.

**Enzyme** — A large catalytic protein molecule. Enzymes are present in all living organisms. They speed up the chemical reactions necessary to sustain life. Two of their essential functions are in the conversion of food to energy and conversion of food to new cell material.

**Enzyme Activity Test** — Test to measure the ability of enzymes to speed up chemical reactions. An enzyme reacts with a substrate for a defined time under controlled conditions of temperature and pH. Reaction products form a colored complex with a color-development reactant. Enzyme activity of an unknown solution is determined relative to standard solutions.

**Epitope** — The site on an antigen that is recognized by an antibody or by an antigen receptor (e.g. on T cells); epitopes in proteins can be linear stretches of amino acids or discontinuous regions of amino acids that form a three-dimensional shape.

**Enzymes Granules** — Enzymes formulated in non-dusting solid form, typically between 200 and 100 microns in diameter. There are many different technologies available for granulating enzymes, including high shear granulation, extrusion/marmerization, spray coating and prilling.

**Histamine** — A vasoactive amine released from mast cells (white blood cells) usually after the binding of an antigen to allergic antibodies bound to the surface of the mast cell; histamine is responsible for many of the symptoms associated with an allergic response to a substance (see vasoactive amine).

**IgE** — Immunoglobulin E, a class of antibody made in response to allergens that mediates the Type 1 hypersensitivity response. IgE can be found in serum or bound to the surface of mast cells distributed throughout the body.

**Immunoassay** — In the context of this document, an in vitro assay that can detect serum antibodies resulting from exposure to antigens (see ELISA); also an assay that can detect antigens.

**Induction of Allergy** — The initial phase of the immune response where the allergen (antigen/immunogen) is engulfed by antigen-presenting cells, processed and presented to T cells that in turn interact with B cells, leading to the generation of specific IgE antibodies to the allergen.

**Irritant** — A substance capable of producing irritation or inflammation as a result of its contact with living tissue. Unlike allergens, the response is not dependent on the immune system, does not require a latency period and has no memory component.

**Kjeldahl Analysis** — A method to analyze total protein by determining the nitrogen content.

**Lipase** — A class of enzymes that speed up the breakdown of fats.

**Lipid** — A class of chemical compounds, including fats and oils, found in plant and animal cells.

**Occupational Asthma (Enzyme Asthma)** — Asthma produced by workplace conditions. Enzyme asthma is a specific type of occupational asthma in which the asthmatic response is triggered in people with allergic antibodies through breathing a high concentration of enzymes.

**Peptide Bonds** — Chemical bonds that attach amino acids together in proteins.

**Polyclonal Antibody** — A preparation of antiserum that contains many different antibodies that recognize different epitopes on an antigen; some of these antibodies can bind to the same epitopes with different binding strengths.

**Potentiation** — An increased immunological response to an enzyme as a result of the simultaneous exposure to another enzyme, detergent matrix or some other adjuvant (see adjuvant).

**Protease** — A class of enzymes that speed up the breakdown of the chemical bonds between connecting amino acids in proteins.

**Protein** — A class of chemical compounds found in plant and animal cells. Proteins are made up of long chains of amino acids.

**RAST (Radio Allergo-Sorbent Test)** — A sensitive laboratory test used for detecting and measuring antibodies in the blood of persons exposed to allergens, widely used in allergy clinical work

**Rhinitis** — An inflammation of the nasal mucosal membrane that can be caused by irritation or by an allergic response. Rhinitis is characterized by runny nose with or without itching, watery eyes, sneezing and congestion.

**Sensitization** — The stimulation of the immune system by an allergen that leads to the development of allergic antibodies to the allergen. This is not a disease. See induction of allergy.

**Skin Prick Test** — An in vivo technique for detecting allergic antibodies in persons exposed to specific allergens. The test consists of pricking the superficial layer of the skin with a solution of the allergen. In an individual with allergic antibody, the allergen binds to the allergic antibodies on the mast cell leading to the release of mediators such as histamine. A raised reddened area with surrounding erythema (wheal and flare) will appear on the skin.

**Subtilisin** — A serine protease derived from Bacillus subtilis or closely related species.

**Substrate** — The substance acted upon by an enzyme, broken down into smaller components.

**Type 1 Hypersensitivity** — A specific form of allergy, also known as immediate hypersensitivity, involving IgE antibodies and mediated by the release of histamine and other pro-inflammatory mediators that lead to the development of symptoms.

**Urticaria** — Another description for hives that is typified by swelling, itching and redness of the skin, can be caused by an allergic reaction to an allergen or by a physiological response to a stimulant.

**Vasoactive Amines** — Substances, including histamine and 5-hydroxytryptamine, that increase vascular permeability and smooth muscle contraction.

# Appendix 1 — Estimation of Exposure to Enzymes from Early Detergent Formulations

PRODUCT ENZYME DUSTINESS COMPARISON: 1970 SWEDISH CONSUMER EXPOSURE VS. CONSUMER EXPOSURES TO 1980, 1984, and 1993 PROCTER & GAMBLE DETERGENTS

## Summary

Detergent products made today are at least 28,000 times less enzyme-dusty than products used by Swedish consumers in the late 1960s and described in the study conducted by L. Belin et al. in 1970 (Belin et al., 1970). Airborne enzyme exposures to Swedish consumers described in the Belin study are estimated to average 212 ng/m$^3$. This conclusion is based on the Belin study description that consumers used unprotected enzyme-containing product; Procter & Gamble (P&G) airborne enzyme analyses taken during hand wash simulations with dusty (micronized) detergent product; and P&G results from several detergent product and enzyme ingredient studies carried out since 1970. Based on improved coatings for enzyme encapsulation and reduced enzyme protein content of enzyme ingredients, the relative exposure obtained through use at recommended doses of today's granular detergents is calculated to be 0.0057 ng/m$^3$. This exposure is 37,193 times less than that estimated to have been experienced by the Swedish consumers reported on in the Belin paper.

## Background

In the 1970s, two papers (Belin, 1970; Zettestrom, 1974) were published describing sensitization of several Swedish consumers to enzyme-containing detergent products and subsequent reaction upon re-exposure to these products. Belin's report is significant as it documents a case where consumer use of a detergent resulted in enzyme sensitization. Unfortunately, while formulation concentration of enzyme was described, actual exposures to airborne enzyme dust concentrations were not determined. There is, therefore, no airborne enzyme level to associate with producing these sensitizations during use.

