



AMFEP/2012/02

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Re. Enzyme and classification of enzyme products in accordance with the CLP regulation

Enzymes are a specific subcategory of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB). According to REACH, the substance identification of enzymes is defined as “The enzyme protein together with the other constituents deriving from the fermentation or extraction process, but excluding any water, which may be separated without affecting the stability of the enzyme protein or changing its composition” (Ref. 1) . This UVCB enzyme substance is composed of the following constituents present under normal fermentation conditions:

- a. Active enzyme protein (aep)
- b. The other constituents from the fermentation:
 - Other proteins + peptides and amino acids
 - Carbohydrates, Lipids, Inorganic salts

a. Active enzyme protein (aep)

REACH defines that the primary identifier for enzymes is catalytic activity of active enzyme protein (aep) defined by Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB, www.chem.qmul.ac.uk/iubmb/enzyme/index.html).

There are approximately 400 enzymes listed in EINECS. Of those, 17 enzymes (13 enzymes on EINECS, 1 enzyme on ELINCS and 3 enzyme groups) are in Annex VI of CLP regulation. The common harmonized classification of the 17 enzymes is as respiratory sensitizers. Subtilisin and other proteases may have additional skin/eye irritancy classifications.

Enzymes regardless of the catalytic activities are potential respiratory sensitizers, whereas the weight of human evidence indicates that enzymes are not skin sensitizers (Ref. 2, 3, 4). All enzymes must therefore be classified as respiratory sensitizers (Ref. 5), “H334: Hazard Category 1: May cause allergy or asthma symptoms or breathing difficulties if inhaled” in accordance with the CLP Regulation.

In 1973 ACGIH (American Conference of Industrial Hygienists) proposed a TLV-CEILING (TLV = Threshold Limit Value) for Subtilisins, IUBMB number 3.4.21.62 (cf. also the example in the REACH Guidance for identification and naming of substances in REACH, section 7.11.1, Subtilisin) of 60 ng aep/m³. The aep was outlined as “100% crystalline active pure enzyme (CAPE)”. This workplace exposure limit was adopted by several national authorities, including the member states in EU. In 2004, the UK authorities (Health & Safety Executive,

HSE) replaced it by an 8-hour TWA (Time weighted average) limit of 40 ng aep/m³. To our knowledge the Subtilisin exposure limit of 60 ng aep/m³ (for UK 40 ng aep/m³) is the only nationally regulated exposure limit for enzymes. It is important to notice, that this limit is defined for the active enzyme protein (aep) constituent of an UVCB enzyme concentrate as well as a finished formulated enzyme product.

b. The other constituents

The Enzyme REACH Consortium (ERC) policy document on “Safety evaluation of technical enzyme products with regards to the REACH legislation” describes how to document sameness of the other constituents based upon objective and transparent criteria. ERC will propose each member of a SIEF to adhere to this policy and to use these criteria in order to define sameness in a SIEF (Ref. 6, 7). Adhering to this policy also effectively ensures that the other constituents do not contribute any toxicological safety concern, and that therefore the aep alone can be used for classification of the UVCB enzyme substance.

The absence of respiratory sensitization potential of the other constituent categories is described below.

Denatured and inactivated enzyme proteins

Denatured and inactivated enzyme proteins typically originate from temperature- or pH-inactivation of the aep or by proteolytic autolysis (“self digestion”) in the case of proteases. In these cases, the tertiary conformation of the enzyme molecule is destroyed, leading to a (partially) random coil structure with substantially reduced respiratory sensitization potential. In general, these alterations in conformation are associated with decrease in the antigenic reactivity in humans. Thus, the reactivity of globular proteins with homologous antibodies is usually destroyed or negligible after denaturation of the protein.

Denatured proteins are in the vast majority of investigated cases much less immunogenic than the corresponding native protein (Ref. 8, 9, 10, 11, 12, 13). Further, heat-treated protein hydrolysates are often regarded as ‘hypo-allergenic’ in line with the above (Ref. 14).

