Enzyme REACH Consortium Guidance: How to populate IUCLID Section 1 for enzymes in inquiry and registration dossiers

Disclaimer

This communication is meant as guidance only. It is published by Enzyme REACH Consortium (ERC) in order to assist its members in their efforts to understand and comply with REACH.

Please be reminded, however, that REACH is the only authoritative legal text and that the present document does not substitute legal or otherwise expert advice. ERC and its members do not accept any liability for use of this communication or for activities contemplated and carried out under or relying on this communication.

Introduction

The purpose of the guidance is to give practical information to Enzyme REACH Consortium (ERC) members based on our experiences with REACH dossiers. The guidance focuses on specific descriptions on enzyme substances which may not be covered by ECHA's support documents. Identifying the enzyme substance accurately is critical in an inquiry dossier for ECHA to assess whether the enzyme has already been registered and to possibly link the new registrant with an already existing SIEF.

ECHA has specifically addressed the issue of identification of enzyme substances, cf. section 4.3.2.3 of ECHA's Guidance on Substance Identification [1]. IUCLID should be populated according to this identification. The relevant paragraphs of ECHA's guidance are inserted below. This guidance does not cover issues related to splitting and/or merging SIEFs.

The enzyme substance should be regarded as a 'UVCB sub-type 1substance' due to its variability and partly unknown composition.

…

Enzyme substances are identified by the enzyme protein (IUBMB nomenclature) and the other constituents from the fermentation.

…

The enzyme substance typically contains 10-80 % (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the ranges indicated in the following table.

[1] Guidance for identification and naming of substances under REACH and CLP. Reference: ECHA-16-B-37.1-EN. Version 2.1, May 2017

Active enzyme protein	*10 - 80%*
Other proteins + peptides and amino acids	*5 - 55%*
Carbohydrates	*3 - 40%*
Lipids	*0 - 5%*
Inorganic salts	*1 - 45%*
Total	*100 %*

Submission of an inquiry dossier is a prerequisite for registration. The information required in the inquiry dossier:

- identity of the requested submitter (REACH-IT account)
- identity and composition of substance (IUCLID section 1.1 Identification, 1.2 Composition)
- analytical data (IUCLID section 1.4 Analytical Information)
- information requirements (IUCLID section 14 Information requirements)

In this guidance document we will provide practical advice on how to fill sections 1.1 Identification, 1.2 Composition and 1.4 Analytical information in IUCLID for enzyme substances.

How to read the guidance

The guidance text is black.
Proposed text populated in IUCLID is blue
Text which is variable depending on enzyme is orange.

Section 1. General Information

Section 1.1 Identification

An enzyme should be identified according to the **Enzyme Nomenclature** defined by the Nomenclature Committee of the International Union of Biochemistry (IUBMB) based on catalytic activities. β-glucosidase is used as an example below.

β-glucosidase
EC: 232-598-7
IUBMB: 3.2.1.21
CAS: 9001-22-3
Reaction: Hydrolysis of terminal, non-reducing β-D-glucosyl residues with release of β-D-glucose

Other substance identifiers + New item Import file

#	Flags	Identifier	Identity
1	None None	CAS number	9001-22-3
2	None None	EC number	232-589-7
3	None None	trade name	FLASHZYME
4	None None	other: Reaction	Hydrolysis of terminal, non-reducing β-D-glucosyl residues with release of β-D-glucose
5	None None	other: Systematic name	β-D-glucoside glucohydrolase
6	None None	other: IUBMB name	3.2.1.21

The substance has to be linked with a reference substance. If the reference substance is not available in the IUCLID *reference substance inventory*, a new reference substance object may have to be created manually.

Identification of substance None None

Reference substance
Beta-Glucosidase | Beta-glucosidase IUBMB 3.2.1.21 | EC 232-589-7 | 9001-22-3

