Why enzyme substances are not nanomaterials

Introduction

As a response to the continued and increasing focus on nanomaterials Amfep wants to communicate its view on whether industrial enzyme products (further called 'enzyme substances')[1] can be regarded as nanomaterials.

The following information has been collected by Amfep members and represents Amfep's interpretation of published legislation and/or information given by competent authorities in connection with this issue.

It represents the current situation to the best of our knowledge.

Amfep position

Enzyme substances cannot be considered as nanomaterials since:

i) enzyme protein molecules cannot be considered as defined particles;
ii) enzyme substance is not on the market as particles in the nanometer scale;
iii) enzyme substance does not have nanomaterial properties.

Definition of nanomaterials and situation in the EU

The European Commission (Commission Recommendation 2011/696/EU) recommends to use the following definition of the term nanomaterial[2]:

"*A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.*

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %".

A specific derogation is given to fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm (Art. 3), which materials are considered as nanomaterials regardless of the size range.

To narrow the interpretation, 'particle', 'agglomerate' and 'aggregate' are defined as follows (Art. 4):

(a) 'particle' means a minute piece of matter with defined physical boundaries;

(b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

[1] Industrial enzyme products are not pure substances. In this document, they are referred to as 'substances' on basis of the REACH definition

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF

(c) 'aggregate' means a particle comprising of strongly bound or fused particles.

The above definition will be reviewed in 2014 in the light of technical and scientific progress.

To resolve if a material meets this definition a multi-step assessment is required including:

1) Determine if the material consists of fullerenes, graphene flakes or single-wall carbon nanotubes.
2) Determine if the material consists of particles (i.e. with physical boundaries), or aggregates or agglomerates containing particles.
3) Determine the external dimensions of the (constituent) particles.
4) Determine the median value of the particle size distribution (corresponds with the 50 % fraction mentioned in the definition.)

The EC's Joint Research Centre (JRC) conclude in their recent report[3] that none of the currently available state-of-the-art techniques for analyzing nanomaterials (e.g. DLS, TEM, SAXS, FFF, XRD) can individually determine whether all kinds of potential nanomaterials fulfill the definition in the EC Recommendation. For the time being it remains necessary to combine different and complimentary methods for each material. The JRC proposed that a revision of the current recommended EC definition of nanomaterials should incorporate this by providing guidance on which techniques are required to assess different types of potential nanomaterials.

The EC has recently stated that the current legislation on chemicals (e.g. REACH) will for the moment be sufficient to cover nanomaterials[4]. To improve availability of information on nanomaterials, the EC will create a web platform with references to all relevant information sources, including registries on a national or sector level, where they exist.

As stated by the Commission[5], nanomaterials are not intrinsically hazardous per se but there may be a need to take into account specific considerations in their risk assessment. Therefore one purpose of the definition is to provide clear and unambiguous criteria to identify materials for which such considerations apply.

In the Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers[6], contains another definition of nanomaterials, specifically focusing on intentionally produced materials engineered to have nanoscale dimensions:

'engineered nanomaterial' means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale.

Properties that are characteristic of the nanoscale include:

(i) those related to the large specific surface area of the materials considered; and/or

[3] http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/26399/1/irmm_nanomaterials%20%28online%29.pdf
[4] http://europa.eu/rapid/press-release_IP-12-1050_en.htm?locale=en
[5] http://ec.europa.eu/environment/chemicals/nanotech/index.htm
[6] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF

(ii) specific physico-chemical properties that are different from those of the non-nanoform of the same material;

This focus on engineered nanomaterials is in line with the opinion[7] of EFSA´s Scientific Committee on "The potential risks arising from nanoscience and nanotechnologies on food and feed safety". EFSA specifically mentions: *'"Natural" nanoscale materials (e.g. micelles) will be considered if they have been deliberately used e.g. to encapsulate bioactive compounds or further engineered to retain their nanoscale properties. "Natural" nanoscale components present as emulsions (e.g. in homogenized milk, mayonnaise, etc.) will not be considered*'.

