amfep
association of manufacturers & formulators of enzyme products

Guide to the safe handling of industrial enzyme preparations

2013

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List of Contents

INTRODUCTION

In this document, Amfep provides information on the safe handling of enzyme preparations for industrial use and on safety assessments in regards to consumer use.

The characteristics of enzymes, inherent hazards of enzyme preparations and possible health risks from exposure are discussed. Methods to minimize these risks are presented.

Included is a review of the requirements on enzyme products set by the European REACH legislation (EU, REACH Regulation, 2006).

For more specific safety information on a particular enzyme preparation, please refer to the relevant Safety Data Sheet and to the warning label required by the applicable legislation (REACH, CLP) (EU, CLP regulation, 2008), and the preceding documents Directive 67/548/EEC on "Dangerous Substances" and Directive 1999/45/EC, on "Dangerous Preparations", all as amended).

Furthermore, the enzyme suppliers can advise or provide more detailed information and support related to the specific enzyme preparation and the handling and consumer use situation.

WHAT ARE ENZYMES?

Enzymes form a special class of proteins, composed of the amino acid building blocks found in all other proteins. Proteins in general are natural substances produced by all living cells. All living organisms – be they man, animal, plant or microorganisms – require enzymes to conduct virtually all physiological processes which are essential for growth and life.

Enzymes act as catalysts, substances which in very small amounts are able to accelerate the rate of specific chemical reactions a million times or more. Consequently, enzymes are able to speed up the building up or breaking down of organic matter such as carbohydrates, fats and proteins. Enzymes are highly specialized in their catalytic properties. Each enzyme acts only on a restricted number of compounds (substances) and, when acting, catalyses only one specific reaction. For example, proteolytic enzymes (proteases), e.g. present in the human digestive system, break down proteins into smaller molecules which can then be absorbed into the blood stream.

The properties of enzymes make them very useful in catalysing desired reactions in industrial processes. Consequently, enzymes are extensively used in several industries within the technical (e.g. detergent, starch, textile, and fuel alcohol), food (e.g. dairy, baking, brewing, wine and juice) and animal feed area.

Commercial microbial enzyme preparations are derived from bacteria, yeasts or fungi. Industry uses pure cultures of selected non-pathogenic strains of microorganisms (Enzyme REACH Consortium, 2009) to produce specific enzymes by fermentation techniques.

POSSIBLE RISKS INVOLVED IN CASE OF EXPOSURE

Industrial enzymes have a low toxicity in humans. Enzymes present no concern for endpoints like acute toxicity, genotoxicity, sub-acute and repeated dose toxicity, reproductive toxicity and carcinogenicity (Basketter, 2012a) (Basketter, 2012c).
The exceptions to the safety profile of enzymes are the ability of some proteases to cause irritating effects at high concentrations, and the intrinsic respiratory sensitization potential of all industrial enzymes. Sensitization may cause respiratory allergy in individuals exposed repeatedly to sufficiently high airborne concentrations of enzyme dust or aerosols.

- **Allergy by inhalation**

Like many other proteins, enzymes may act as allergens. For development of an allergy a two-step process will have to take place: sensitization and elicitation (Basketter, 2010a). When allergens are inhaled in the form of dust or aerosols they may give rise to the formation of antibodies specific to them. This process is called sensitization. At this stage the sensitized individuals do not suffer from any allergy symptoms. Sensitized individuals may develop an allergy via the elicitation step, if they are exposed to sufficiently high airborne concentrations of enzyme (Basketter, 2012b). At this stage the individuals will have developed the typical symptoms for respiratory allergy such as hay fever or asthma. When this condition is due to exposure in the working environment, it is called occupational allergy.

The respiratory symptoms from allergen exposure may include itching of nose and eyes, nasal and sinus congestion and sneezing. Coughing, hoarseness, tightness of the chest and shortness of breath are indicators of asthma. These symptoms may occur during or after working hours and they will disappear within hours or a few days after the exposure has ceased. Allergy symptoms may be similar to those of the common cold, and if such symptoms occur frequently at the work place and only rarely in week-ends or during holidays, they may be the result of enzyme exposure.