From in-house research on industrial enzymes, it is confirmed that the antigenic activity of heat treated enzymes is reduced considerably compared to the native/active protein source.

In conclusion, denatured proteins are not regarded as a risk factor under the exposure conditions of industrial enzymes.

Peptides and amino acids

Peptides and amino acids typically originate from temperature- or pH-inactivation of the aep or by proteolytic autolysis (“self digestion”) in the case of proteases. This is the same situation as for the denatured and inactivated enzyme proteins described above. This part of the dry matter enzyme concentrate also has only insignificant (negligible) respiratory sensitization potential.

Carbohydrates, Lipids and Inorganic salts

The carbohydrates, lipids and inorganic salts which are present in the dry matter of an enzyme concentrate are not respiratory sensitizing substances.

Conclusion

When enzymes meet the criteria laid down in “Safety evaluation of technical enzyme products with regards to the REACH legislation”, the non-enzymatic constituents are considered safe and do not contribute to classification. Therefore active enzyme protein shall be used as basis for classification and labeling of enzyme products. The UVCB enzyme substance defined under REACH shall be used for tonnage calculation. The Enzyme Reach Consortium provides guidance (Ref. 15).

References

1) ECHA, “Guidance for identification and naming of substances under REACH“, 2007, section 4.3.2.3. , <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-the-different-methods-under-reach>

2) HERA risk assessment on Protease, <http://www.heraproject.com/RiskAssessment.cfm?SUBID=22>, and HERA risk assessment on Amylase/Lipase/Cellulase, <http://www.heraproject.com/RiskAssessment.cfm?SUBID=38>.

3) Basketter D.A. et al.: Defining occupational and consumer exposure limits for enzyme protein respiratory allergens under REACH. Toxicology. 268:165-170, 2010.



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4) Basketter D.A. et al.: Enzymes, detergents and skin: facts and fantasies. Br. J. Dermatol. 158, 1177-1181, 2008.



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5) AMFEP (the Association of Manufacturers and Formulators of Enzyme Products) policy on classification of enzymes as “Respiratory Sensitisation Category 1” in accordance with the EU Regulation on classification, labelling and packaging of substances and mixtures (EC No 1272/2008, “CLP Regulation”) (Amfep/09/73)



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6) Manufacturers and importers of enzymes have created an open consortium, The Enzymes REACH Consortium (“ERC”) with the overall purpose of facilitating a smooth REACH implementation: www.enzymes-reach.org.

7) The Enzymes REACH Consortium document. Safety evaluation of technical enzyme products with regards to the REACH legislation (ERpC/09/06)



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8) Valenta, R., and D. Kraft. 2002. From allergen structure to new forms of allergen specific immunotherapy. Curr. Opin. Immunol. 14: 718–727.

9) Valenta, R. 2002. The future of antigen-specific immunotherapy of allergy. Nat. Rev. Immunol. 2: 446–453.

10) Takai, T., et al. 1997. Engineering of the major house dust mite allergen Der f2 for allergen-specific immunotherapy. Nat. Biotechnol. 15: 754–758.

11) Takai, T., et al. 2000. Unlocking the allergenic structure of the major house dust mite allergen Der f 2 by elimination of key intramolecular interactions. FEBS Lett. 484: 102–107.

12) Nakazawa, T., et al. 2005. Multiple-mutation at a potential ligand-binding region decreased allergenicity of a mite allergen Der f 2 without disrupting global structure. FEBS Lett. 579: 1988–1994.

13) Kikuchi Y. , et al. 2006. Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p 1 to sensitization towards IgE and IgG responses. J Immunol 177:1609-1617.

14) Høst A,. and Halcken S. 2004. Hypoallergenic formulas--when, to whom and how long: after more than 15 years we know the right indication!. Allergy 59 Suppl 78:45-52.

15) The Enzymes REACH Consortium document. Calculation of Tonnage for Enzyme Substances (ERpC/09/05)



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