REACH
Data waiving argumentation for technical enzymes
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1. Introduction

Enzymes are used as e.g. processing aids for production of chemical substances or as ingredients in chemical products. Such enzymes (hereinafter referred to as technical enzymes) are within the scope of registration under REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemicals).

According to the Enzyme REACH Consortium (http://www.enzymes-reach.org/) document "Safety evaluation of technical enzyme products with regards to the REACH legislation", the safety documentation of an enzyme consists of two main elements:

- Safety of the enzyme
- Safety of the production strain

The main safety issue of concern regarding the enzyme protein itself is respiratory allergy and in a very few cases primary irritations. These are well known and described for the different enzymes produced. A review of the extensive literature which is concerned with the safety of enzymes from microbial sources strongly supports the general assumption that enzyme preparations from non-toxigenic, non-pathogenic organisms are safe. Historical toxicity data confirm this [1;2].

As a consequence, it is the safety of the production strain that is the primary concern and thus, it is necessary to establish the safety of the production strains before enzymes within the same IUBMB number produced by different production strains can be considered to be the same substance from a safety point of view. This is an important prerequisite for applying the concept of data waiving [3].

In the following, data waivers for both human health and environmental hazards will be presented. Data waivers are generally applicable for all enzymes, unless otherwise described.

2. Physico-chemical data (all enzymes)

- Melting point: Enzymes are polymers of 20 different naturally occurring amino acids in various length and order. The amino acids sequence determines the structure of the enzyme since at biological conditions the structure of this polymer is defined by the energy minimum fold. The enzyme fold and structure is held together primarily by hydrogen bonds, hydrophobic interactions, ion bonds and van der Waals forces between the different amino acids and cofactors. When heating the enzymes above the biological conditions, typically 80°C and higher, the attracting forces within the enzymes are broken, the fold is disrupted and the enzyme denatures. Denatured the enzymes lose their activity and typically coagulate. Thus a classical melting point is not relevant for enzymes.
• Boiling point: As just mentioned, heating leads to denaturation/coagulation of the enzyme and it is thus not possible to determine the boiling point for enzymes.

• Vapor pressure: < 1x10⁻⁶ Pa (25°C) [4;5]

• Surface tension: Enzymes are proteins and have no surface tension. The surface tension study needs only be conducted if based on structure, surface activity is expected or can be predicted.

• Water solubility:
  ▪ Subtilisin: 100 – 800 g/L [5]
  ▪ Amylase, lipase, cellulase: < 800 g/L [4]

• Dissociation constant (only required for tonnages > 100 Ton/year): It is not technically feasible to determine the dissociation constant of enzymes.

• Log $K_{ow}$ (Octanol-water partition coefficient):
  ▪ Subtilisin: -3.1, indicating high hydrophilicity and low lipophilicity (21°C Henkel 2005 according to HERA - Human and Environmental Risk Assessment on ingredients of household cleaning products)[5]
  ▪ Amylase, lipase, cellulase: -2.95, indicating high hydrophilicity and low lipophilicity (Henkel 2005, according to HERA [4])

• Flash point: In the pure form, enzymes are solids at room temperature and through a wide range of ambient temperature, and therefore this parameter is not relevant. The flash point is only a relevant property for liquids, thus it does not need to be measured for substances that are solids or gases at room temperature.

• Flammability:
  o Flammability on contact with water: Enzymes are easily soluble in water and from experience of handling enzymes together with consideration of the structure of enzymes it can be concluded that enzymes are not flammable on contact with water.
  o Pyrophoricity: As they are proteins produced by fermentations, i.e. usually in solution, enzymes are not pyrophoric. In the pure, lyophilized form, they might be pyrophoric, but will in such case only exist in very small quantities for research purpose.

• Explosive properties: As enzymes are proteins produced by fermentation, i.e. usually in solution, they are not associated with explosive properties.

• Self-ignition temperature: Enzymes are proteins and do not possess the ability to ignite themselves.