During that same time period, M.H. Hendricks published a paper (Hendricks, 1970) describing exposure levels reached when consumers use enzyme-containing detergents. These detergents were made from enzyme stocks that had undergone new granulation processes and reduced enzyme protein dust generation during handling of these products. Substantial improvements have continued to be made in the reduction of enzyme dust generation during use of P&G detergent products up to 1993. These include improvements by the enzyme supplier on the coating and prilling processes and by the P&G manufacturing/handling processes used during production and packaging.

To date, it is not known what level of airborne enzyme the Swedish consumers in the 1970 study were exposed to. Further, a comparative assessment that indicates how much less dusty products today are versus those used in the Swedish consumer study has not been done. This summary, then, provides a basis for both estimating the exposure level experienced by the Swedish consumers and comparison of the exposure levels generated during use of the old detergents versus detergents of today.

## Approach

An airborne enzyme concentration was estimated for the Belin (1970) study. Using this value, several separate studies were combined to obtain relative dustiness values for the 1970 product used by Swedish consumers and products used in 1997.

Specifically, the studies conducted by the Procter & Gamble Company utilized in this assessment were:

1. Non-protected enzyme product studies (4). Airborne enzyme concentration during a 50-gram dispense of powdered product into a sink was determined to be 212 ng enzyme protein/$m^3$. The finished product used in this determination was micronized, thereby removing enzyme protection normally provided by enzyme granulation processes. This micronized form closely simulates the product form used by consumers in the Belin study.

2. 1970 granulated enzyme product studies (3). After the change to a granulated enzyme stock form in the 1970s, Hendricks determined an exposure to consumers during a one-cup pour of the newly granulated enzyme-containing product. Several assumptions are detailed in this appendix and are used to derive a calculated exposure of 1.01 ng/$m^3$.

3. 1984 product studies (5). Comparison of 1984 products to the product used in the Hendricks study showed that the 1984 products were 30 to 70 times less enzyme-dusty.

4. 1992 to 1993 enzyme supplier prilling improvements. A number of enzyme suppliers have changed to improved coating and double-coating techniques that significantly reduced enzyme dust generation in the product by a factor of 3 to 10 times lower than the 1984 product.

## Materials and Methods

Reagents. Bovine Serum Albumin (BSA), Dimethylsulfoxide, trishydroxymethyl aminomethane (Tris), calcium chloride dihydrate, sodium thiosulfate pentahydrate, N-succinyl-l-alanyl-L-alanyl-L-prolyl-L-phenylalanyl-p-nitronalide (PNA), and phenylmethylsulphonyl fluoride (PMSF) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Whatman type GF/C glass fiber filters, Falcon type disposable 50 mL conical centrifuge tubes, and a Cel-Gro Tissue Culture Rotator (1640 Lab-Line) were obtained from Fisher Scientific Company (Cincinnati, OH, USA). The Tecan RSP 5051 was obtained from Tecan US (Hillsborough, NC, USA). The Bio-Tec EL 312 microplate reader was obtained from Bio-Tec Instruments, Inc. (Wintooski, VT, USA).

Enzyme. Prilled Alcalase® 2.0T (Bacillus licheniformis, EC 3.4.21.14) containing 6.7% total protein and prilled Savinase 6.0T (Bacillus subtilis lentus, EC 3.4.21.14) containing 4.2% were obtained from Novozymes, Denmark.

Finished product. Several granulated detergent formulations were used in these studies and were designated as XK (1970), L, W (1984), and Z (1993). XK, L and W formulas all contained Alcalase. The Z was a non-enzyme-containing blank product that was then spiked with Savinase during the study.

These detergents were products manufactured by the Procter & Gamble Company and contained enzymes, anionic and nonionic surfactants, silicate builders,and perborate bleach.

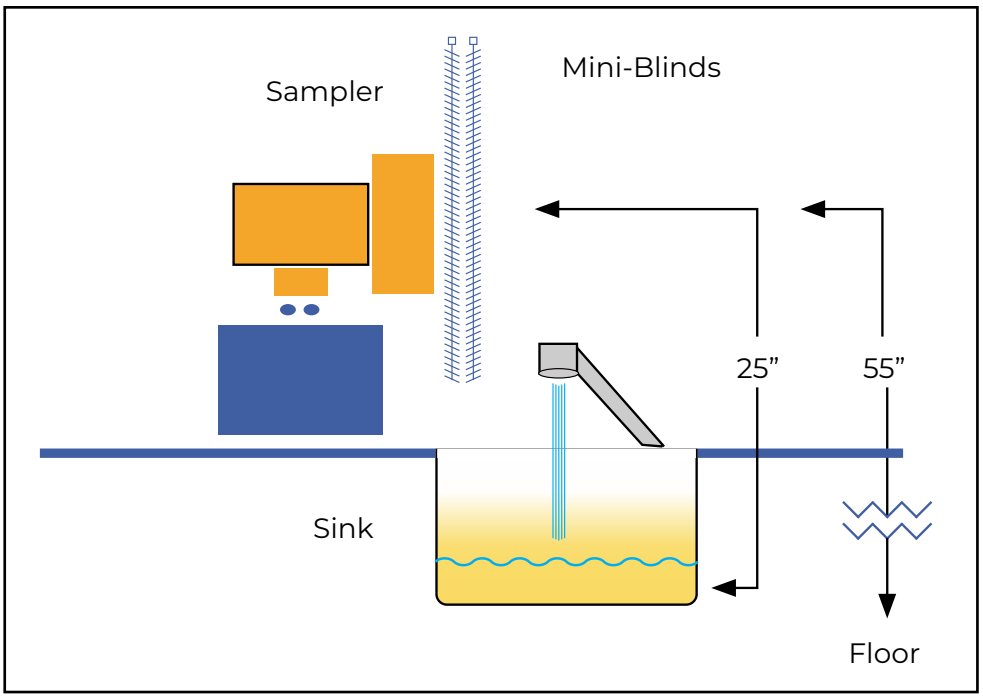
Product micronization. Approximately 100 grams of detergent product was micronized (finely ground) to a powdered material with a uniform 1-micron particle size using an Ultra Centrifugal Mill Type ZM1 (Brinkman Instruments, Inc., Westbury, NY, USA) with a 1.0 mm stainless steel sieve. Prior to the micronization, 100 grams of non-enzyme-containing Z product was spiked with 1.2% Savinase 6.0T, giving a protein concentration of 667 µg/gram of detergent product. This micronized sample was then used in the detergent dispensing studies.

