Amfep Guidance in a Nutshell Classification of enzymes according to the CLP Regulation

1. Introduction

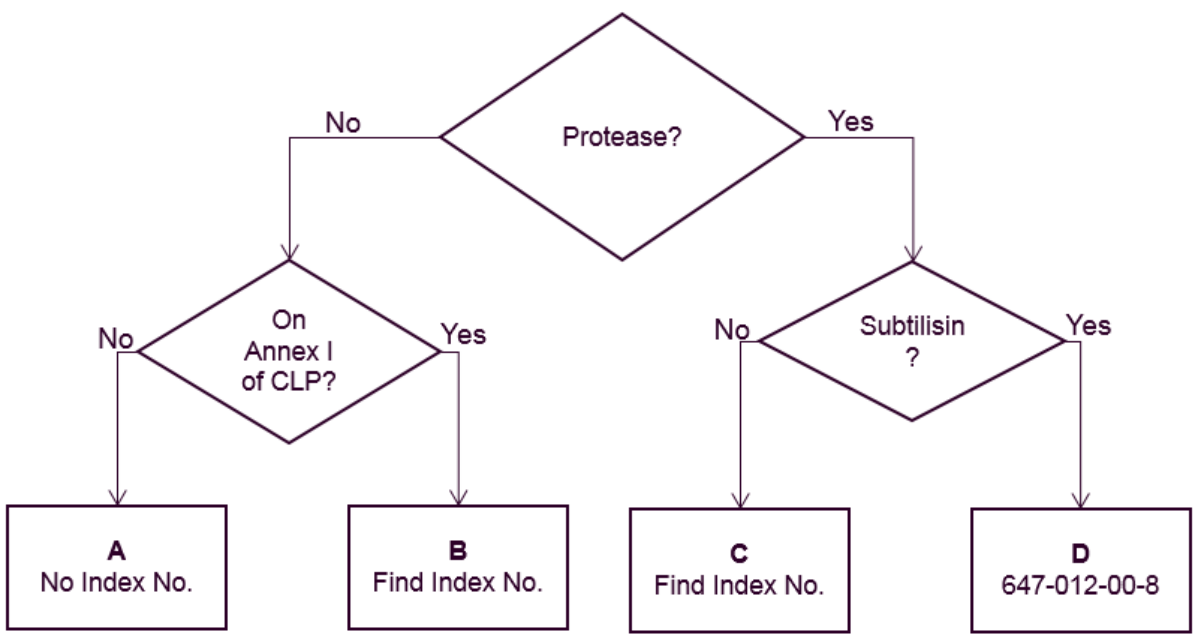
Enzymes on their own or in mixtures should be classified in accordance with Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (the 'CLP Regulation'). This guidance provides practical information how to classify enzymes and enzyme mixtures in accordance with the Guidance on the Application of the CLP Criteria document issued by ECHA (European Chemical Agency), available at https://echa.europa.eu/documents/10162/13562/clp_en.pdf.

Definitions of terms are attached in the last page.

For more detailed information, please read Amfep Document “Enzymes and the implementation of UN GHS in EU via the CLP Regulation”

2. Classification of enzyme

In this section, a process for classification of an enzyme is described.
Many enzymes are listed on Annex I to the CLP, and have mandatory (harmonized) classification for certain end-points. Proteases have additional classification due to their catalytic activities.



	Enzyme classification	CLP classification	Index No. in Annex VI
A	Non-protease(s)	Resp Sens. Cat 1	N/A

B	Non-protease(s)	Resp Sens. Cat 1	647-001-00-8 647-002-00-3 647-003-00-9 647-004-00-4 647-015-00-4 647-016-00-X 647-017-00-5	glucosidase, β- cellulase cellobiohydrolase, exo- cellulases with the exception of those specified elsewhere in this Annex amylase, α- amylases with the exception of those specified elsewhere in this Annex laccase
C	Protease(s)	Resp Sens. Cat 1 Skin irrit Cat 2 Eye irrit Cat 2 STOT SE Cat 3	647-005-00-X 647-006-00-5 647-007-00-0 647-008-00-6 647-009-00-1 647-010-00-7 647-011-00-2 647-013-00-3 647-014-00-9	bromelain, juice ficin papain pepsin A rennin trypsin chymotrypsin proteinase, microbial neutral proteases with the exception of those specified elsewhere in this Annex
D	Protease, Subtilisin	Acute Tox Cat 4* Resp Sens. Cat 1 Skin Irrit Cat 2 Eye Dam. Cat 1 STOT SE 3 Aquatic Acute 1* Aquatic Chronic 2 *	647-012-00-8	subtilisin

*From REACH registration dossier, additional to harmonized end-points.

