Enzyme REACH Consortium Guidance: Application of read-across for enzyme substances

1. Introduction

Enzymes are protein with catalytic activities and belong to Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCB). Enzyme substances are enzyme concentrate, dry matter obtained after the manufacturing process and they are identified based on catalytic activities defined in International Union of Biochemistry and Molecular Biology (IUBMB). Enzyme concentrates with the same IUBMB number can be regarded as the same substance, despite using different production organism, provided that the hazardous properties do not differ significantly and warrant the same classification. (ECHA's guidance for identification and naming of substances under REACH and CLP, Section 4.3.2.3 and Section 5). Enzyme REACH Consortium (ERC) further developed industrial guidance how to document the same classification within the same IUBMB (ERC Safety evaluation of technical enzyme products with regards to the REACH legislation [1]).

Enzymes registered under REACH regulation must fulfil information requirements that depend on the tonnage for which the substance is registered. Information requirements can be fulfilled by conducting testing on the substance itself. Other options include using existing data (generated for purposes other than REACH), weight of evidence approach, Qualitative or Quantitative structure-activity relationship (QSAR) modelling or grouping of substances and read-across approach. In some cases, it is also possible to waive the information requirement if the testing is not technically or scientifically possible (see ERC document on Data waiving argumentation for technical enzymes).

1.1. Selecting read-across approach

For enzymes, read-across can be utilised for physico-chemical, toxicological and environmental toxicity endpoints. Depending on the endpoint and the data availability, either **analogue** or **category approach** can be used for read-across.

In the **analogue approach** test data from a structural analogue (*source* substance) is used to predict the properties of the registered substance (*target* substance) i.e., one-to-one read-across. For this approach it is recommended to use an enzyme in the same IUBMB sub-sub-class, or at least sub-class, as a source substance (e.g., source substance alpha-amylase IUBMB 3.2.1.1 for target substance cellulase IUBMB 3.2.1.4) to support the argument of structural similarity.

In the **category approach** read-across is applied between the registered substance (target substance) and a group of structurally similar substances, i.e., many-to-one read-across. Substances in the group should be grouped together on defined structural similarities and/or differences. With the category approach it is proposed that the properties of the substances are either similar or they follow a pattern that can be applied also to the target substance. Furthermore, it is recommended to build the category from enzymes that at least belong to same IUBMB class to support structural similarity.

Whether an analogue or category approach is selected for the read-across, the justification for the selection needs to be documented in a read-across assessment report. Templates for reporting are made

available by ECHA in their Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals section R.6.2.6 [2].

For further general information on read-across approach, see ECHA guidance documents Read-Across Assessment Framework (RAAF) [3] and RAAF: Considerations on multi-constituent substances and UVCBs [4] (March 2017) and Advice on using read-across for UVCB substances [5] (May 2022).

2. Physico-chemical properties

Most of the physico-chemical properties are waived for enzymes (see document Data waiving argumentation for technical enzymes). However, for a few endpoints read-across is a valid option to fulfil information requirements. With regards to physico-chemical properties, all enzymes in different IUBMB classes can be grouped together, as the physico-chemical properties of enzyme concentrate, dry matter are independent to the catalytic activity of the enzyme but based on the fact that all enzymes are proteins. The dry concentrate form of all enzyme substances is shown to act similarly in the tests required for REACH.

2.1. Density

Density data have been generated for various protein families. The data published in the cited book comes from 20 different protein families and they all have densities in the range from 1.32 g/mL to 1.42 g/mL. The density for the enzyme has not been determined but since it is a protein it is expected, with very high reliability that it has density in this range.

Thomas E. Creighton. (1993). Proteins: structures and molecular properties. W.H.Freeman and Company. ISBN 0-7167-2317-4

2.2. Vapour pressure

Enzymes are protein and vapor pressures of 4 different enzymes (alpha-amylase, cellulase, glucoamylase and subtilisin were measured with freeze-dried test samples. The vapor pressures of the 4 enzymes were indeed very similar from 0.00195 to 0.00689 Pa. Other enzymes are also protein, thereby vapour pressure is expected to be very similar to the 4 enzymes, therefore these data are used for read-across.

2.3. Partition coefficient n-octanol/water

Because all enzymes are built up of the combination of the same 20 common amino acids, the physical and chemical characteristics are very similar for different enzymes, and hence, read-across from other enzymes should be fully applicable. Enzymes have been analysed and the LogPow from literature studies was found to be between -3.1 to -2.95. In addition, the octanol-water partition coefficient (logPow) for glucoamylase was measured <= -1.3 at 20°C.

Due to the similar nature of enzymes, this value can also be extrapolated to other enzymes.