Some individuals are more prone to become sensitized than others. Atopic individuals, i.e. persons already allergic to one or more of the common allergens like grass pollen or cat dander, may develop an enzyme allergy more easily than others. Not all atopic individuals will become allergic to enzymes, and conversely, non-atopic individuals may develop an enzyme allergy if exposed to sufficiently high airborne concentrations of enzyme.

Smokers have a markedly increased risk of becoming sensitized and developing allergy symptoms (Johnson, 1997).

As the risk of sensitization correlates to the concentration of inhalable enzyme particles, the formulation of the enzyme product and how the enzyme product is handled is very important. Liquid enzyme products can be handled with low release of inhalable enzyme particles, but improper handling processes or inappropriate equipment may create significant levels of airborne enzyme particles in aerosols. Granulated enzyme products must be handled very carefully to ensure the integrity of the granulates. If the granulates are broken, this may result in airborne exposure levels that may give rise to the development of sensitization and allergy. Powdered formulations of enzyme products inherently have the potential to give rise to airborne concentrations of enzymes that will result in the development of allergies, unless such enzyme products are handled in closed processes with the use of local exhaust ventilation and respiratory protection for product transfers and equipment cleaning.

Apart from respiratory allergy, brought about by inhalation, people can in general become sensitized to certain substances by skin contact or acquire an allergy by ingestion (food allergy). However, there is no scientific evidence that enzymes are associated to allergy caused by skin contact or via ingestion (Basketter, 2008), (Bindslev-Jensen, 2006).

- **Irritation**

Enzyme preparations containing proteolytic enzymes are classified as irritating to eye and skin. Other enzyme classes; e.g. amylases, lipases and cellulases are essentially free from any irritating effects to both eye and skin (Basketter, 2012a), (Basketter 2012c).

The risk of developing such irritation depends on a number of factors such as the duration of the exposure, the enzyme concentration in the material, and on the size of the body area that is exposed. In addition, body areas where perspiration occurs, such as hands, armpits, groin and feet or under a face mask are areas where irritation is most likely to appear. The combination of proteolytic enzymes with surfactants may results in a stronger irritative effect than what can be accounted for by the individual contributions from the constituent parts (HERA, 2005), (HERA, 2007).

OCCUPATIONAL AND CONSUMER EXPOSURE LIMITS UNDER REACH

Within the European legislation commonly referred to as REACH (Registration, Evaluation, Assessment and Restriction of Chemicals), there is a requirement to define acceptable exposure limits in order to ensure safe application (REACH, 2006). Such an exposure limit is often established as a derived no effect level (DNEL), however, where a DNEL cannot be established, which is the case for respiratory sensitizing substances like enzymes, a derived minimal effect level (DMEL) is recommended. Contrary to a DNEL limit, the DMEL identifies a level at which exposure may result in a limited degree of response; it does not define a no adverse effect level (Basketter, 2010a).

The occupational DMEL for enzymes in general has been established based on the success of the exposure limit of 60 ng/m^3 for pure enzyme protein suggested by the American Conference of Governmental and Industrial Hygienists (ACGIH, 2004). This exposure limit is also adopted by several national authorities (AISE, 2013), however, it should be noted, that in the UK, an occupational exposure limit of 40 ng/m^3 is required for proteases (HSE, 2002).

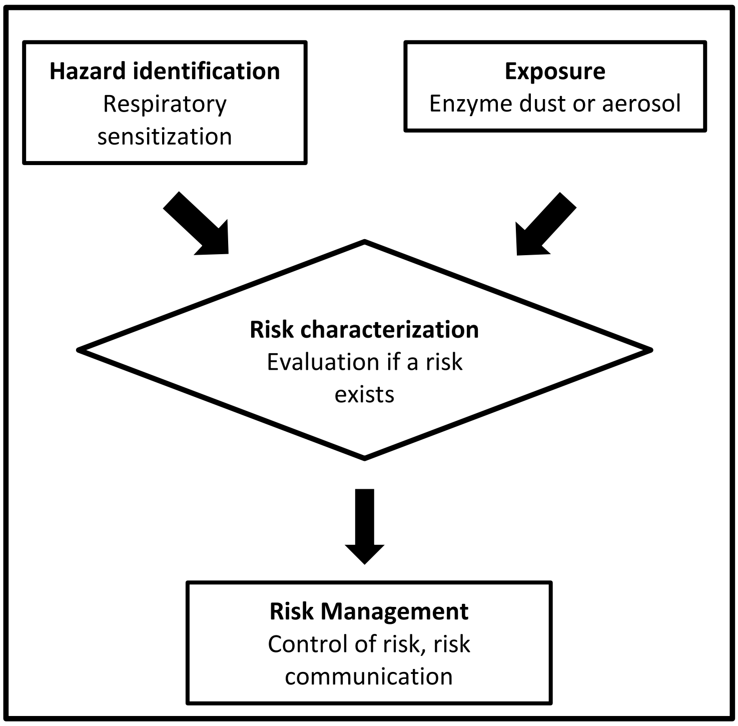
For consumers a DMEL of 15 ng/m^3 for pure enzyme protein can be adopted as the starting point for new and existing enzymes for which there are no other data to indicate that a different value may be more appropriate (Basketter, 2010a).

