Hazard classification of industrial enzymes

The objective of this leaflet is to clarify the general basis for the CLP classification of enzymes and enzyme mixtures, especially for the hazard class 'Respiratory sensitiser'.

Enzymes are a group of substances that can be derived from a source of biological origin. They are widely used in industry, but also in consumer products, leading to a need to clarify the basis of their hazard classification.

Under REACH, enzymes are identified as UVCB substances. Most enzymes are manufactured by fermentation and, if not purified, they can contain the following constituents:
a. The active enzyme protein(s)
b. Other constituents from the fermentation process:
- Other proteins, peptides and amino acids
- Carbohydrates, lipids, inorganic salts

The enzyme industry recommends in general only using production organisms, which have a long history of safe use.

An enzyme is named and identified in accordance with the IUBMB enzyme nomenclature convention[1)]. If the identities of constituents other than the enzyme protein are not known, they can be indicated in a grouping approach (i.e., using general terms such as proteins, peptides, amino acids, carbohydrates, lipids and inorganic salts). However, constituents have to be indicated if their identities are known and they have to be identified if their typical concentration exceeds 10 % or if they are relevant for classification and labelling or PBT assessment.

Enzymes may possess respiratory sensitisation potential regardless of the type of catalytic activity. Therefore, it is recommended to consider classifying all enzymes as respiratory sensitiser Category 1, H334, in accordance with the CLP Regulation, unless there is scientific evidence from e.g. immunochemical/immunological testing that they do not induce a specific response. The use of the sub-categories for respiratory and skin sensitisers, which discriminate between strong sensitisers and other sensitisers (categories 1A and 1B, respectively) for respiratory allergens, should be done where data allows. In other cases, respiratory sensitiser Category 1 would be more appropriate.

CLP Annex VI currently includes a harmonised classification for 17 enzymes. All these are classified and labelled as respiratory sensitiser Category 1: H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. In addition to the Resp. Sens. 1 classification, proteases in Annex VI have additional classifications, namely STOT SE 3, Skin Irrit. 2 and Eye Irrit. 2 (except subtilisin, which is classified as Eye Dam. 1). The REACH registration dossier for e.g. subtilisin includes additional self-classification as Acute Tox. 4, Aquatic Acute 1 and Aquatic Chronic 2. These classifications are due to the *proteolytic activity* of proteases.

1) http://www.chem.qmul.ac.uk/iubmb/enzyme/

The classification of a UVCB substance in general can be based on the available test data on the UVCB substance as a whole. However, where such data allowing appropriate hazard classification is not available, the hazard classification of the substance can be derived from the classification of the constituents of the UVCB substance. If there is not enough data on the constituents, read-across may be applied. The constituent-based approach and read-across also apply to enzymes.

A mixture containing several enzymes must be classified as a respiratory sensitiser when at least one ingredient has been classified as such and is present at or above the appropriate generic concentration limits, unless sufficient data on the mixture itself indicating otherwise is available and bridging is not possible. Substances that are classified as sensitisers may elicit a response, when present in a mixture in quantities below the generic concentrations or specific concentration limits for classification, and must thus be indicated on the label at the lower concentrations established in Table 3.4.6 of Annex I to CLP. Thus, for an enzyme (solid or liquid) that is a Category 1 respiratory sensitiser in a mixture, the general threshold concentration is 1% for the classification of the mixture (Table 3.4.5), and 0.1% for the inclusion of the supplementary statement EUH208 — 'Contains (name of sensitising substance). May produce an allergic reaction' on the label (Table 3.4.6). The threshold is considered on the basis of active enzyme protein per individual type of catalytic activity.

Where enzymes of similar catalytic activity (i.e. belonging to the same IUBMB subclass or belonging to the same enzyme type as listed in Annex VI to CLP*) are used in a mixture, the levels of these enzymes can often be considered as 'additive'. The situation can for example be that enzymes derived from different organisms that act on the same substrate might be similar enough to elicit an 'additive' response. On the other hand, when the enzymes in the mixture have a different structure and different immunologically available epitopes, the 'additivity' might not apply. Therefore, when there is evidence from immunochemical/immunological testing that each enzyme induces specific responses, the enzymes need to be considered as separate entities.

It may be possible that an enzyme mixture is placed on the market in a form or state in which the classification as respiratory sensitiser is not relevant and can thus be omitted.

* The IUBMB subclass specifies the type of reaction and the sub-subclass defines the specific enzyme.

A single CAS/EC number can cover the same enzyme from several different sources. Enzymes from different organisms can be very different in structure. This could potentially lead to different antigenicity even though the activity is the same.