Type of substance

Type of substance
UVCB

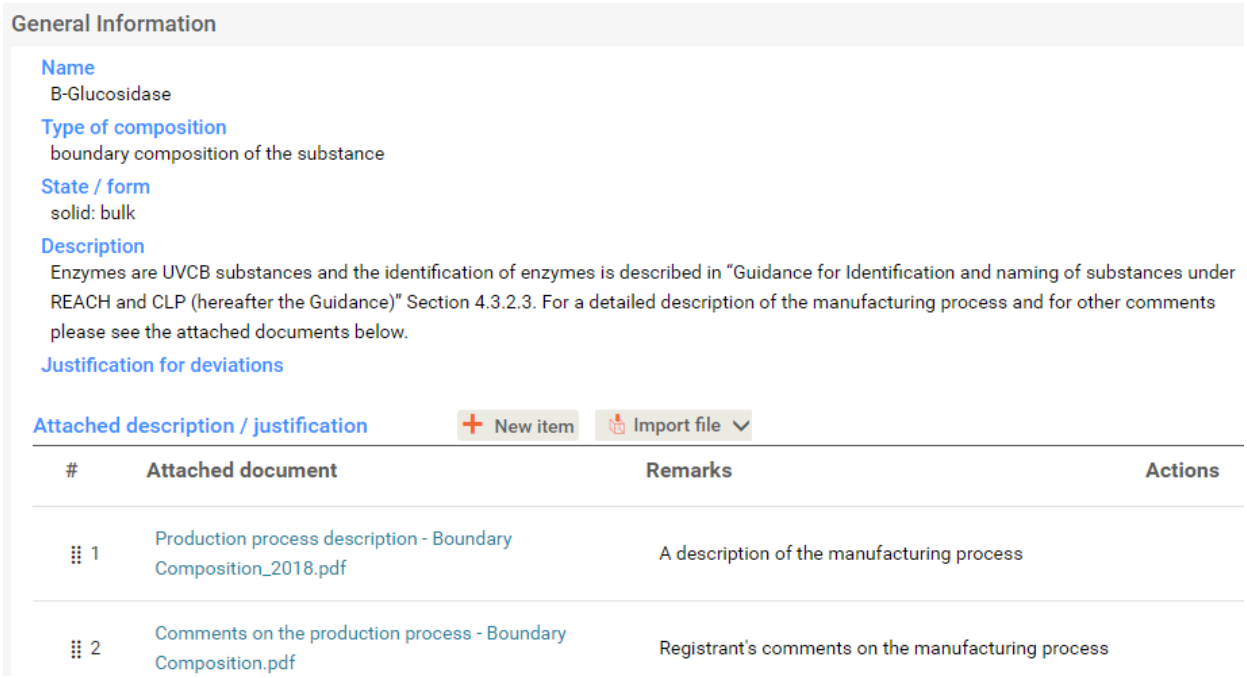
Origin
other: Enzyme

Section 1.2 Composition[2]

In this section, the manufacturing processes and a source should be provided if a substance is UVCB (Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials). An enzyme is a UVCB substance, however for enzymes it is not necessary to provide a source when the classification condition is met, according to the ECHA's Substance

[2] This section only covers the Boundary Composition. The Legal Entity Composition information can be filled out in the same way as the boundary.

Identification description "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". If a production strain(s) meets the criteria in ERC's document on "Safety evaluation of technical enzyme products with regards to the REACH legislation" then this classification condition is met. It is advisable to document this clearly in this section or as an attachment. A generic description of the production process should also be provided as an attachment.



General Information

Name
B-Glucosidase

Type of composition
boundary composition of the substance

State / form
solid: bulk

Description
Enzymes are UVCB substances and the identification of enzymes is described in "Guidance for Identification and naming of substances under REACH and CLP (hereafter the Guidance)" Section 4.3.2.3. For a detailed description of the manufacturing process and for other comments please see the attached documents below.

Justification for deviations

Attached description / justification + New item Import file

#	Attached document	Remarks	Actions
1	Production process description - Boundary Composition_2018.pdf	A description of the manufacturing process	
2	Comments on the production process - Boundary Composition.pdf	Registrant's comments on the manufacturing process	

Boundary composition is required for lead registration dossiers in addition to Legal Entity composition. For member registrations, only Legal Entity composition is required. ERC recommends populating the composition section according to ECHA's guidance on substance identification Section 4.3.2.3 (page 1 of this guidance).

Constituent (Reference substance should be created accordingly)	Range	Typical concentration
Active enzyme protein of the enzyme	10 - 80%	50 %
Other proteins + peptides and amino acids	5 - 55%	30%
Carbohydrates	3 - 40%	10 %
Lipids	0 - 5%	1 %
Inorganic salts	1 - 45%	9 %
Total	100 %	100 %

Please see Remarks for each component below.

Active enzyme protein of the enzyme

Remarks: In accordance with "GUIDANCE FOR SUBSTANCE IDENTIFICATION AND NAMING IN REACH Section 4.3.2.3", the enzyme substance consists of 1) the active enzyme protein and 2) constituents other than the active enzyme protein.