According to REACH, it is a requirement that a Chemical Safety Report (CSR) including exposure scenarios is made for all technical applications of REACH registered enzymes which are produced in volumes more than 10 tons per year. The exposure scenario must document the safe use of the enzyme in the specific application, which in relation to respiratory allergy means that it must be documented that the airborne enzyme exposures are kept below the DMEL values.

DMEL for occupational exposure: 60 ng pure enzyme protein/m^3
DMEL for consumer exposure: 15 ng pure enzyme protein/m^3

RISK ASSESSMENT AND RISK MANAGEMENT

A risk assessment and risk management process can be illustrated in the below process flow (AISE, 2006):



Hazard identification: As stated previously the most significant hazard identified is allergy due to exposure via inhalation. However, irritation may also need to be addressed if proteolytic enzymes are applied.

Exposure assessment: Exposure assessment establish the amount of enzyme to which the user may be exposed during the handling process or intended use. Also foreseeable misuses and accidents should be addressed.

Risk characterization: Risk characterization is the examination of the relationship between the exposure and the exposure limit or DMEL value. The exposure limit may be different than the DMEL value due to regulatory conditions or due to information from various benchmark exposures.

Risk Management: The risk management process is the process of reducing the risk by lowering the exposure and by communicating the risk efficiently to the appropriate audience.

- **Occupational**

Precautions against respiratory sensitization

Very detailed guidelines for control of enzyme exposure in production facilities can be found in "The international Association for Soaps, Detergents and Maintenance Products. Guiding principle for the safe handling of enzymes in detergent manufacture" (AISE, 2013) and in (US SDA, 1995). These guidelines were developed for the detergent industry, but the principles stated are generally applicable. An example of how to conduct a control program for enzyme exposure in a detergent manufacturing plant is given in (Schweigert, 2000). The overall conclusion in this example is, that the

containment of enzymes via encapsulation, i.e. the use of granulate formulation of the enzyme product, combined with improved hygiene is a prerequisite for controlling airborne enzyme particles at the work place. Documentation of health problems as a result of failed exposure control and hence failed risk management is given in (Cullinan, 2000).
For the occupational conditions the exposure limit will in most instances be the DMEL of 60 ng/m^3, but other limits may be valid due to regulatory conditions or internal policies. To ensure that the exposure is below the DMEL it has to be controlled, and this can be done by a number of precautions:

1. Technical measures ("engineering controls")

Airborne dust and aerosols may be formed through high-energy operations, such as mixing, grinding, washing with water-pressure or steam and when using compressed air for cleaning operations[1]. Dust or aerosol formation can be limited by using equipment designed to minimise damage to enzyme granulates. Rooms and locations where enzymes are handled should be well ventilated. At mixing and filling sites and other operations that can create dust and aerosols, local exhaust ventilation should be installed, and ventilation systems should be equipped with filters or other proper control of the exhaust. Process equipment should be enclosed to limit aerosol spreading.

Cleaning should be conducted exclusively with low pressure water and equipment should be designed to exclude splashing. For "dry" cleaning, vacuuming with equipment fitted with high efficiency filters (HEPA-filters) is required.

Monitoring of the enzyme exposure in the air can be used to evaluate the effectiveness of the engineering controls. Monitoring can be conducted either regularly or at certain intervals. The enzyme supplier can provide guidance and assistance on how to monitor enzyme exposure in production facilities.

