EFSA publishes final guidance on the risk assessment of the application of nanoscience and nanotechnologies in food and feed chain

After the public consultation on the scientific opinion regarding the draft guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain, the final version of the document was published on the 10th of May 2011 (http://www.efsa.europa.eu/en/efsajournal/pub/2140.htm). The aim of the opinion is to give practical guidance for the risk assessment of applications, involving the use of Engineered Nanomaterials (ENM) in the food and feed chain. The guidance also is clearly stating that reduced information can be provided when absence of exposure to the ENM can be proved by data indicating no migration from food contact materials, or in cases where complete degradation/dissolution is demonstrated with no absorption of the ENM as such.

Although the final version of the guidance document does not introduce any fundamental change to the draft version endorsed for public consultations in January 2011, a number of sections have been reorganised and clarified in the final version. More specifically, the final version of the guidance document:

- clarifies the scope of substances that are to be considered ENM
- specifies the need to take into account the presence of ENM in biological fluids and tissues and the difficulties related to this task
- clarifies the strategic approach for toxicity testing with a new classification into 6 general cases
- provides an additional decision tree for toxicity testing (Figure 3), and
- provides changes with regard to some of the recommended toxicity tests.

We discuss below the main elements of the final guidance documents and highlight some of the new elements that were not present in the January draft version of the document.

Engineered nanomaterials in the scope of the guidance

In the final draft guidance the term engineered nanomaterial (ENM) "refers to a nanomaterial produced either intentionally or unintentionally (due to the production process) to be used in the food and feed area." The term in the context of the guidance should be equivalent to "manufactured" or " processed" as used in the previous reports of SCENIHR from 2009 and 2010. **An important clarification available in the final version of the opinion is that "natural compounds are considered within the context of this ENM guidance, only if they have been deliberately produced to have nanoparticles, or used e.g. to encapsulate bioactive compounds."**

Characterisation and identification of ENM

The risk of the ENM is determined by its chemical composition, physico-chemical properties, interactions with tissues and its potential exposure. The characterisation of ENM by its physico-chemical form in food/feed products is important in order to assess whether the material tested is representative of the form in which it is intended to be used. In the initial state of the risk assessment it should be considered whether the structure and properties of the ENM can possibly be affected due to its addition into the food/feed matrix. After such assessment it is important to carry out the tests in an adequate environment. The change in the physico-chemical parameters should be considered in five stages:

- ENM, as manufactured
- ENM, as delivered for use in food/feed products
- ENM, as present in the food/feed matrix
- ENM, as used in toxicity testing
- ENM, as present in biological fluids and tissues (particularly important for the ADME studies)

As in the draft guidance, the parameters for characterisation and identification of ENM are presented in table 1 of the document. Again, as in the first draft, the size characterisation is required to be determined by two methods, one of which being electron microscopy. Primary and secondary particles should be characterised by size range and number size distribution. **The characterisation of ENM as present in biological fluids and tissues is added as a separate point in the guidance (p. 3.1.4).** In these studies, it is essential that the measuring system be able to detect the nanomaterial itself or its elemental composition in the biological sample. Concerning the performance criteria for characterisation methods the new guidance is reminding that "it may be expected that regulatory authorities will systematically require routine methods for monitoring compliance with the specification of the ENM. "

Exposure Assessment and Toxicity Testing

As in the first draft of the document, the exposure scenarios logic chart is illustrated in Figure 2 of the document, which aims to help on the decision regarding the extent of the hazard characterisation and to provide parameters for the exposure assessment required for the risk assessment. The guidance is specifying that "where it can be demonstrated that the ENM are completely solubilised in the food/feed matrix, no human exposure is expected apart from exposure to resulting degradation product (non-nanoform fraction). On the other hand, "when ENM are present in an indirect way, e.g. due to migration or transfer of non-nanoform degradation products of the ENM, ... its type and amount should be determined".

The strategy for toxicity testing is determined by the ENM in the food/feed matrix and by information on the non-nanoform of the substance. **The strategic approach in the first draft document has now been clarified to distinguish between 6 general cases**:

- **Case 1: No presence of ENM in preparations/ formulations as marketed** ENM is completely degraded/solubilised to non-nanoform – the EFSA guidance on non-nanoforms for the specific intended use should apply
- **Case 2: No migration from food contact materials (i.e. no exposure)**

"Where evidence is provided convincingly demonstrating, by appropriate analytical methods that there is no migration, the risk assessment could be based on the information that there is no exposure to the ENM via food and therefore there is no toxicological concern."

- **Case 3: Complete ENM transformation in the food/feed matrix before ingestion**

Complete transformation (before ingestion) of the ENM into a non-nanoform in the food/feed matrix – the EFSA guidance on non-nanoforms for the specific intended use should apply.