• Oxidizing properties: As for all other proteins this parameter is not relevant for enzymes. However, oxidases might catalyze oxidation
reactions, given that the proper substrate and physico-chemical conditions are present.

- Granulometry: The study does not need to be conducted if the substance is marketed or used in a form which does not give raise to the danger of exposure e.g. non solid or granular form. Enzymes are primarily marketed in such a form; therefore this parameter is not relevant. Chemical Safety Report of REACH enzyme dossiers will describe physical form of products placed on marked and provide documentation that exposure from inhalation route is below safety concern.

- Stability in organic solvents and identity of relevant degradation products: Enzymes are insoluble in most organic solvents and usually form suspensions when added to organic solvents, in which typically they also denature. To the extent that they are soluble they will not contain their active fold (see argument in melting point) and thus they will be inactive. Therefore this parameter is not relevant.

- Viscosity: Enzymes are solids in the pure form and this parameter is therefore not relevant. However, enzyme solutions typically have a viscosity slightly higher than water.

3. Health hazard identification

Enzymes are in general considered to be of low toxicity with the exception of the allergenic potential of enzymes via inhalation and the limited irritation effects of some proteases at high concentration. Toxicological knowledge has been collected during the last 40 years from in vivo animal studies as well as in vitro studies. This, in combination with experience from human exposure, provides the basis for data waiving. In cases of known enzyme class(es), the majority of toxicological studies required under REACH would not provide any additional knowledge. The safety of technical enzymes especially enzymes used in detergents has been evaluated before by HERA [4;5] and AMFEP (www.amfep.org). Based on these evaluations and toxicological evidence from literature as well as industrial in-house reports, weight of evidence for data waiving of the individual toxicological parameters will be provided in the following sections.

3.1 Toxicokinetics

Toxicokinetic studies performed on enzymes are very limited, but toxicokinetic information can be derived from the structure of enzymes combined with knowledge available for proteins in general since enzymes are proteins with catalytic activity.

Absorption

Skin:
The physico-chemical properties of a compound are decisive for the potential percutaneous penetration, in particular factors like ionization, molecular size and lipophilicity. In general, non-ionized molecules easily penetrate the skin, with small molecules penetrating more easily than large molecules. Lipophilicity also
facilitates penetration. Investigations of percutaneous absorption of peptides, proteins and other molecules of large size revealed that percutaneous absorption of proteins is extremely low and of no toxicological relevance [6;7].

Gastrointestinal tract:
Proteins are digested into amino acids by pancreatic proteolytic enzymes in the lumen of the gastrointestinal tract [8]. As enzymes are simply a class of proteins, it can be expected that enzymes will undergo the same process, simply representing a tiny food source.

Furthermore, enzymes have been used for decades in treatment of both adults and children with exocrine pancreatic insufficiency. Typical enzymatic drugs (e.g. Creon® from Solvay Pharmaceuticals or Pancrease Microtabs from Jansson/Cilaq) are a combination of the enzymes amylase, lipase and protease – enzymes, which are also used in a wide range of industrial applications. These drugs are typically administered orally at therapeutic concentrations i.e. concentrations where a digestive effect can be expected. Clinical trials and crossover studies confirmed the safe use of these compounds in patients, both adults and children, confirming the low toxicity of enzymes [9-20].

Additionally, in a study investigating the gastrointestinal absorption of enzymes in pigs with pancreatic insufficiency and treated with Creon®, analysis of plasma samples taken in the period of 0.5 to 48 hours after oral administration of the drug, did not result in treatment related changes of plasma enzyme levels indicating no gastric absorption of the administered enzymes [21].

Furthermore, a variety of enzymes are added to animal feed with the purpose to increase nutrient digestibility in the gastrointestinal tract. The safety of such enzymes active in the gastrointestinal tract are thoroughly evaluated as part of their approval process in the EU and elsewhere.

In conclusion, from the available data combined with the knowledge of the fate of proteins in the gastrointestinal system, it can be concluded that absorption of enzymes in toxicological significant amounts through the gastrointestinal tract is unlikely.