2. Careful handling

In order to avoid exposure to enzyme preparations it is important to use handling practices that do not generate dust or aerosols. Dust or aerosols can develop during spillage, material transfers, spraying, cleaning with high pressure water, milling or grinding of large particles into smaller ones, exposure of larger particles to mechanical force, pumping of air though a liquid, vigorously stirring of a liquid, allowing a liquid to dry out after spillage, etc.
Sweeping, blowing, splashing, spraying, steam cleaning and high pressure water flushing must therefore be avoided. Any operation which might create dust or aerosols should take place in areas that are provided with adequate exhaust or other forms of mechanical systems.
In terms of handling granulated enzyme products, the most important measure to avoid enzyme exposure is to ensure the integrity of the enzyme granulates. In essence, if the granulates remain intact, there will be no exposure from them.

3. Cleanliness

Even if no dust is visible, it is important that containers, surfaces and equipment that have been in contact with enzymes are cleaned by wet washing or vacuum-cleaned with a high efficiency filtered vacuum system.
High pressure cleaning should not be used. Work clothes should be changed daily and whenever they are grossly contaminated (e.g. after a spill) with enzyme material.

4. Personal protective equipment

Primary protection methods against enzyme exposure are the use of ventilation, engineering control and work processes to control exposures below the DMEL. If the primary protection against

[1] Compressed air should not be used for cleaning operations; instead use wet cleaning with low water pressure or vacuum equipped with high efficiency filters.

respiratory enzyme exposure is in place face mask equipped with P3 filter will not be necessary. However, certain operations and situations require the use of respiratory protection such as spill clean-up, during cleaning or repair of machinery contaminated with enzymes or when air monitoring has indicated a high enzyme level. In such cases, respiratory protection approved and selected for use against dust or aerosol should be used; i.e. face masks equipped with P3 filters. The respiratory protection must be checked for proper fit and function each time it is used. Gloves and goggles may be appropriate protective measures in such situations as well.
Personal protective equipment should be worn during the clean-up or repair, and until the end of the clearing time, or until air monitoring data show that the exposure is below the DMEL. Clearing time can be defined according to the size of the room and according to the capacity of the ventilation system in the room.

Precautions against skin and eye irritation

In order to minimize the risk of irritation to eyes and skin, the following precautions should be taken:

1. <u>Personal protective equipment</u>

Under normal handling procedures, safety glasses and simple cotton clothing is recommended and will give the necessary protection. When handling slurries and during maintenance, repair and cleaning-up major spillages under wet conditions, protective clothing, safety glasses, impermeable gloves and respiratory protection (face mask equipped with P3 filter) is recommended.

2. <u>Careful handling</u>

It is important to use handling practices that prevent direct contact with the skin. Operations which may create spillage and splashing must therefore be avoided. Rubbing of the face and eyes should be avoided when wearing protective gloves that are soiled with enzyme.

3. <u>Cleanliness</u>

Equipment, containers and surfaces that have been in contact with enzymes should be cleaned. Personal cleanliness is also essential to prevent irritation of skin and eyes. Therefore, hands should be washed with plenty of water and mild soap before and after each visit to the lavatory, before leaving the work area and immediately after coming into contact with the enzyme materials. A plan for changing clothes and undergarments should be in place and adhered to.

Measures in case of accidents

<u>Spillage</u>
Spilled enzymes must be removed immediately by vacuum equipped with a high efficiency filter, mopping or washing. In order to avoid dust or aerosol formation during cleaning, do not sweep and do not use high water pressure, steam, or compressed air on spills. Use plenty of water to flush all enzyme material away in order to prevent enzyme dust being generated from dried-up material. Depending upon the place and extent of the spill, respiratory protection and protective clothing should be used during cleaning. The area should be evacuated during the clean-up of the spill and until the exposure is below the DMEL or until the end of the clearing-time.

<u>Machinery malfunction and repair</u>
In case of machine maintenance, cleaning should be arranged before repair work is commenced. Respiratory protection and protective clothing may be needed. Cleaning of enzyme-contaminated components should be performed by adequately trained personnel and not by any mechanic means.

Medical treatment in case of exposure

In case of symptoms indicate the possible development of respiratory allergy, a physician should be consulted. The physician can test whether the symptoms are likely to be due to enzyme allergy via a skin test or via a RAST/CAP test (blood-sample).The enzyme supplier can be contacted for test material to conduct skin test or for arranging a RAST/CAP test.