Product dustiness comparisons — Galley Dust Box (5). Five trials were conducted, each consisting of pouring from a 49-ounce box of formula W poured from a height of 28 inches into a shallow pan within a Galley Dust Box. The Galley Dust Box has a holding area for the detergent granules, allowing product to drop down onto a shallow pan. A pump is positioned on top of the box near one end. Dropping the product onto the pan created a dust-laden atmosphere from which an air sample was collected. A Gast vacuum pump calibrated at a flow rate of 17.7 liters per minute was used to draw the airborne dust onto a Whatman GFC filter. The level of the airborne enzyme collected on the dust pad was measured by the protease activity method described by Rothgeb et al. (1988).

Airborne protease levels by activity measurement (Galley Dust Box study). The protease activity method described by Rothgeb et al. (1988) was used to measure Savinase and Alcalase proteases in airborne dust. In this method, dust-pad extract solutions are mixed with a chromogenic peptide paranitroanalide (PNA). Proteolytic hydrolysis of the PNA produces a yellow color measurable at the 410 nm visible range. This method is easily automated on an Abbott VP Autoanalyzer (Abbott Labs, Dallas, TX, USA) at 37°C. Calibration is conducted using a Savinase or Alcalase material previously analyzed by the method of Anson (1938).

Enzyme raw material dustiness measurements (Heubach measurements). Granulated (prilled) raw material enzymes were evaluated for relative dustiness using a Heubach dustiness meter (Rothgeb et al., 1988). Briefly, the method measures dustiness by analyzing airborne enzymes collected on a filter positioned above a porous bottom cup. This cup contains the enzyme prill which undergoes physical breakage as ball bearings roll over the material by means of a rotating lever parallel to the cup bottom. During this process, nitrogen gas is passed through the bottom of the cup and through the raw material. Generated attrition dust is collected on a dust pad as the nitrogen passes up through the collection filter. The level of enzyme dust collected is measured by ELISA using the protocol described below. Comparison of Heubach data for each enzyme determines if the material is more or less dusty than the reference material. All comparisons are conducted on the basis of equal volume in the cup and not on an equal weight basis.

Product dispensing, sink filling and air collection while using unprotected enzyme product. Five samples of 50 grams each were weighed from the micronized finished detergent product into 100-mL disposable beakers. Using the setup shown in the adjacent figure, the faucet was turned on and water was allowed to come to 45°C without blocking the sink drain. One second before




dispensing the product, a General Metal Works type HV2000P air sampler (General Metal Works, Inc., Cleves, OH, USA) fitted with a 10-cm diameter GF/C glass fiber filter and calibrated to a flow rate of 0.33 $m^3$/minute was turned on. The sink drain was then blocked by use of a stopper and immediately one of the 50 gram samples of micronized detergent was dispensed into the sink at a height of 12 inches above the sink bottom and a time equal to zero minutes. The sink was allowed to fill to a volume of 8 liters, at which point the air sampler was turned off and the time recorded. The faucet flow rate was about 8 liters per minute. The air sampler was positioned at breathing zone height, perpendicular to the front of the person performing the dissolution task, and facing the water-dispensing area of the sink. The sampling height was 55 inches from the floor to the center of the sampler and 25 inches from the bottom of the sink. Two sets of adjustable mini-blinds were positioned immediately in front of the air sampler. One set of blinds was opened at a 315-degree angle relative to the air sampler intake. This set of blinds touched the air sampler. The other set was positioned next to and touching the first set but with the blinds angled in an opposite direction at 45 degrees relative to the sampler intake. In this manner, the blinds would act as a deflector for splashing or splattering of diluted product as washing and rinsing occurred but without interrupting air flow. Four more trials with 50-gram samples were conducted. In between each trial, the sink was cleaned and the room cleared of airborne enzyme by room exhaust and the use of high-powered fans. In addition to room checks after clean-out, one additional test was run to demonstrate the splatter-prevention effectiveness of the mini-blind set-up by dispensing product following the same procedure but without turning the sampler on. This would show if any enzyme solution was splattering onto the open-face pads due to filling the sink and potentially biasing results high.

Air sample extraction for analysis. Upon collection, each pad was removed, placed in a 50-mL conical tube and immersed in 25 mL of an enzyme extraction buffer consisting of 500 mM NaCl, 20 mM Tris, 0.1% BSA, 20 mM thiosulfate, 1 mM calcium chloride, and 0.1% Tween 20, pH 8.2. Each tube sat a minimum of 18 hours at 10°C prior to analysis. At the time of analysis, the pads were removed and discarded. The extract solution in the tube was analyzed for enzyme concentration. Previous work has shown that maximum extraction is obtained within one hour of sitting in this solution or 20 minutes if rotating along the longitudinal axis of the tube at about 25 rpm.

Enzyme measurement. Solutions prepared from air collections were analyzed by an Enzyme-Linked Immunosorbent Assay (ELISA) to quantitate enzyme protein present. The ELISA method is a modification of the method described by Miller et al. (1994) and was used to measure Savinase in these solutions. Antibodies and antibody conjugate were produced according to protocols outlined in the Miller et al. (1994) publication. Briefly, this is a two-site enzyme immunoassay. Microtiter plates are coated with 100 µL of rabbit-generated antibodies specific for a detergent enzyme. The coating concentration of the antibody was 2 µg/mL 0.015M bicarbonate buffer, pH 9.6. The plates are allowed to sit overnight at 10°C, then washed three times with phosphate buffered saline (PBS) buffer and patted dry on a paper towel. Next, 250 µL of 2% BSA in PBS, 0.1% Tween is added and allowed to sit for 1.5 hours at room temperature. The wells are washed three times with PBS and then patted dry. The procedure continues with the addition of 50 µL ELISA assay buffer and 100 µL of the dust-pad extract solutions to the microtiter plate. Next, 50 µL of conjugate antibodies (same antibody used to coat the plate but conjugated with the detecting enzyme, alkaline phosphatase) at 2 µg/mL ELISA assay buffer is added to the well and incubated at 37°C for 2 hours, then washed six times. Para-nitrophenyl phosphate solution is then added to each well and the rate of production of yellow color due to release of para-nitrophenol by the alkaline phosphatase is measured at 405 nm. For each run, standards are included for calibration. These standards are prepared using a Savinase material previously analyzed for protein content. Six standards are used ranging from 200 pg/mL to 20 ng/mL. As conducted in the Procter & Gamble Company labs, this assay system in combination with the above air collection procedure had an effective measurement range of 6 to 597 + 6 ng/$m^3$ Savinase protein in airborne dust for the five-minute sampling period.