Of interest in this respect is the recent draft revision[8] of Regulation (EU) No 1169/2011 as regards the definition of 'engineered nanomaterials' in order to align it with the Commission Recommendation 2011/696/EU on the definition of 'nanomaterial'. The proposed adapted definition retains the reference to the intentionality of the manufacture, and includes a further definition of "intentionally manufactured" to mean: *" that the material is manufactured to fulfil/perform a specific function or purpose;"*. The proposed adapted definition also includes a further derogation, apart from the fullerenes etc., for food additives:

"*food additives covered by the definition set out in the first paragraph shall not be considered as engineered nanomaterials, if they have been included in the Union lists referred to in Article 4 of Regulation (EC) No 1333/2008 byCommission Regulations (EU) No 1129/2011 and (EU) No 1130/2011;*".

Definition of nanomaterials and situation in the US

In 2001, a federal R&D program, the National Nanotechnology Initiative (NNI), was established to coordinate multiagency efforts in nanoscale science, engineering, and technology in the US. The NNI collaborates with more than 20 US agencies including the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). The participating agencies follow the NNI's definition for nanotechnology, which is[9]:

1) *Research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1 - 100 nanometer range.*
2) *Creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.*
3) *Ability to control or manipulate on the atomic scale.*

This definition is very broad and appears to be more focused on commercial claims rather than strictly categorizing a potential material as a nanomaterial (it is indeed far less 'quantitative' compared to the EC definition). Currently, EPA and FDA regulate nanomaterials under their existing legislation (e.g. TSCA).

Of particular importance in relation to biological materials like enzymes is the following text from FDA's draft guidance[10]:

[7] Scientific Opinion of the Scientific Committee on a request from the European Commission on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. The EFSA Journal (2009) 958, 1-39

[8] SANCO/11616/2013, see http://www.nanotechia.org/news/news-articles/ec-revises-nano-elements-regulation-provision-food-information-and-labelling

[9] Definition stated on the US EPA website: http://epa.gov/ncer/nano/questions/index.html (see also the NNI website: http://www.nano.gov/)

[10] http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm

"B. Rationale for Elements within the Points to Consider

1. Engineered material or end product

This term is used to distinguish between products that have been engineered to contain nanoscale materials or involve the application of nanotechnology from those products that contain incidental or background levels of nanomaterials or those that contain materials that naturally occur in the nanoscale range. FDA is particularly interested in the deliberate manipulation and control of particle size to produce specific properties, because the emergence of these new properties or phenomena may warrant further evaluation. This is distinct from the more familiar use of biological or chemical substances that may naturally exist at small scales, including at the nanoscale, such as microorganisms or proteins."

Why industrial enzyme substances are not nanomaterials

The industrial manufacturing of enzyme substances is fundamentally different from how man-made and natural (e.g. clay particles and sod from volcanoes) nanomaterials are being produced. Most commercial nanomaterials (e.g. inorganic nanoparticles, carbon-based nanostructures and quantum dots) are synthesized through several chemical reactions (e.g. changing pH and salt concentration to make ions in solution to form nanoparticles). Contrary, enzyme proteins are synthesized by all living organisms e.g. bacteria and fungi as the result of normal metabolic activity. Industrial enzyme substances are produced from plant or animal material but notably from microorganisms by fermentation. Following fermentation of the production organism the target enzyme protein is retrieved through different filtering steps. The resulting 'enzyme substance' consists of the active enzyme protein plus other constituents from the fermentation. The substance matter can be divided into biological matter normally referred to as TOS (Total Organic Solids, consisting of proteins, peptides and amino acids, carbohydrates and lipids) and soluble mineral matter normally referred to as Ash (inorganic salts).