- **Consumer**

Consumer enzyme exposure is expected to be significantly lower than the exposure in occupational settings both in terms of airborne concentration and duration of the exposure (Basketter, 2012c). However, contrary to the occupational situation the consumer exposure will not be subject to the same control mechanism as for the occupational exposure, and hence, it is critical that a risk assessment which considers the complete picture of the potential for consumer enzyme exposure is made.

A detailed guideline on how to conduct a risk assessment on enzyme containing consumer products is given in (SDA, 2005), whereas a more general introduction to the subject is given in "The international Association for Soaps, Detergents and Maintenance Products; Guiding principle for the safe handling of enzymes in detergent manufacture" (AISE, 2013).

The use of enzymes in detergent products is by far the most widespread source for consumer exposure, and the consumer safety in this enzyme application has been documented in various papers, e.g. (Rodriguez, 1994) and (Sarlo, 2010). Studies of the exposure from the earliest enzyme containing detergent products and the detergent products of today have shown that the consumer enzyme exposure has been reduced dramatically from more than 10 times the DMEL (15 ng/m^3) to an exposure below 1 ng/m^3. The main reason for this reduction is the change from powdered enzyme product formulations in the early detergent products to the use of encapsulated granulated enzyme formulations in today's products (SDA, 1995).

Enzyme containing spray products for various household care applications have become very popular in the recent years. Especially for these products it is of vital importance that a thorough risk assessment is performed before the product is launched, in order to ensure an exposure below the DMEL. If the product has a low viscosity or if the nozzle of the spray device produces very fine particles, the DMEL may be exceeded. Unfortunately, the level of enzyme exposure from spray products depends on a number of parameters, which makes it very difficult to translate data from one spray product to another. A very good example of how to conduct an assessment of enzyme exposure from a spray product is given in (Weeks, 2011). Most enzyme suppliers will be able to support the risk assessment of enzyme containing spray products.

History shows that some proposed consumer uses of enzymes were not placed on the market because the risk assessment showed an unacceptably high enzyme exposure, and in some cases it even led to sensitization (Basketter, 2012c). Examples of such uses and the risk assessment of these can be found in (Johnsen, 1997) and (Kelling, 1998). Most of these examples come from the suggested use of enzymes within personal care products. It has been shown that the use of enzymes in personal care products can lead to enzyme exposure from both the direct source and in addition also from a wide number of indirect sources (Basketter, 2012c).

- **Professionals**

Professional uses of enzyme containing products covers a wide range of uses, which in some instances are very similar to consumer uses whereas other cases show great similarities to occupational use.
It is as important to provide a proper risk assessment for professional uses as it is for consumer and occupational uses, and for each use it will have to be discussed whether the consumer DMEL (15 ng/m^3) or the occupational DMEL (60 ng/m^3) would be applicable for the specific situation. In this discussion a number of parameters will have to be accounted for e.g.:

- Use-settings (e.g. defined workspace as in institutional kitchen or institutional laundry, or open area where other persons may be present; inside or outside)
- How will the enzyme containing product be applied (e.g. using spraying)
- Any potential option for residual enzyme material to be settled on surfaces
- Level of training and information of the staff handling the enzyme containing products

It is well known that within the segment of professional use of enzyme containing products, several of these products are applied via spraying, and several products are used for hard surface cleaning. Because of enzyme aerosols generated during spraying or residual enzyme dust on the hard surface after cleaning both spraying and hard surface cleaning calls for special attention.

CONCLUSIONS

Industrial enzymes are widely used in industry already for decades with a long history of safe use. Except for the intrinsic sensitizing potential of all enzymes and the irritating potential of some of those enzymes that hold proteolytic activity, they are of limited significance from a toxicological point of view. The capacity to cause respiratory allergic reactions in humans is, however, one which must be subject to stringent control both in occupational, professional and consumer settings. Past experience demonstrates that enzymes can be used safely by ensuring that the exposure is kept below the DMEL, and that this can be obtained by providing thorough risk assessment and risk management.
This is also a key issue for the expansion of enzymes into new product categories, where the risk assessment and risk management process is absolutely vital for such new business areas.

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