Protein assessment. Protein was assessed by the Kjeldahl Total Nitrogen Method (Bradstrut, 1965). This method has proven to be the most practical protein method for application across a wide variety of enzyme classes in detergent enzyme ingredient form. All enzyme measurements are based on standards calibrated by this protein method.

Protective equipment for participants. Due to the fine particle size of the enzyme-containing product, all participants conducting the dissolution trials wore eye protection. Also, everyone in the test room, including analysts collecting the air samples, wore respiratory personal protective equipment to filter out airborne enzymes.

## Experimental Results

Exposure while dispensing powdered detergent into a sink and filling with water. Results of air collections conducted following procedures outlined above for the product dispensing tasks are shown below.

| Test | Airborne Savinase Concentration (ng/m³) | |
|---|---|---|
| Pre-trial area check | none detected | |
| Product Dispense Trial 1 | 118 | |
| Cleanup Check | none detected | **Average** 212 ng/m³ ± 104 Standard deviation |
| Product Dispense Trial 2 | 218 | |
| Cleanup Check | none detected | |
| Product Dispense Trial 3 | 165 | |
| Cleanup Check | none detected | |
| Product Dispense Trial 4 | 387 | |
| Cleanup Check | none detected | |
| Product Dispense Trial 5 | 170 | |
| Cleanup Check | none detected | |
| Dispensing without sampler on | none detected | |

The airborne Savinase concentration measured during product dispensing and sink filling ranged from 165 to 387 ng/m³ with an average of 212 ng/m³. No airborne enzyme was detected after cleanup, indicating there is no carryover from test to test. The splatter test also indicated that the mini-blinds were effective in preventing enzyme contamination from splatter as the sink filled with water.

## Evaluation of Belin et al. Study and Previous Work by the Procter & Gamble Company

Swedish consumer exposure during dissolution of non-protected enzyme-containing product. The product used by Swedish consumers reported in the Belin paper was described as being "powdered" so it was different from the less-dusty detergent containing "granular enzyme." One can conclude from this information that the enzyme stock put into the detergents the Swedish consumers were using was the non-protected, powdered form of the enzyme used by the industry in the 1960s and early 1970s. Zetterstrom (1977), who later carried out follow-up work in an attempt to help evaluate the clinical history of the occurrences reported by Belin and others, gives a little more detail on the wash conditions he was using. In his studies, Zetterstrom used 50-gram doses of detergent in 14-liter capacity laundry wash basins containing 8 to 10 liters of water.

Based on this information, measurement of airborne enzyme during detergent dispensing into a sink was set up using non-protected enzyme, 50-gram detergent dispenses, and sink-fill volumes of 8 liters. The non-protected enzyme came from a micronization process typically used for lab preparation of granular detergents for analysis. During the micronization process, the product is

ground to a fine powder, reducing particle diameters to about 1.0 micron. Micronized enzyme could, therefore, be used to simulate the powdered enzyme-containing detergent formulations of the 1960s and early 1970s.

The air-flow rate used in the dispensing study is also a point that requires some perspective. The pumps used are air samplers that sample at about 330 to 400 liters per minute (min). For use with a 10-cm diameter filter, the cross-sectional flow rate through the filter is 300 to 400 liters/minute/filter area, or 300 to 400/((10 cm/2)$^2$ x 3.14), or (3.8 to 5.1 liters/min)/cm$^2$. Compared to the breathing rate and flow rate through the nose per area and assuming a 1-cm diameter for the nostril, a 10- to 16-liter-per-minute breathing rate per area would equal 10 to 16/((1 cm/2)$^2$ x 3.14) x 2 nostrils), or (6.4 to 10.2 liters/minute)/cm$^2$. The 10- to 16-L/min breathing rate is the rate specified by Hendricks (1970) for housewives doing light work during the laundering process. Clearly, the cross-sectional flow rate is slower for the air sampler at 3.8- to 5.1-liter/min/cm$^2$ than air flow through the nose at 6.4- to 10.2-liter/min/cm$^2$. Further, the room in which these studies were conducted had a total volume of 304,483 liters. Only 0.11 to 0.13% of total room air was sampled per minute using this sampling set up. A slower cross-sectional flow rate than the nose and sampling a tenth of a percent of the total room volume supports that using these pumps would not bias collected enzyme levels higher than what a person actually captures during breathing.

On the basis of the dispensing measurements, it is reasonable to assume that the Swedish consumers were exposed to enzyme concentrations at about the 212 ng/m$^3$ level.

Translation of Hendricks consumer exposure data to enzyme protein exposure terminology. There is a significant modification that must be made when evaluating the data presented in the Hendricks (1970). This change deals with the early assumption applied in the paper that respirable enzyme dust of 20 microns and smaller is the only material to be concerned with in regards to sensitization. Based on this occurrence, the Hendricks estimate for consumer exposure to enzymes must be expanded to include all particles that are collected at the breathing zone by the air sampler. Since there are no further data available on what percentage of total enzyme dust was collected at larger particle sizes, a best estimate is needed. This can be done by assuming that a proportional formula ratio of the enzyme protein is maintained in the total dust collected. Hendricks indicates that the level of 20 microns and smaller size enzyme dust particles collected during pouring is 1/8 lower than the level expected for the amount of detergent collected. Given this, the total enzyme ingredient dust that would be present in the detergent dust collected is 8 x 0.5 ng, or 4 ng enzyme ingredient @1.5 AU/gm (Note that enzyme activity is expressed here in the Anson Unit, AU). This translates to enzyme protein for a two-minute pour as (4 ng enzyme ingredient) x (1.5 AU/gm ingredient)/(30 AU/gram enzyme protein), or 0.2 ng airborne enzyme protein per two-minute pour. (Note that in calculating this number, the 30 AU/gm enzyme protein value comes from experimentally measured total protein determination of the ingredient as 5% and, thus, (1.5 AU/gm ingredient)/(0.05 gm protein/gm ingredient) = 30 AU/gm protein).