Respiratory tract:
Enzymes can be inhaled in the form of small dust particles or aerosols i.e. adhered to solid dust particles or as droplets of fluid. Absorption of hydrophilic substances such as enzymes by lung tissue is determined by diffusion and depends on molecular size. The transport channels in the alveolar membrane have a size of 1 nm (10Å) [8], which excludes the absorption of enzymes, since their size is above 1 nm. Removal of deposits depends on the site of deposition. In the alveoli where the main removal is via phagocytosis [8], the macrophages carrying the deposits can move to the interstitium, the ciliated epithelium or the lymphatic system indicating that there could be a risk of systemic exposure to enzymes by this route. However, due to the fact that enzymes are potential respiratory allergens, stringent risk management strategies have been introduced for the working environment leading to very low pulmonary exposure excluding any chance of toxicologically significant absorption.

Consumer
exposure is even lower. Furthermore, no bioaccumulation will occur after absorption due to rapid biological degradation and enzymes hydrophilic nature. In conclusion, absorption of enzymes by the respiratory tract can be considered insignificant.

**Bioavailability**

Due to the combined information that skin absorption of enzymes is at a toxicologically insignificant level, that enzymes are degraded in the gastrointestinal tract and that they are only absorbed to a very low extent by the respiratory tract, the total bioavailability of enzymes can be concluded to be extremely low. This is further supported by the physico-chemical data, i.e. enzymes have a low logKow value, indicating that they have no bioaccumulation potential and can be anticipated to be readily biodegraded. Thus, systemic exposure following enzyme exposure at occupational and consumer exposure levels is without toxicological significance.

### 3.2 Skin and eye irritation

There are substantial *in vivo* animal data on all industrially applied enzyme classes, as well as *in vitro* data performed before formal validation, for both skin and eye irritancy. These data document that enzymes are not irritating, with the exception of proteases. Non-proteases lack the potential to be skin and eye irritants but proteases show a range of effects from no irritation to moderate irritation. It is, however, only the most concentrated and reactive samples which show an effect which is transient in nature and commonly is classified as mild irritation (3; 4; in-house industrial data). This is also the outcome of clinical investigations (6; 23; 26), confirming that enzymes at the product use concentration, even with exaggerated exposures, do not give rise to any occupational or consumer risk of skin or eye irritation.

Given the above, ‘*in vitro*’ irritation testing is not considered to add any scientific value nor lead to a change in the classification adopted from existing data (in-house industrial data).

### 3.3 Skin sensitization

The skin sensitization potential of enzymes has recently been reviewed by Basketter et al. [6] and HERA [4;5] revealing that enzymes should not be considered skin sensitizers. In addition, there is an unequivocal statement from AMFEP ([www.amfep.org](http://www.amfep.org)) on this topic showing that enzymes do not have skin sensitizing potential. The lack of skin sensitizing potential is substantiated by evidence from robust human experimental data and extensive in-use human studies performed with detergents containing enzymes [22-26]. All of these studies confirmed that the presence of enzymes in the detergents did not result in contact skin sensitization, including those conducted with atopic individuals.

However, in spite of clear evidence that enzymes should not be considered skin sensitizers, animal skin sensitization models might give rise to positive results. This is because, just like the previously used guinea pig skin sensitization
models, the Local Lymph Node Assay (LLNA), (OECD Test Guideline 429) is inappropriate for the assessment of proteins. These animal models are validated for the testing of small chemicals, not for water soluble protein-based materials, known to be human respiratory allergens. The LLNA does not discriminate between chemical and respiratory sensitizers [27], leading to the real risk of false-positive results with proteins, particularly those already known to be sensitizing by the respiratory route, such as enzymes. Indeed, in our experience, all foreign proteins can be made to generate skin reactions in suitably treated animals, including the OECD recognized guinea pig tests and the LLNA [28]. This makes the available animal models inappropriate when used with proteins. Therefore, the assessment of enzymes in any of the existing animal models can be predicted not to provide new and useful knowledge. This conclusion is based on the following considerations:

- The results of predictive testing in man demonstrate that enzymes do not have skin sensitization potential for man.
- In clinical settings, enzymes have only very rarely been suggested as a possible cause of allergic contact dermatitis (ACD). Even in these few cases, a causal relationship has never been proven. Further, several clinical studies have demonstrated that enzymes are not a cause of ACD [23;26;29-34].
- ACD has never been reported in the detergent enzyme industries where there has been extensive occupational enzyme exposure which, in the past, led to respiratory sensitization and/or irritant dermatitis. For more than 40 years, billions of consumers have had regular, often daily, skin exposure to enzymes during laundry by hand but there is no evidence that this exposure has given rise to skin sensitization.
- The available skin sensitization test methods are not suitable for enzymes. No animal model has been developed or validated for assessing proteins as contact skin sensitizers. So far, no in vitro models exist either.

Since enzyme products are well documented not to be skin sensitizers in man and because no suitable animal model or in vitro assay for protein skin sensitization exists, we consider testing enzymes in animal models developed for chemical contact allergens as both scientifically and ethically unjustified. Finally, the precautions recommended in the material safety data sheets should be sufficient to prevent even a theoretical hazard of skin sensitization.

### 3.4 Acute toxicity

In general, enzymes are of very low toxicity due to ready biodegradability and very low bioavailability. In traditional acute toxicity testing, mortality has been the endpoint. However, because enzymes show very low toxicity, extremely high doses that are far above human exposure levels typically have been applied. Therefore, acute toxicity studies are not considered to provide appropriate knowledge and are as such not a relevant test system for enzymes.

**Dermal route**

Systemic exposure by the dermal route is unlikely based upon the existing toxicokinetic knowledge of enzymes, which due to their relatively large molecular
weight, are not expected to be absorbed through the skin. Therefore, it can be safely assumed that technical enzymes do not exert any acute dermal toxicity.

This conclusion is confirmed by the toxicological data available. Sub-acute dermal toxicity studies with protease in rabbits (Novozymes, unpublished data) did not provide evidence for systemic effect to enzymes. This finding is confirmed by data from acute dermal toxicity studies (Novozymes, unpublished data) of enzyme products in both rats and rabbits. None of these studies revealed any acute toxic effect through the dermal administration route. No clinical signs or adverse effects due to systemic exposure could be observed.

Data waivers will further be established through exposure scenarios, i.e. no significant dermal exposure to consumers and professionals due to the toxicologically insignificant enzyme concentrations in end products and in the case of workers due to occupational hygiene measures associated with the prevention of respiratory allergy which includes protective clothing.

**Inhalation route**

Due to the fact that enzymes are respiratory allergens, DMEL (Derived Minimum Effect Level) values have to be established to ensure that enzymes can be used safely. Appropriate exposure limits are being established to protect consumers, professionals and workers. Respiratory allergy is considered the most sensitive toxic endpoint for enzymes and thus exposure limits will ensure that exposure levels are low and without any toxicological relevance. Commonly, occupational exposure limit (OEL) values for workers are between 40-60 ng enzyme protein/m$^3$ (8 hour time-weighted average values) for occupational settings in EU countries. More than 30 studies on acute inhalation toxicity in rodents revealed that for the majority of enzymes, no harmful effect could be detected at concentrations up to several mg/l air or g/m$^3$ representing the highest possible concentrations administered and equivalent to nuisance dust levels. Only for a few proteases could LC50 values for inhalation be determined. LC50 values for proteases are typically in the range of 0.05g/m$^3$ up to 1g/m$^3$ [5]. Thus most acute inhalation toxicity studies of both proteolytic and non-proteolytic enzymes in rodents showed a very low order of toxicity indicated by the fact that LC50 values could not be established (LC50 values above the highest applicable dose). In the few cases where LC50 values could be established, the values were more than a factor of $10^6$ above the actual OEL value (Consortium in-house data), indicating that concentrations used in acute inhalation toxicity studies are irrelevant to all known exposure scenarios. It has to be remembered also that acute inhalation toxicity studies are designed to test for lethality, which is not relevant for enzymes and that the real endpoint of concern, allergenicity, cannot be evaluated in this type of studies.