The majority of enzyme protein molecules have a molecular weight within the range of 20-160,000 kDa (average of ~50 kDa); which converted to nanometers corresponds to a globular diameter of approximately 3-7 nm in size[11]. Although, this size is still falling within the nanoscale as defined by the EC and NNI, is at the periphery and not unusual for biological substances (see FDA statement above). Most biological matter (DNA, organelles, proteins/enzymes) falls within the nanoscale of 1-100 nm.

In their Questions and Answers on the Commission Recommendation on the definition of nanomaterial[12], the Commission specifically mentions that proteins are non-particular and do not fall under the definition of nanomaterials. This is also in line with the scientific advice[13] from the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), that says "*Obviously most substances will have internal structures that individually could be considered as being at the nanoscale, for example molecules, crystals or domains, but these do not, a priori, qualify for classification as nanomaterials. For example, simply because a polymer may have individual molecules of nanometre dimensions does not necessarily confer nanomaterial status on that substance".*

[11] Erickson HP (2009). Biological Procedures Online 11:1.

[12] MEMO/11/704, http://europa.eu/rapid/press-release_MEMO-11-704_en.htm?locale=en or http://ec.europa.eu/environment/chemicals/nanotech/questions_answers.htm

[13] http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf

Enzyme proteins should also not be considered as nanomaterials because their characteristics are completely different from the commercially produced nanomaterials. An important aspect of nanomaterials is their specific physicochemical properties, most likely due to their large specific surface area[14]. This characteristic is important because many physical and chemical processes take place at the surface of solids. These surface-specific processes can be associated with health effects. In contrast, the characteristics of enzyme proteins are located inside the molecule: the respiratory sensitization potential of enzyme proteins is associated to specific amino acid sequences (=epitopes), and the catalytic activity of enzyme proteins depends on the catalytic site consisting of a specific three-dimensional setting of amino acid residues. In this respect it is of importance to note that the nature of enzyme proteins is to catalyze biochemical reactions and under 'normal operating conditions' they will not agglomerate as this would deteriorate the formation of the enzyme-substrate complex; and thus reduce the catalytic power of the enzyme protein. Thus, enzymes are dispersed as individual molecules throughout the media in which they operate.

The soluble mineral matter consists of normal electrolytes from the cells of the organism which is used as an enzyme source, and nutrients from the fermentation medium. Either way, these salts are present in the enzyme substance mixed with the organic matter and are not expected to crystallize but rather to be present in an amorphous state when the enzyme substance is dried, or to be fully dissolved when the enzyme substance is in solution.

Enzyme preparations on the market are enzyme substances which have been either formulated as liquid products containing free flowing molecules, or as granulates. The latter are particles with defined physical boundaries, however far bigger than the scales defined as nanomaterial. The enzyme industry policy is to avoid enzyme preparations able to form inhalable or respirable dust[15], i.e. with size < 10 µm.

Conclusion

Amfep concludes that enzyme substances cannot be considered as nanomaterials because: i) enzyme protein molecules cannot be considered as defined particles; ii) enzyme substance is not on the market as particles in the nanometer scale; and iii) enzyme substance does not have nanomaterial properties. Enzymes should, together with many other biological structures, be referred to as (bio)material and not as nanomaterial. Nanomaterials are never referred to as molecules, but rather as a (nano)particle, rod, ring, tube, fiber or composite (to mention a few).

Overall, there appears to be less concern about enzyme substances being categorized as nanomaterials in the US, especially as preparations have been on the (global) market for decades without any claims of using nanotechnology. The same argument should apply to the EU and other international markets. However, to avoid confusion Amfep strongly discourages any of its members to start using nano-claims in the marketing of their enzyme products, unless the product in fact contains a non-biomolecule nanomaterial formulation ingredient.

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[14] http://ec.europa.eu/health/scientific_committees/opinions_layman/nanomaterials/en/l-3/2.htm#1
[15] http://www.osha.gov/dsg/topics/silicacrystalline/dust/chapter_1.html