Converting this amount into a more meaningful concentration per cubic meter is difficult. Unfortunately, the Hendricks publication never directly indicates the volume of air corresponding to the detergent dust collections. Looking closely at some of the descriptions of the Bendix air sampler and taking into account Hendricks' emphasis of the importance of breathing rates throughout his publication, it is likely that the pump was chosen and set up to get closer to a breathing air sampling rate. He does indicate flow rates of 3 to 4 cubic feet per minute (CFM) for measuring collection efficiency and to compare to bellows-derived flows versus constant flow. Bellows-derived air flows were used to simulate breathing. Hendrick concluded that there is no difference in the airborne concentrations determined by either sampling system and that constant flow would suffice. This work was also done with Bendix air samplers. Therefore, it is likely that the air flow rates used to collect detergent dust were rates of 3 to 4 CFM. An average of 3.5 CFM was used in this assessment. This average flow rate translates to 3.5 CFM/(35.31 CFM/meter), or 0.0991 $m^3$/min. While the 3.5 CFM flow rate is still faster than the breathing rate emphasized in the Hendricks paper (0.0991 $m^3$/min. vs. 0.016 $m^3$/min.), the information in the paper does not indicate that the pump ever sampled at a lower rate. For a pouring time of two minutes, a total of 0.198 $m^3$ was sampled. Given this information, the airborne enzyme concentration during a two-minute product scooping and pouring of XK calculates to be 0.2 ng enzyme protein/0.198 $m^3$ or 1.01 ng/$m^3$.

1984 Procter & Gamble Company product comparison to Hendricks product data. Improvements to the encapsulation techniques used on enzyme stocks by suppliers and to the manufacturing handling process of enzyme stocks during detergent formulation dramatically reduced enzyme dustiness in P&G detergent products in 1984. Products manufactured during that time were evaluated for enzyme dustiness to dimension this reduction in potential exposure to the consumer. Studies were conducted on L and W formulations through pouring experiments in a galley dust box-type set-up. Results of these studies showed that enzyme levels generated during pouring were 30 to 70 times lower compared to the XK formulation used in the Hendricks study (4). For comparison purposes below, the more conservative value of 30 was chosen for the dustiness reduction factor provided by 1984 products.

Improved, less dusty enzyme ingredient materials available in 1993 and later. Around 1993 enzyme suppliers made improvements to the coating techniques used in enzyme prilling processes. These improvements substantially reduced the generation of enzyme airborne dust by these stock materials. Measurements of double-coated materials using the Heubach dust meter have shown that at least a 3- to 10-fold reduction of enzyme dust compared to the 1984 product was realized from use of enzymes prilled with these new techniques.

Product enzyme protein level calculation and protein dose per laundry use.

Belin Publication The level present in products used by the Swedish consumers was 0.3 to 1.0% of enzyme ingredient that was 2.0 AU/gram. A 2.0 AU/gram stock material contains (2.0 AU/gm stock material)/(30 AU/gm enzyme protein) = 0.0667 gm enzyme protein/gm enzyme ingredient material. The consumer product contained (0.3 to 1.0 gm enzyme ingredient/100 gm

detergent product) x (0.0667 gm enzyme protein/gm enzyme ingredient) = 200 to 667 µg enzyme protein/gm detergent product. Zetterstrom (1977) refers to 50-gram doses for laundry wash basin skin soak tests and other tests so the assumption is a minimum of 50 grams per dose. The calculated protein dose in a 50-gram detergent use is 10,000 to 33,500 µg protein. In the airborne enzyme measurements, the product used had 667 µg enzyme protein/gm detergent so the total dose = 667 µg protein/gm detergent x 50 grams detergent = 33,350 µg enzyme protein.

1969 – 70 Product, XK: The level of enzyme in XK was 1.2 % of a 1.5 Anson Unit protease per gram of detergent product. This translates to (1.2 X $10^{-2}$ grams)(1.5 AU/gm detergent product)/(30 AU/gm enzyme protein) = 600 µg enzyme protein/gm detergent product. The Hendricks (1970) refers to a 1-cup dose. The density of the product (HK formulation) was 0.33 gm/cc, which translates to 78.1 gm/cup. The total protein per laundry use is (78.1 gm product/use) x (600 µg protein/gm product) = 46,860 µg protein.

1984 Product, L and W: The level of enzyme in L and W was 1.7 X $10^{-2}$ Anson Units per gram of detergent product. This translates to (1.6 X $10^{-2}$ AU/gm detergent product)/(30 AU/gm enzyme protein) = 567 µg enzyme protein/gm detergent product. Recommended laundry dose was 1 cup for normal wash loads. Product density was 3.4 oz./cup, which translates to 96.4 gm/cup. The total protein per laundry use is (96.4 gm product/use) x (567 µg protein/gm product) = 54,659 µg protein.

1993 Procter & Gamble products: In 1990, P&G began reporting the enzyme protein concentration in all detergent products and used micrograms per gram of detergent product as the units. The detergent products in 1993 contained up to 340 µg enzyme protein/gm detergent product with a scoop assisted delivery of about 65 grams recommended for normal load. The recommended dose then was equivalent to 340 µg enzyme protein/gram detergent x 65 grams = 22,100 µg protein use.

Comparison of dustiness and exposures per use. The data below can be used to compare airborne enzyme exposures of Swedish consumers in the publication to consumers using P&G products since 1970. Using the most conservative data from each comparison above (e.g. L is 30 x less dusty than XK, not 70 times), the relative enzyme dustiness is shown below. Note that this is relative dustiness based on the assumption that in cases where dispensing the same amount of product but with a higher level of enzyme or dispensing different amounts of product, the enzyme dose differences per use will result in proportionally different enzyme dustiness responses. For example, if you use twice the amount of enzyme per dose then the dustiness will be twice as much.

**A comparison of relative dustiness from recommended or published uses**

| Time Period | Enzyme Form | Product Level (µg Enzyme Protein per gm Product) | Relative Decreased Dustiness* |
|---|---|---|---|
| mid 1960s to early 1970 | unprotected, powder | 200 to 667 (used 667) | 1 x |
| 1970 | granulated | 600 | 189 x |
| 1984 | prilled | 567 | 5,670 x |
| 1993 | double-coated prill | 340 | 28,367 x |

* Dustiness Time Period X/Dustiness in mid-1960s to early 1970s

The relative dustiness for the Swedish consumer exposure, compared to the dustiness in the Hendricks consumers' exposure scenario, was calculated by comparing the exposure values for each and factoring in the dose relation. Thus, (212 ng/m$^3$)/(1.01 ng/m$^3$) x (enzyme level in Hendricks/enzyme level from Swedish consumer product) = 133.3 x (600/667) = 189. The product used by the consumers in the Hendricks study was 189 times less enzyme-dusty than that used by the Swedish consumers in the Belin study. The 1984 product's relative dustiness was calculated using the 30-times-less-dusty determination only. This is due to the enzyme level differences between the Hedricks and L/M formulas being compensated for by their direct comparison of dustiness via equivalent product weight use in the Galley Dust Box measurements. Thus 1984 detergent is (189 factor) x 30 = 5,670 times less dusty than the Swedish consumer product. Concentration is factored into calculating relative dustiness for the 1993 product. The 1993 detergent is (5,670 factor) x 3 x 567/340 = 28,367 times less dusty than the Swedish consumer product.