Additionally, industry has documented that respiratory irritation due to enzyme preparations is a very rare phenomenon which will not occur at the low concentrations of enzymes found in consumer products as for example detergents. The risk to consumers is thus considered very low and regarded as toxicologically insignificant. This is supported by the positive safety outcome of a clinical study of the highest reported consumer exposure level with spot cleaning by spray [35-37].
The industry has taken measures to minimize occupational exposure. Subtilisin preparations, depending on atmospheric concentration, may be irritating to the respiratory tract, but due to the risk of sensitization, these enzyme preparations are now specifically formulated to avoid exposure by inhalation. Worker safety is further assured through current proper work practices, effective cleaning, engineering controls, and use of personal protective equipment. Acute inhalation toxicity studies with lethality as endpoint makes this animal test system inappropriate in relation to relevant exposure to enzymes by inhalation and therefore lacking any capacity to provide new and/or useful knowledge.

Conclusions

Toxicokinetic data together with evidence from animal studies and historical human experience derived from the use of detergent enzymes for decades confirm that exposure to technical enzymes will not result in any toxicologically relevant uptake by dermal or inhalation route. Acute systemic exposure to a toxicologically significant amount of enzymes by those routes can therefore be excluded and will further be prohibited by the obligatory setting of a DMEL value for enzymes, resulting in negligible exposure to enzymes. In vivo acute dermal and/or inhalation toxicity studies will not add any value and cannot be expected to provide valuable knowledge and are thus considered scientifically and ethically unjustified.

3.5 Reproductive toxicology

From the toxicokinetic information available, it can be concluded that the bioavailability of enzymes is low due to the fact that no significant absorption can be expected through the respiratory and/or gastrointestinal tract and/or through the skin. Exposure to enzymes will be limited because of the DMEL (derived minimum exposure levels) settings for workers, professionals and consumers to prevent respiratory allergy (supported by exposure scenarios and DMEL values). Apart from the irritation potential of some proteases, respiratory allergy is generally considered to be the only human health hazard of enzymes indicating that this is the most sensitive endpoint considering enzyme toxicity. Concentrations that are not expected to result in respiratory allergy will certainly not result in any other toxic effect. This conclusion is substantiated by the material that follows.

Although endocrine disrupting chemicals are a broad group of chemicals consisting of man-made and natural compounds it is unlikely that enzymes have the potential to cause endocrine disruption. The enzymatic structure is different from any endocrine disrupter known to date [38]. Indeed, enzymes are much larger than endocrine disrupters in general excluding mechanisms such as direct action on hormone receptors (EDSTAC (Endocrine Disruptor Screening and Testing Advisory Committee, US EPA), [39]). Due to the high biodegradability of enzymes, it is highly unlikely that they could reach target organs or sites to any significant amount or of any significant period of time. Testing of enzymes in currently available screening assays typically based on hormone receptor binding cannot be expected to provide any evidence for endocrine disruption due to the specific features of enzymes.
Data from acute and subchronic oral toxicity studies provide evidence that enzymes are of very low toxicological activity \([18;40-90]\). Typically, the derived NOAEL values are significantly higher than the maximum doses applied. None of the oral toxicity studies performed by members of the consortium in the past 40 years, as well as published data from other studies revealed any effect that indicates that enzymes could have an adverse effect on the reproduction system in males or females.