3. Classification of enzyme mixtures

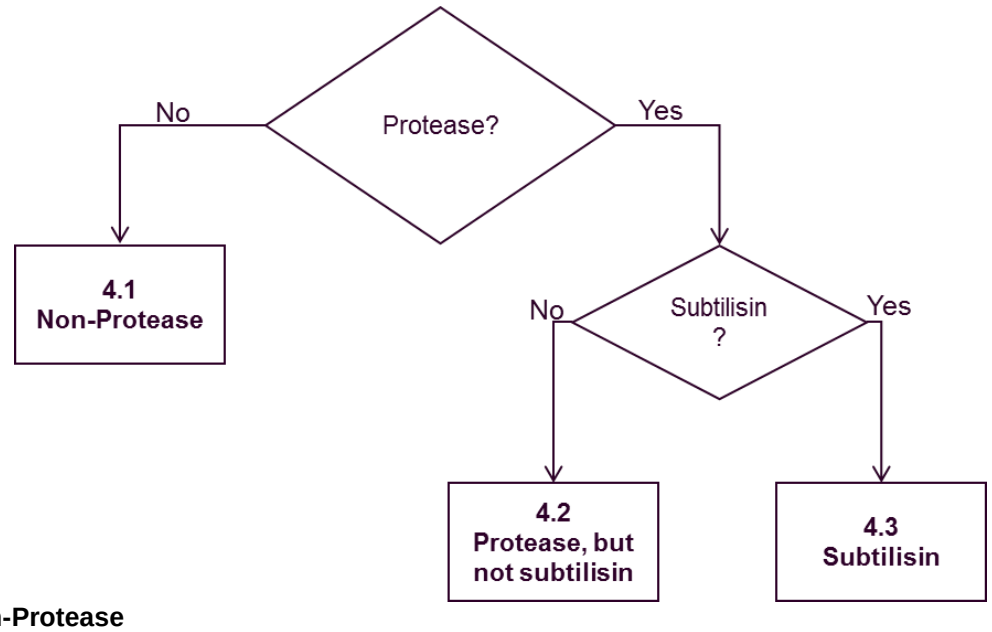
CLP classification of mixtures follows a tiered approach:
Tier 1: Classification based on data for the mixture as a whole
Tier 2: Classification using 'bridging principles' (essentially using data for similar mixtures)
Tier 3: Classification using concentration thresholds and calculation/summation

This guide will focus only on the third method, as this is the most common approach to classifying mixtures. For information on the other methods, please look in the ECHA guidance referred to in the Introduction section.

4. Classification of enzyme mixtures using concentration thresholds and calculation/summation

In this section, we focus on only enzyme classification of mixtures containing enzymes and according to the Guidance document (see link in Introduction section). Please note if other ingredients are classified, they should also be taken into account.

It is also a critical step to determine or estimate concentration of aep since classification of an enzyme mixture is dependent on it, see the Amfep Document "Enzymes and the implementation of UN GHS in EU via the CLP Regulation" referred to in the Introduction section.



4.1. Non-Protease

Resp. Sens. Cat 1

Classification of an enzyme mixture is based on Section 3.4.3.3.1. in the ECHA guidance.

This hazard class is not 'Additive', meaning that different enzymes in the same mixture are evaluated independently against the values below. If, however, these enzymes are known to be *immunochemically identical*, the concentration of aeps should be summed for classification.

(Likewise, enzymes with the same catalytic activity (same IUB name and number) should be viewed additively unless they are known to be *immunochemically different)*

Concentration of aep	Classification
C ≥ 1 %	Resp. Sens. Cat 1
0,1 % ≤ C < 1%	No classification, but the following statement should be on a label EUH 208 – 'Contains (name of sensitising substance). May produce an allergic reaction'*
C < 0,1%	Not classified. No CLP labelling. Other labelling may still be required due to other legislation, e.g. Detergent Regulation.

*According to Section 3.4.4.2 of the Guidance.

4.2 Protease, but not subtilisin

Skin irrit. Cat 2 Eye irrit. Cat 2 STOT SE Cat 3 Resp. Sens. Cat 1 – see also 4.1

Each of the end points for irritation (eye, skin and respiratory irritation) are 'Additive', meaning that e.g. all substances in a mixture that are classified for skin corrosion or skin irritation must be evaluated together to derive the mixture classification.

In the case of only one protease in a mixture and no other ingredients influencing classification:

Concentration of aep	Classification
C ≥ 20%	Skin irrit Cat 2

	Eye irrit Cat 2 STOT SE Cat 3 Resp Sens. Cat 1
10% ≤ C < 20%	Skin irrit Cat 2 Eye irrit Cat 2 Resp Sens. Cat 1
1% ≤ C < 10%	Resp Sens. Cat 1
0,1 % ≤ C < 1%	No classification, but the following statement should be on a label EUH 208 – 'Contains (name of sensitising substance). May produce an allergic reaction'
< 0,1%	Not classified. No CLP labelling. Other labelling may still be required due to other legislation, e.g. Detergent Regulation.

For the end points on skin and eye irritation it is possible to use bridging principles when classifying mixtures. Reference to the guidance.