**A comparison of calculated exposures from recommended or published uses**

| Time Period | Enzyme Form | Detergent Dose (grams) | Protein Dose/use (µg) | Exposure During use (ng/m$^3$) |
|---|---|---|---|---|
| mid 1960s to early 1970 | unprotected, powder | 50 | 33,350 | 212 |
| 1970 | granulated | 78.1 | 46,860 | 1.01 |
| 1984 | prilled | 96.4 | 54,659 | 0.042 |
| 1993 | double-coated prill | 65 | 22,100 | 0.0057 |

The Swedish consumer exposure value of 212 ng/m$^3$ and the Hendricks consumer exposure value of 1.01 have been discussed. The exposure from use of the 1984 product is calculated by factoring in the 30 times less enzyme dusty result but with more product being used. The factor of 30 takes into account the protein concentration differences such that only the amount of product being dosed impacts further the exposure calculation. Thus, the 1984 product use exposure to enzyme is calculated as being (1.01 ng/m$^3$) x (96.4/78.1)/30 = 0.042 ng/m$^3$. The exposure from use of the 1993

product is calculated by factoring in the 3-times-less-dusty enzymes used in 1984 and the protein dose ratio of the 1984 to 1993 products. The exposure calculated for a recommended use of the 1993 product is (0.042 ng/$m^3$) x (22,100/54,659)/3 = 0.0057 ng/$m^3$.

## Discussion

Several papers have been evaluated in order to discern a best estimate of exposure to enzyme aerosol during product use. In particular, the Belin (1970) paper reports on consumers in the 1960s who were sensitized to enzymes in the detergents they were using for laundering. As this is a recorded sensitization event, an exposure level would be very useful in carrying out risk assessments. The work reported here simulated those use conditions with a product containing non-protected enzyme as was used at the time of Belin's Swedish consumers' experiences and determined a value of 212 ng/$m^3$ as the best estimate of exposure. The Hendricks (1970) paper also provides an opportunity to assess exposure and relative product dustiness at the time that enzyme encapsulation processes were applied to reduce enzyme dustiness. While several assumptions had to be made in order to derive an exposure in ng/$m^3$ terms, a value of 1.01 ng/$m^3$ was determined. From these exposure values and through use of relative product dustiness measurements and dust reductions with improved encapsulation processes, the relative dustiness of today's products and exposures from their use by consumers can be calculated. Comparisons show that, relative to what Swedish consumers were using in the 1960s, today's granulated detergents are about 28,000 times less enzyme-dusty and, at recommended uses, the consumer will be exposed to approximately 0.0057 ng/$m^3$ of enzyme, that is, about 37,000 times less than in the 1960s.

Finally, it should be pointed out that the comparison above is based on the most conservative assessment of changes in dustiness. This includes using:

1. A factor of 8 applied to the Hendricks result for measured enzyme exposure during 1-cup dispensing to compensate for his reporting only enzyme dust that was 20 microns in size or smaller.
2. Applying the 30-times-less-dusty factor of L and W relative to XK even though results showed range from 30 to 70 times less dusty.
3. Applying the 3-times-less-dusty factor for the improved enzyme prilling process even though results show improvements range from 3- to 10-times-less-dusty prills.

# Appendix 2 — Enzyme Risk Assessments of Hand-Laundering Practices

This appendix describes approaches that were employed by the Procter & Gamble Company to assess risk from potential exposure to proteases during hand laundering. This work was conducted as part of a program to determine if enzymes could be safely introduced into products used in hand laundry applications.

## Summary

The risks from using an enzyme-containing product for hand laundering were evaluated prior to market introduction. Two types of products commonly used for hand laundering were developed to meet consumer expectations, a granular product and a bar product. Potential risk was evaluated from estimates of exposure of worst-case hand-laundering practices, comparison of the exposure to known benchmarks to characterize risk, and a clinical study to confirm safety under actual use conditions. The results of the exposure estimate, risk assessment and clinical study led to the conclusion that the product was safe and could be marketed (Cormier et al., 2004).

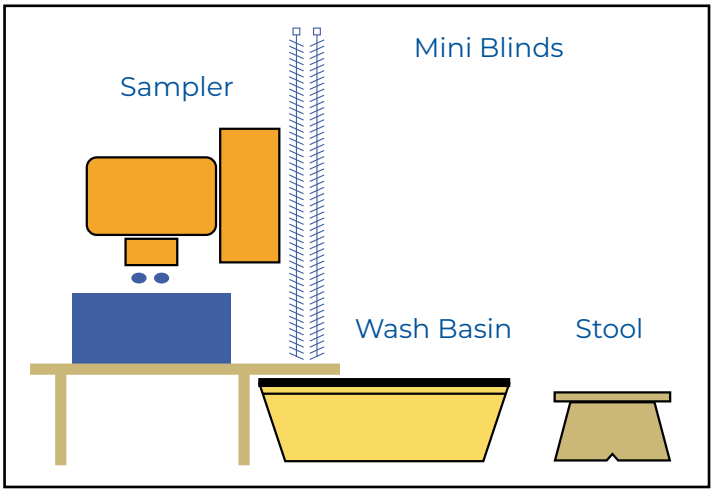
Estimated exposure ranged from 0.06 to 0.18 ng protein/$m^3$ for granular products and 0.004 to 0.026 ng/$m^3$ for laundry bar products. These estimated exposures were about one-fifth that of a relevant no-effect benchmark of 1 ng/$m^3$, which had been established for machine-laundering applications. Based on this comparison, the risks from intended use of these products were determined to be acceptable. A clinical study was then conducted to confirm this risk decision for the intended use of the products and to assess potential unintended uses. In the clinical study, nearly 500 subjects used the products in their homes as they normally would use their non-enzyme-containing product. After two years of use, none of the study subjects developed IgE antibodies to the enzymes used in the enzyme-containing laundry product. These results confirmed the risk decision based on the analytical estimate of exposure and provided additional reassurance for unintended use. Based on the assessment of the intended use and the results of the clinical study, the product was judged to be safe and the product was introduced into the market.