Complementing the above information is data from 26 industrial studies (Novozymes, unpublished data) on fertility and/or teratogenicity and/or reproduction studies primarily in rodents but also other species like dogs and rabbits which did not identify any evidence for reproductive toxicity of enzymes. Both proteolytic and non-proteolytic enzymes have been investigated for their teratogenic and reproductive toxicity potential. Several of these studies have been published in peer reviewed articles [61;66;70;91]. Enzymes have been produced and used for many years without any evidence for reproductive potential in humans. OEL for workers is set to be 60ng/m\(^3\) to protect against respiratory sensitization. Considering that endocrine disrupting chemicals in general are a factor of 100 000 less potent than physiologically relevant hormones [92], the low worker exposure to enzymes due to rigorous application of airborne limit and very low exposure to consumers (below 15ng/m\(^3\), which is the highest known consumer exposure and only the case when using pre-spotters [37]) and the low bioavailability together with the high biodegradability of enzymes, no reproductive toxicity effect can be expected in humans. Furthermore, enzymes have been used for decades to treat pancreatic insufficiency in both children and adults without any evidence of reproductive toxicity [93].

In conclusion, toxicokinetic data together with the enzymatic structure and the weight of evidence from animal studies and human exposure provide no evidence for reproductive toxicity of enzymes. Investigations of enzymes in \textit{in vitro} screening systems for endocrine disrupters cannot be expected to provide any new knowledge due to the nature of enzymes, especially since the majority of screening studies deals with receptor mediated mechanism. It is most unlikely that new animal studies will provide any new knowledge, because the results and conclusions of numerous animal studies, investigating effects on fertility and teratogenicity and the repeated dose oral toxicity studies \textit{in vivo} have not identified any adverse effects related to reproduction. Thus, reproductive toxicity studies are considered scientifically and ethically unjustified.

### 3.6 Genotoxicity and carcinogenicity

Enzyme proteins are not regarded as either genotoxic and/or carcinogenic. Genotoxicity testing is in general performed to confirm that the production strain does not produce any genotoxic or carcinogenic metabolites. Basically all enzyme substances have therefore been tested in the Ames test and in the chromosome aberration test \textit{in vitro} and a few enzyme substances also in the mouse lymphoma test [40;42;44;45;48-50;52;53;55-61;63;66-68;70;71;78;81;82;84;85;91;94;95]. In none of these test systems did enzyme proteins show evidence of genotoxicity.
Enzymatic drugs have been used since the 19th century without providing any evidence of a genotoxic or carcinogenic effect [9-11;15-20;75-77;96] confirming the results of large amounts of in vitro and in vivo data available.

It is our view that the inclusion of in vivo assay(s) for microbially produced enzymes is unjustified for scientific as well as ethical reasons, which is further supported by the following arguments [3]:

Technical enzymes are produced by fermentation and contain not only the principal enzyme protein but also residual growth medium from the fermentation and metabolites from the production strain. As with other proteins, the enzymes are not genotoxic.

The enzymes are produced by microbial strains, which have been thoroughly characterized as non-pathogenic and non-toxigenic and in most instances with a history of safe use in food enzyme manufacture. Therefore the primary concern, when evaluating the genotoxic potential of an enzyme preparation, is the highly theoretical (albeit highly unlikely) possibility of the expression (and hence the presence) of a hitherto unknown microbial metabolite with genotoxic potential and at a concentration of genotoxicological importance.

Within the field of drug development, a standard battery of genotoxicity tests is required, including at least one in vivo test. During the conduct of these tests, blood samples are collected at pre-determined time points and the plasma is analyzed for the concentration of the test article and metabolites to establish evidence of adequate concentration and duration of exposure. Without such data the study is considered completely inappropriate by the regulatory authorities.

Given the above, it is clear that in any test, it is the demonstration of adequate in vivo exposure to the target organs or target cells which must be a fundamental prerequisite. Thus to adopt an assessment of genotoxicity similar to that employed in drug development would be unsuitable for technical enzymes for the following reasons:

- Enzymes dosed orally to rodents are readily digested and decomposed in the gastrointestinal tract and only a negligible fraction, if any at all, of the intact enzyme molecule is absorbed systemically. The constitution, the kinetics and the dynamics of the enzyme decomposition products and possible impurities from the fermentation are completely unknown. Therefore in the field of enzyme development, exposure data is never collected because it is considered meaningless.
- Further, a review of the extensive literature, concerned with the safety of enzymes from microbial sources, strongly support the general assumption that enzymes from non-toxigenic, non-pathogenic organisms are safe. Numerous tests for in vitro genotoxicity have failed to reveal the presence of a single mutagen or clastogen. These aspects were reviewed by Pariza and Johnson.
[1], who presented a compelling argument for the position that tests for genotoxic potential of enzyme preparations produced by well-characterized non-toxigenic microorganisms are unnecessary for safety evaluation.