- **Case 4: Transformation during digestion**

ENM completely dissolves/degrades in the gastro-intestinal tract – data for the non-nanoform substance, "as long as the possibility to ENM absorption before the dissolution/degradation stage can be excluded". In such case, limited set of tests consisting of in vitro genotoxicity, in vivo local effects and/or other appropriate in vivo tests may be deemed sufficient. If it is proven that the systematic toxicity profile of the dissolved ENM is similar to the soluble form, no further testing is required. However, in all cases data on the non-nanoform is required according to the EFSA guidance for the intended use.

- **Case 5: Information on non-nanoform available**

Information of a non-nanoform of the same substance is available, and some or all of the ENM persist in the food matrix and in gastrointestinal fluids - a testing approach should be used, "based on comparison of information on absorption; distribution, metabolism and excretion (ADME), toxicity and genotoxicity of the non-nanoform with, in first instance, ADME, repeated-dose 90-day oral toxicity study in rodents and genotoxicity information of the ENM."According to the guidance, if the differences from the comparison of the two forms show increased hazard, then more toxicity testing will be required. If the differences indicate less hazard, then any request to waive further testing should be scientifically justified.

- **Case 6: No information on non-nanoform available**

No information of the non-nanoform, some or all of the ENM persist in the food/feed matrix and in gastrointestinal fluids, the approach should follow the relevant EFSA guidance for the intended use with the modifications in the opinion to take into account the nano-properties.

The ENM toxicity testing strategy is presented in Table 2 of the document and the new decision tree for toxicity testing is included in Figure 3 of the final guidance.

Changes in recommended toxicity tests

In vitro digestion models can simulate the conditions of the human gastrointestinal tract and can be used to demonstrate dissolution of the ENM but to which extent the different in vitro models lead to conclusions regarding dissolution/degradation of nanomaterials has not yet been studied. For the determination of the genotoxicity, two in vitro tests are required by the guidance:

- A test for induction of gene mutation in mammalian cells (preferably the mouse lymphoma tk assay with colony sizing) (OECD test guidance 476)
- An in vitro micronucleus assay (OECD test guideline 487)

Other in vitro studies, like cytotoxicity, oxidative stress, potential for inflammation and immunotoxicity, may give more information on the mechanism of action of ENMs.

In contrast to the first draft of the guidance document, the in vitro chromosomal aberration test (OECD test guideline 437) is removed from the final version of the scientific opinion and thus the final guidance requires two tests: OECD test guideline 476 and OECD test guideline 487. Also, in opposition to the draft document, in the final opinion the use of bacterial reverse mutation test for detection of genotoxicity of ENMs is not considered to be appropriate because the bacterial cells do not have the ability to phagocytose particles. In vivo genotoxicity test should be considered if:

- At least one of the in vitro tests indicates positive results
- It is impossible to test the ENM in vitro
- If both in vitro tests are negative but there are indications that reactive radical species are generated

The in vivo studies are essential to determine toxicokinetic profile of the ENM using the information received from ADME. "Tissue distribution, accumulation/persistency and elimination from tissues is considered to be more relevant than blood plasma levels". The new version of the guidance is insisting on the special attention that should be paid to the typical target organs that have increased capacity for intake particles such as liver, spleen and lung. The OECD test guideline 417 is cited as a description of "the general methodologies with multiple measures and endpoints for performing ADME studies."

Further, "for ingested ENM, the minimum requirement is a repeated-dose 90-day oral toxicity study in rodents (OECD test guideline 408), modified to include assessment of some additional parameters described in the more recent guideline on repeated-dose 28-day oral toxicity study in rodents (OECD test guideline 407). "

If a follow-up by in vivo genotoxicity testing is required, the choice of an appropriate in vivo genotoxicity tests requires expert judgement and any of the tests referred in the scientific opinion can be used:

- An in vivo micronucleus test (OECD test guideline 474)
- An in vivo Comet assay (no OECD test guideline at present; internationally agreed protocols available, e.g. see hptt://cometassay.com)
- A transgenic rodent gene mutation assay (draft OECD test guideline)

The consideration on exposure assessment stays basically without any significant changes in comparison to the first draft of the guidance as well as the risk characterisation.

In the final version of the guidance, the uncertainty analysis is placed in a separate chapter (Chapter 8 of the document). It is divided in 4 parts: uncertainties in the physico-chemical characterisation of ENM, uncertainties in the hazard characterisation of the ENM, uncertainties in exposure assessment and uncertainties in the risk characterisation.

The guidance is acknowledging that "the field is under fast development and consequently this guidance document will be revised as appropriate".

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