## Estimates of Potential Exposure

Hand-laundering practices vary significantly from region to region according to local practices and conditions. After evaluating the range of practices where market introductions were being considered, it was determined that Philippine hand-laundering practices were worst case by virtue of frequency, duration and practice.

Consumer-relevant airborne enzyme exposure was determined by air collection at the user's breathing zone and measurement of the enzyme concentration present in the volume of air collected during the experiment. Air collections were taken while the user washed a sample of clothes using typical Philippine hand-laundering practices. Enzymes were measured using immunospecific detection and quantitation of the detergent enzyme in the collected air samples.

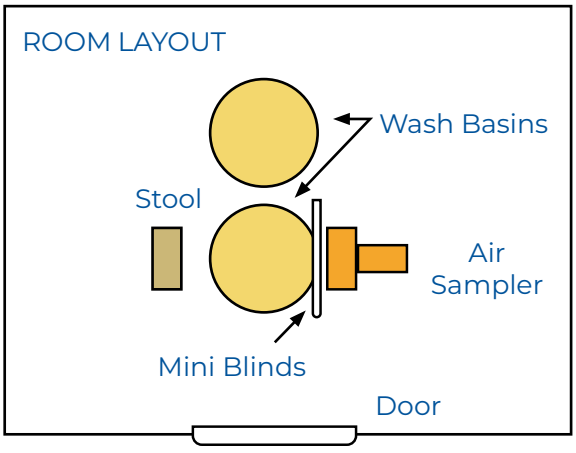
Air collection and splatter protection. Air collection was carried out using a General Metal Works type HV2000P air sampler (General Metal Works, Inc., Cleveland, OH, USA) fitted with a GF/C glass fiber filter and calibrated to a flow rate of 0.67 $m^3$/min. The air sampler was positioned at breathing zone




height, perpendicular to the front of the person as they sat on the stool performing the wash trial and facing the wash basin area. Two sets of adjustable mini-blinds were positioned immediately in front of the air sampler. One set of blinds was opened at a 315˚ angle relative to the air sampler intake. This set of blinds touched the air sampler. The other set was positioned next to the first set and touching but with the blinds angled in an opposite direction at a 45˚ angle relative to the sampler intake. In this manner, the blinds would act as a deflector for splashing or splattering of diluted product as washing and rinsing occurred without interrupting air flow. This set-up has been used effectively in a number of washing scenarios.

Enzyme measurement. Solutions prepared from air collections were analyzed by an Enzyme-Linked Immunosorbent Assay (ELISA) to quantitate enzyme protein present. The ELISA method was a modification of the method described by Miller et al. (1994).

Hand laundering. Trials were conducted to simulate habits and practices of Philippine hand-laundering practices. The trials took place in a square shaped room (10 ft. x 8 ft. x 9 ft.) with little to no air circulation. Hand laundering was conducted in a wash basin while squatting or sitting on a stool. The layout on the right shows two wash basins. Air sampling began with the panelist starting the laundering process and continuing through the 10-minute wash period.



Results of Trials to Estimate Exposure

A total of eight hand-laundering trials were evaluated for airborne enzyme production under Philippine hand-laundering conditions. These trials took place using five panelists.

| Product | Estimated Enzyme Protein Exposure (range of values obtained) |
|---|---|
| Laundry Bar | 0.004 to 0.026 ng/m$^3$ |
| Laundry Granule | 0.06 to 0.18 ng/m$^3$ |

The maximum airborne concentration achieved was generated with the granular laundry product. The reasons for this include: 1) the dosing concentration of granular enzyme per laundering event was higher than that for the laundry bar, and 2) the rate of total enzyme delivery was immediate for the granular enzyme versus slow for the bar as it was used.

Risk Assessment of Hand-Laundering Use

The enzyme used in the hand-laundering formulation was a subtilisin protease used in machine laundry detergent formulations. Thus, comparison of exposures to machine use were relevant to exposure estimates for the hand wash formulation. The maximum exposure to enzymes during hand laundering was 0.18 ng/m$^3$, which was one-fifth the value of the no-adverse-effect benchmark of 1 ng/m$^3$ established from previous experience with machine laundry products and practices.

Based on this comparison, the conclusion was that the use of enzymes formulated into hand-laundry products would not pose a higher risk than existing uses of enzyme-containing laundry products.

Clinical Evaluations of Hand-Laundry Product

In hand-laundering geographies, the product used for cleaning clothes is often used for other purposes in the household as well. These alternative uses could also generate exposures to enzymes that may ultimately have an impact on the risk decision. Though these uses are generally infrequent, they are difficult to predict and simulate in a laboratory setting. To address these potential alternative uses, the products were tested in a clinical study where panelists were encouraged to use the product as they normally would and the health status was followed with clinical endpoints.

For this study, 500 atopic Philippine women were enlisted and followed over two years of use of the enzyme-containing product. Results of the study showed that none developed enzyme-specific IgE antibodies, even among women with compromised skin caused by the harsh laundering practices of the Philippine population (Cormier, 2004).

## Conclusion

The results from the estimates of potential exposure compared favorably with a relevant no-effect benchmark of exposure from machine laundering. The worst-case exposure from hand-laundering practices was one-fifth that of the no-effect benchmark. Based on this assessment, the risk for potential exposure to enzymes from the intended use of this product was determined to be acceptable. A clinical study confirmed this decision and also established that the risk from alternative uses of the product would also likely be safe. The product was introduced to the market with a surveillance program and no adverse reports occurred after introduction.

This appendix summarizes a risk assessment example for a specific enzyme-containing laundry pre-treater product. A variety of laundry pre-treaters containing enzymes have been produced and sold worldwide since the mid-1990s. Although there have been no indications of allergic symptoms among consumers, previous work had indicated the potential to produce significant concentrations of enzyme in the air using trigger sprayers (Battelle, 1999; Battelle, 2000). A study was conducted by the Soap and Detergent Association (SDA) to characterize aerosols to which a consumer could be potentially exposed from a trigger spray containing a prototype enzyme laundry product. For the purpose of this study, a prototype, non-commercial water-based formulation containing 0.5% protease enzyme was used. Total enzyme aerosol from product application and product bounce back was measured. Exposure of protease in the air ranged from 67 to 121 ng/$m^3$ over a 10.5-minute period of simulated product use (Battelle, 2000).