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- Basketter D, Berg N, Broekhuizen C, Fieldsend M, Kirkwood S, Kluin C, Mathieu S, Rodriguez C (2012a). Enzymes in Cleaning Products: An Overview of Toxicological Properties and Risk Assessment/Management. Regul. Toxicol. Pharmacol., 64(1):117-123.

2.4. Flammability

Read-across data on substance cellulase (IUBMB 3.2.1.4) has been made available in 2020 for the endpoint vapour pressure for all ERC members who act as lead registrants. As there is only one source substance data, an analogue approach is suggested for this endpoint.

Cellulase was freeze-dried and tested for flammability. The test showed that cellulase is not flammable.

Most of the enzymes are produced by fermentation, typically in solution. After the fermentation, enzymes are recovered as enzyme concentrate in a liquid form and further formulated as liquid products or granules. The substance in contact with water is not considered to be highly flammable according to Regulation (EC) No 440/2008. Based on this data, other enzymes are also expected to be Non-flammable (Not Classified).

Enzymes are globular proteins produced by fermentation i.e. typically in solution. After the fermentation, enzymes are recovered as enzyme concentrate in a liquid form and further formulated as liquid products or granules. Decades of experience in production, handling and use of enzymes show that the substance does not react with water (e.g. the substance is manufactured with water or/and washed with water). The substance is soluble and stable in water. Therefore, the substance in contact with water is not considered to be highly flammable according to Regulation (EC) No 440/2008. Under REACH, enzymes are defined as enzyme concentrate, dry matter [6]. As dry matter, proteins in general are not considered to be highly flammable according to Regulation (EC) No 440/2008. This is supported by the chemical structure of the proteins. Proteins contain reactive groups such as hydroxyl, carboxylic acid, amines, thiols groups etc. During combustion, carboxylic acid groups may go through decarboxylation and hydroxyl groups may be released as water vapor [7]. The carboxylic acid may also promote char formation [7, 8]. Proteins also contain nitrogen and sulfur which form a disulfide bond helping in contributing to its non-inherent flammability [7, 8]. These properties have in fact led to the use of proteins as flame retardants [7, 8]. Additionally, several proteins have already been tested using recognized method under REACH and CLP (e.g. UN Test N.1) and found not to be highly flammable according to Regulation (EC) No 440/2008, e.g. Protein hydrolyzates, rice bran (EC number: 305-224-5), Protein hydrolyzates, animal (EC number: 309-203-1), Insulin DesB30 (EC number: 944-550-8).

3. Toxicological properties

In general, enzymes exhibit the same toxicological properties and besides the fact that they are respiratory sensitizers are of low toxicity, which is confirmed by toxicity studies performed in the industry and published safety evaluations for a variety of enzymes [9-53].

Read across for toxicological endpoints can be applied for enzyme substances that belong to same IUBMB sub-sub-class or sub-class, provided that the safety of the other constituents has been established (safe-strain lineage) and that the toxic effect with regards to the selected endpoint can be considered comparable. However, read-across between enzymes of different IUBMB class should only be considered and performed when there are no studies available for the specific enzyme. In general, read-across between enzymes of different IUBMB numbers can be performed for the majority of enzyme substances, except for proteases due to the intrinsic properties of proteases to catalyse protein degradation. In order to perform read-across between enzymes of different IUBMB classification for health hazard identifications, except genotoxicity, enzyme substances can be divided into two groups, proteases and non-proteases. For genotoxicity, all enzymes can be grouped together since the overall conclusion is that enzymes are not mutagenic or clastogenic.

For acute skin and eye irritation as well as genotoxicity, qualitative read-across can be applied since there is typically no dose response relationship. Qualitative and quantitative read-across can be applied for acute oral and repeated dose oral toxicity.

4. Ecotoxicological properties

In general, enzymes exhibit the same ecotoxicological properties which are confirmed by ecotoxicity studies performed in the industry.

In general, the same rules for read-across for health hazards identification described previously can be applied for environmental hazard identification. However, to perform read-across between enzymes of different IUBMB classes for environmental hazards, enzyme substances should be divided into three groups, proteases, oxidoreductases and all other enzymes. With regards to biodegradability all enzymes can be grouped together.

Qualitative read-across will be applied for both aquatic toxicity and ready biodegradability since the PNEC (predicted no effect concentration) values for the majority of enzymes, except for proteases and oxidoreductases, is considered the highest dose tested for a given end point. As far as biodegradability is concerned all enzymes are considered readily biodegradable according to the current OECD guidelines indicating a simple yes or no answer without a quantitative relationship. Aquatic toxicity tests should be performed according to current OECD guidelines and under consideration that enzymes are readily biodegradable i.e., semi-static system when possible.

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