Based on these considerations, we conclude that within the field of enzyme development, the conduct of in vivo genotoxicity provides no added value. Such a requirement suffers from an obvious lack of scientific rationale and is considered scientifically and ethically unjustified.

In conclusion, the large amount of data on genotoxicity available together with structural knowledge, toxicokinetic and human data provide no evidence for genotoxic or carcinogenic potential of enzymes. From this and from the lack of scientific rationale it can be concluded that neither the mouse lymphoma test nor in vivo mutagenicity tests (micronucleus) can be expected to provide any new knowledge and will only result in the unnecessary use of animals.

3.7 Carcinogenicity

The low bioavailability of, as well as the low exposure to enzymes, the lack of genotoxic potential and the consequent absence of any evidence of carcinogenic properties from both human and animal data do not justify any requirement for conducting carcinogenicity studies.

4. Environmental hazards

4.1 Enzymes and the eco-systems - potential for establishment of waivers

Enzymes

Enzymes are found in every living organism as an essential component of the digestive systems, and as a key tool in metabolism by breaking down proteins, carbohydrates and lipids and as such, they are the basis for all life. Enzymes are proteins, hydrophilic and readily biodegradable. In nature, they are involved at any level of the food chain and in the microbial biodegradation.

For more than 40 years enzymes have been used in industrial processes to replace chemicals and reduce requirements for water and energy. Furthermore they are widely distributed in household articles for laundering.

Achieved knowledge on hazard by testing

The widespread use of enzymes has been followed by investigations relevant for ecotoxicity. Investigations are mainly performed to establish knowledge on short-term aquatic toxicity and on the rate of biodegradability of the enzyme.

The main results achieved can be listed as follows:
1. All enzyme classes are readily biodegradable. Degradation products are primarily peptides and amino acids, then carbon dioxide and water which cause no harm to nature.

2. Non-proteolytic enzymes should not be classified as harmful or toxic to the environment.

3. Proteolytic enzymes are acutely harmful or toxic, but should not be considered environmentally hazardous due to their rapid elimination in the environment.

Caution should be exercised for enzymes producing harmful by-products, e.g. hydrogen peroxide or ammonia from substrates. The classification of such enzymes cannot be based on analogy, but only on data obtained by relevant tests and analyses. These tests have to consider that such enzymes are normally stored and transported separately from their substrates or in an inactivated form, preventing the premature release of such by-products.

Knowledge based on scientific rationale

The rapid elimination from the eco-systems has made long-term testing less relevant. One long-term dynamic study with a protease on early life stage fish was carried out, but revealed only dose related but no long-term effects.

1. Due to the hydrophilic properties and ready biodegradability of proteins, bioaccumulation tests are considered unnecessary.

2. Since all enzyme classes are readily biodegradable, enzymes are considered as having a low toxic potential to microorganisms.

3. Enzymes are primarily active in aqueous environment. Adverse effects to nature in dry environments are thus not expected, adverse effects to nature in wetlands etc. are demonstrated by the testing for acute aquatic toxicity.

Application of data waivers

Below, an overview is given of the studies that can be waived, including a reason why.

1. Long-term aquatic tests due to the rapid degradation of the enzyme molecule resulting in short exposure time

2. Terrestrial tests due to the low activity of the enzyme in non-aqueous environment. Use in irrigation areas, wetlands etc. will be covered by the aquatic tests.
3. Microbial sludge tests for Sewage treatment plants (OECD 209). The shown readily biodegradability shown demonstrates the low toxicity of enzymes to microorganisms.
5. Reference List


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