Spray Pre-Treater Case Study (Weeks et al., 2011): A specific product was designed using components that were intended to reduce aerosolization of enzyme, in addition to other requirements intended to assure efficacy in order to address the airborne level enzymes generated in the SDA study. The enzyme Savinase was used in the new formula at less than half the enzyme level used in the prototype product discussed above. Dynamic viscosity measurements were used to monitor changes in the formula, and a sprayer was chosen to provide a relatively coarse spray with few small droplets. Direct measurements of the spray (using a laser-based instrument) produced by the product gave a preliminary indication that few small droplets were produced, but an exposure study was required to investigate the level of exposure under use conditions.

The exposure study protocol included the following elements:

- A chamber measuring 8 ft. x 8 ft. x 8 ft. was constructed to simulate a small laundry room (14.5 $m^3$).
- Ventilation was not allowed during experiments, but a large volume of air was used to flush the chamber after each run.
- To simulate heavy use, the product was applied to a series of six fabric targets held vertically over a washing machine. Five sprays (each a single stroke of approximately 1 g) were applied to each target at a distance of 6 inches. The sprays were applied at a rate of 1 per second, with a 10-second lag between targets. Total time of application was 1.3 minutes. The vertical position of the fabric was to maximize bounce back of enzyme aerosol into the potential breathing zone of the user.
- Particle size distributions were recorded with an aerodynamic particle analyzer.
- Enzyme concentrations in the air were sampled by drawing the air through PTFE membrane filters. The enzyme was measured via ELISA.
- Sampling began 1 minute before application, and ceased 10 minutes after applications began to capture bounce back.

- Each experiment was replicated eight times.
- This type of product was typically expected to be used once or twice per week. The expected duration of exposure ranged from seconds per use to a few minutes per use.

## Exposure Results

Although the trigger sprayer and formulation were chosen to minimize the formation of small particles, a few small particles were still produced through a combination of the break-up of larger particles in the spray stream and impact on the fabric target. Measurement of the particles that reached the expected breathing zone of the user showed that the mean particle diameter was less than one micron. The particle sizes in the air also rapidly decreased over time, because of the settling of larger particles and evaporation of the water carrier. The mean concentration of enzyme in air was 14.5 ng/m$^3$ (+/– 1.06 ng/m$^3$) (Weeks et al., 2011). This level was approximately 4 to 9 times lower than the range of exposures measured for the higher enzyme-containing product tested in the SDA study. In addition, this level of enzyme aerosol is specific for the tested product and may not represent aerosol levels that can be generated by other spray pre-treat products.

## Comparison with Other Enzyme Exposures

Comparison of the measured aerosol level from the spray pre-treat products with several other enzyme exposure scenarios is presented in Table 1. The enzyme used in the new laundry pre-treater product, Savinase, has essentially the same antigenic potency as the benchmark enzyme, Alcalase, and it has a safe history of use in detergent products (Pepys et al., 1973; Schweigert et al., 2000; Zetterstrom, 1977). The proposed pre-treater product tested by Weeks et al. (2011) generated aerosol levels that were significantly less than the estimated aerosol level associated with use of a dusty detergent product known to cause adverse effects in an occupational setting, and slightly higher levels compared to the high end of exposures estimated for laundry detergents with an acceptable safety record when used by consumers. Currently marketed detergent products generally produce much lower enzyme aerosols upon use (<1 ng/m$^3$) than the maximum values presented here (Weeks et al., 2011). Note also that inhalation exposure to the consumer products typically occurs for a period of seconds to several minutes, while workplace exposures tend to extend over longer periods of time.

| Case | Enzyme Concentration in Air (ng/m$^3$) |
|---|---|
| Laundry Pre-Treater (Battelle, 2000) | 67 to 121 |
| Laundry Pre-Treater (Weeks *et al.*, 2011) | 14.5 |
| ACGIH (TLV) | 60 |
| DMEL | 60 |
| Early (Unacceptable) Detergents | 212 |
| Improved Detergents (~1970+) (max.) | 1 |

## Clinical Experience with the Spray Pre-Treat Product

A six-month prospective clinical study was conducted to confirm the safety profile of the spray pre-treat product (Weeks et al., 2001b). Approximately 100 subjects with verified allergies to common substances (atopics) were included in the study. The subjects were asked to use the product on a daily basis and in a manner similar to what was tested in the laboratory (hold fabric in vertical position, 5 sprays/fabric). Subjects were tested for the presence of allergen-specific antibodies to the protease at three and six months by skin prick testing. At the conclusion of the study, no subject became prick-test positive to the enzyme.

In addition, the use of other marketed enzyme-containing spray pre-treat products by consumers has not indicated any potential for allergic symptoms as reported in the literature. Although data of this type cannot be expected to be very sensitive, the years of experience indicate that these products are not causing allergic symptoms in the general public.

## Conclusion

In conclusion, the relatively low level of enzyme aerosol generated by use of the spray product (14.5 ng/m$^3$) in combination with the lack of sensitization to enzyme observed in the six-month clinical study supports the safety of this enzyme-containing product. In addition, the overall lack of literature reports of allergy associated with previously marketed spray pre-treat products further supports the safety of this product for commercialization. Post-market surveillance, including a toll-free number for consumer comments, questions and complaints, and a database including information on all consumer calls, provides the manufacturer with a mechanism to monitor for any indication of allergic symptoms or other problems.

## Industry Initiatives

Based on the above studies the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) prepared a protocol for how to do risk assessment of enzyme-containing spray products (A.I.S.E., 2013).

The potential enzyme exposure (expressed as concentration of airborne enzymes) of consumers and professionals derived from the use of household cleaning spray products should be evaluated to demonstrate safety prior to marketing. Experience from more than 10 years of testing such products show that the exposure is dependent on a number of different parameters, e.g. formulation, enzyme concentration in product, habits and practices of the consumer and nozzle device. High viscosity formulations and foam-sprays would be expected to generate lower enzyme exposure than liquid formulations of low viscosity. However, each product and application of use will need an individual safety assessment based on actual exposure data, independent of such considerations.

**For more information please go to:**

american cleaning institute®

www.cleaninginstitute.org


www.aise.eu