1) Enzyme substance: The enzyme substance is identified according to catalytic activity defined by IUBMB (INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (http://www.chem.qmul.ac.uk/iubmb/)).
IUBMB name: alpha-amylase; Enzyme Class No.: 3.2.1.1; Reaction: Endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units

2) Constituents other than enzyme protein. The substance does not contain a constituent which is ≥ 10 % (w/w) or relevant for classification and labelling and-or PBT assessment. The enzyme substance typically contains 10-80 % (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the following ranges: Active enzyme protein 10 - 80%, Other proteins plus peptides and amino acids 5 - 55%, Carbohydrates 3 - 40%, Lipids 0 - 5%, Inorganic salts 1 - 45%. The enzyme substance is produced by organisms which meet the criteria for "Safe Strain Lineage Concept" in "Safety evaluation of technical enzyme products with regards to the REACH legislation" dated September, 2021, published by Enzyme REACH Consortium (http://www.enzymes-reach.org/). The constituents other than enzyme protein produced by the organisms meeting the above criteria do not contribute to classification, thereby the enzyme substance having the same catalytic activity from such safe organisms are considered as the same substance.

Protein as a constituent of enzyme deriving from the fermentation or extraction process

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various proteins and peptides.

Carbohydrates

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various carbohydrates.

Lipids

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various lipids.

Inorganic salts

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various inorganic salts.

Set values

Reference substance

Active enzyme protein of β-Glucosidase | Active enzyme protein of β-Glucosidase (EC no. 232-589-7, CAS no. 9001-22-3, EC name: Glucosidase, β-, Enzyme Class no. 3.2.1.21)

Typical concentration

50 % (w/w)

Concentration range

ca. 10 - ca. 80 % (w/w)

Remarks

In accordance with "GUIDANCE FOR SUBSTANCE IDENTIFICATION AND NAMING IN REACH Section 4.3.2.3", the enzyme substance consists of 1) the active enzyme protein and 2) constituents other than the active enzyme protein.

1) Enzyme substance: The enzyme substance is identified according to catalytic activity defined by IUBMB (INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (http://www.chem.qmul.ac.uk/iubmb/)).

IUBMB name: Beta-Glucosidase, Enzyme Class No.: 3.2.1.21

2) Constituents other than enzyme protein. The substance does not contain a constituent which is ≥ 10 % (w/w) or relevant for classification and labelling and-or PBT assessment. The enzyme substance typically contains 10-80 % (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the following ranges: Active enzyme protein 20 - 60%, Carbohydrates 10 - 40%, Lipids 0 - 1%, Inorganic salts 0 - 10%. The enzyme substance is produced by organisms which meet the criteria for "Safe Strain Lineage Concept" in "Safety evaluation of technical enzyme products with regards to the REACH legislation" dated March 25, 2009, published by Enzyme REACH Consortium (http://www.enzymes-reach.org/). The constituents other than enzyme protein produced by the organisms meeting the above criteria do not contribute to classification, thereby the enzyme substance having the same catalytic activity from such safe organisms are considered as the same substance.

Section 1.4 Analytical Information

ERC recommend that assay method(s) for each constituent is populated. Spectra data are not relevant for active enzyme protein and other constituents.

Constituent (Reference substance should be created accordingly)	Analytical method
Active enzyme protein of the enzyme	Enzyme activity assay
Other proteins plus peptides and amino acids	Any method e.g. Kjeldahl to determine protein, peptides and amino acid
Carbohydrates	Any method e.g. colorimetric assay to determine carbohydrates or calculation
Lipids	Any method to determine lipids
Inorganic salts	Determination of ash

In each assay, a method should be uploaded and results should be populated. Below, an example of Kjeldahl protein analysis for β-glucosidase is shown.

Set values

Purpose of analysis
identification and quantification

Analysis type
✓ other: Crude Protein (Nx6.25) (Kjeldahl) Method NMKL 6; Amino acids (acid hydrolysis) Methods ISO 13903:2005 and EU 152/2009

Type of information provided
methods and results

Attached methods/results
Protein_amino_acid_analysis_NMKL6_description.pdf

Rationale for no results

Justification
Spectral data not relevant for active enzyme protein and other constituents.

The total protein concentration was analysed by the Kjeldahl method based on total nitrogen. This method is the standard method for estimating protein content in food forms part of a suite of analyses for foodstuffs.

Remarks
Crude protein Kjeldahl (Nx6,25): 11,9 (± 1,2) g/100 g (NMKL 6)

The results for residual protein can be calculated as follows:
Total protein – active enzyme protein = residual (other) protein
11,9 g/100 g – 7,4 g/100 g = 4,5 g/100 g

The results for residual protein reflect the amount of other proteins plus peptides and amino acids in the UVCB substance: taking dry matter into account (17,5 g/100 g dry matter), the result translates as 26 % of other proteins plus peptides and amino acids in the water-free composition. Such a result for other proteins is consistent with the typical compositional ranges for enzymes and for beta-glucosidase.

For results on separate amino acids, see attached result & method file.