4.3 Subtilisin

Acute Tox Cat 4* (1800 mg/kg)
Eye Dam. Cat 1
Aquatic Acute 1* (M-factor 1)
Aquatic Chronic 2 *
Resp Sens. Cat 1
Skin Irrit Cat 2
STOT SE 3

*From REACH registration dossier

The end points for irritation (skin and respiratory irritation), eye damage, acute toxicity, aquatic acute and aquatic chronic are 'Additive', meaning that e.g. all substances in a mixture that are classified for these effects must be evaluated together to derive the mixture classification.

In the case of only Subtilisin in a mixture and no other ingredients influencing classification:

Concentration of aep	Classification
C ≥ 90%	Acute Tox Cat 4 Eye Dam. Cat 1 Resp Sens. Cat 1 Skin Irrit Cat 2 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 2
25% ≤ C < 90%	Eye Dam. Cat 1 Resp Sens. Cat 1 Skin Irrit Cat 2 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 2
20% ≤ C < 25%	Skin irrit Cat 2 Eye Dam. Cat 1 STOT SE Cat 3 Resp Sens. Cat 1 Aquatic Chronic 3
10% ≤ C < 20%	Skin irrit Cat 2 Eye Dam. Cat 1 Resp Sens. Cat 1 Aquatic Chronic 3
3% ≤ C < 10%	Eye Dam. Cat 1

	Resp Sens. Cat 1 Aquatic Chronic 3
2,5% ≤ C < 3%	Eye irrit Cat 2 Resp Sens. Cat 1 Aquatic Chronic 3
1% ≤ C < 2,5%	Eye irrit Cat 2 Resp Sens. Cat 1
0,1 % ≤ C < 1%	No classification, but the following statement should be on a label EUH 208 – 'Contains (name of sensitising substance). May produce an allergic reaction'
< 0,1%	Not classified. No CLP labelling. Other labelling may still be required due to other legislation, e.g. Detergent Regulation.

The Aquatic chronic 2 classification was added due to 2nd amendment to CLP (Regulation 286/2011) and is based on NOEC data, which is the reason why it is not the same category as Aquatic Acute. When labelling a mixture containing 25% or more of Subtilisin as active enzyme protein, it is allowed, but not mandatory, to combine the hazard phrases H400 ("Very toxic to aquatic life") and H411 ("Toxic to aquatic life with long-lasting effects") and instead use the phrase H410 ("Very toxic to aquatic life with long-lasting effects")

It is important to note that the classification Aquatic Acute 1 and Aquatic Chronic 2 are also covered by the international regulations on the transport of dangerous goods. Therefore, any mixture containing 25% or more of Subtilisin as active enzyme protein will be dangerous goods in class 9 and fall under either designation UN3077 (solids) or UN3082 (liquids).

5. Definition

aep (active enzyme protein): enzyme protein which has a catalytic activity. There are various way to determine aep. For example, aep can be calculated by dividing total activities by the enzyme's specific activity.

Annex VI of the CLP Regulation: A list of substances with mandatory classifications – all other end points than those listed in the Annex are liable to self-classification.

classification: The process of evaluating available test or other data on a substance or mixture in order to determine if the substance or mixture meets any of the criteria for assigning a class (type) and category (severity) of hazard to the substance or mixture. Description of relevant classifications:

Acute Tox. Cat 4	Acute toxicity in category 4. For Subtilisin the route of exposure is oral intake. The toxicity data behind it (1800 mg/kg) is used for classification of mixtures.
Resp. Sens. Cat 1	Respiratory sensitiser in category 1. CLP also includes the possibility to subcategorise into 1A and 1B, however this is not possible for enzymes.
Skin Irrit Cat 2	Skin irritation in category 2. (moderate skin irritation)
Eye Dam. Cat 1	Eye damage in category 1. (severe and irreversible eye damage)
Eye irrit Cat 2	Eye irritation in category 2. (serious, but reversible eye irritation)
STOT SE 3	Specific target organ toxicity – single exposure in category 3. For proteases the relevant in effect in this category is respiratory irritation.
Aquatic Acute 1	Acute toxicity towards aquatic organisms in category 1 (short term environmental hazard). For classification of mixture it is also important to know that the "M-factor" is 1.
Aquatic Chronic 2	Chronic toxicity towards aquatic organisms in

	category 2 (long term environmental hazard).

enzyme: Enzyme concentrate as defined as “substance” by REACH including constituents from manufacturing processes but excluding solvent e.g. water. In this guidance, “enzyme” refers to this definition.

enzyme mixture: A formulated product with an enzyme and other formulation ingredients.

Index No.: Number assigned to substances in Annex VI.

Immunochemically identity: if two enzyme protein cross-react with the same antibody, they are immunochemically identical.

IUBMB name and number: Enzyme nomenclature defined by Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) http://www.chem.qmul.ac.uk/iubmb/enzyme/