Overview of the EFSA Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain

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The EFSA ‘NanoGuidance’ provides applicants and risk assessors with a **structured pathway** to assess potential risks of

- **Engineered nanomaterials** (as per legal definition)

- **Any other type of substance** falling under the food law that might present hazards related to the nanoscale, independently from regulatory definitions

- **Size-dependent properties and biological effects** of potential concern for human health, e.g. toxicokinetic behaviour and particle–cell interactions, are *not rigidly related to specific (legally defined) size thresholds*

- Whereas physical, chemical and biological properties of materials may change with size, there is *no scientific justification for a single size limit* associated with these changes that can be applied to all nanomaterials

- **Potential risks** arising from specific properties related to the nanoscale have to be assessed focusing on such properties and potentially related hazards, which may be independent of the proportion of particles constituting the material with a size below 100 nm
Physicochemical characterisation: the first stage of the scientific assessment

It **answers the first question**: is nano-specific risk assessment (starting with hazard identification and characterisation) needed or not?

**Physicochemical characterisation**

1. **Is the material a nanomaterial (as legally defined)?**
   - No: Follow safety assessment according to the relevant EFSA Guidance for conventional materials.
   - Yes: Continue to the next step.

2. **Characteristic of the nanoscale**
   - Does the material have properties that are characteristic of the nanoscale?
     - No: Follow safety assessment according to the relevant EFSA Guidance for conventional materials.
     - Yes: **Hazard Identification and Characterisation**
       - Starting with *in vitro digestion*.
Characterisation of **particle size and size distribution** is the first step to deciding whether the material has to be considered for nanospecific risk assessment.

It is required that the size parameter should always be measured by **at least two independent techniques**, one being electron microscopy.

If electron microscopy is not applicable (e.g. for some organic nanomaterials), it is recommended to use **another imaging technique** instead of electron microscopy.

For materials with a median particle size above 100 nm, **the presence of properties characteristic of the nanoscale** has to be assessed by the phys-chem characterisation.

Where a material is regarded to fall within the scope of the Guidance, a detailed phys chem characterisation is required for **unambiguous description of the material’s identity** in pristine form and **relevant physicochemical properties**.
Oral Exposure Assessment

Follow safety assessment according to the relevant EFSA Guidance for conventional materials

Type of nanomaterial application (e.g. ingredient/additive/pesticide/food contact material)

Information on the characteristics of the pristine nanomaterial and the amount added to food/feed item or FCM

Does the material fully degrade in the food/feed matrix?

Does the material quickly and fully degrade in digestive tract conditions?

Yes

There is nanomaterial present

No

Direct exposure via food/feed

(potential) Indirect exposure, i.e. after release from FCM/via carry-over

Is there migration/transfer of nanomaterial to food/feed?

Yes

Exposure estimation in various population groups based on consumption data, average and high exposure, and the presence of the nanomaterial and any nanosized degradation products in food/feed, food simulant and/or in vitro digestive tract conditions

No

Quantification and characterisation (particle size distributions) of the nanomaterial and any nanosized degradation products in food/feed, food simulant and/or in vitro digestive tract conditions

Follow safety assessment according to the relevant EFSA Guidance for conventional materials
A stepwise framework for nano-related hazard identification and characterisation is outlined in the Guidance to avoid any unnecessary testing.

Even around or within the nanoscale, there may be considerable fluctuation in the toxicity of a given nanomaterial due to variations in particle size: it is therefore crucial that there is complete correlation between the material as produced and as tested, and that the size and properties of the manufactured material used in the specific application lie within the narrow range covered by the risk assessment.

In this light, batch-to-batch variation is of special concern and strict criteria should be followed to ensure the manufactured material consistently presents constant physicochemical parameters (i.e. those considered in the risk assessment).
Hazard identification and characterisation

Follow safety assessment according to the relevant EFSA Guidance for conventional materials

**Step 0** In vitro gastrointestinal digestion
- Does the nanomaterial degrade quickly and fully under GI tract conditions?

**Step 1a** Review existing information
- All existing phys-chem/toxicological information

**Step 1b** Generate new in vitro data
- Dissolution under lysosomal conditions, in vitro genotoxicity, in vitro cell toxicity

**Is the nanomaterial non-persistent AND no indication of potential toxicity is observed?**

**Step 2a** Pilot in vivo study
- For dose finding and assessment of absorption/tissue distribution/accumulation and elimination phases (≈ 14 days)

**Do these results warrant further testing?**
- Indications for slow elimination/accumulation & distribution to specific tissues may warrant further testing

**Step 2b** In vivo studies
- In vivo genotoxicity
- Modified 90-d oral toxicity study + Satellite group for assessment of absorption/tissue distribution/accumulation (≈ 14 d, 90 d, elimination phase) incl. assessment of key GI sites, liver, brain, testis and spleen by histopathology and relevant endpoints

**Step 3** Targeted in-depth investigations
- E.g. additional toxicokinetic study (optionally human studies), reproductive and developmental toxicity, additional immunotox, neurotox, carcinogenicity/mutagenicity, endocrine effects, gut microbiome

An argument for waiving in vivo studies might be put forward (to be assessed by EFSA on a case-by-case basis)

Go to Risk Characterisation
Hazard identification and characterisation

Follow safety assessment according to the relevant EFSA Guidance for conventional materials.

Step 0: In vitro gastrointestinal digestion
Does the nanomaterial degrade quickly and fully under GI tract conditions?

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All existing phys-chem/toxicological information

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Step 2a: Pilot in vivo study
For dose finding and assessment of absorption/tissue distribution/accumulation and elimination phases (≈ 14 days)

Step 2b: In vivo studies

In vivo genotoxicity

+ Modified 90-d oral toxicity study + Satellite group for assessment of absorption/tissue distribution/accumulation (≈ 14 d, 90 d, elimination phase)

incl. assessment of key GI sites, liver, brain, testis and spleen by histopathology and relevant endpoints
Hazard identification and characterisation

...continues from Step 2 (*In vivo* testing)

Do these results warrant further testing?
Indications for slow elimination/accumulation & distribution to specific tissues may warrant further testing

Yes

Step 3  Targeted in-depth investigations
E.g. additional toxicokinetic study (optionally human studies), reproductive and developmental toxicity, additional immunotox, neurotox, carcinogenicity/mutagenicity, endocrine effects, gut microbiome

No

Go to Risk Characterisation
Conclusions

- The existing risk assessment paradigm for chemicals is also applicable to nanomaterials. However, testing of nanomaterials needs consideration of certain nanospecific aspects that are addressed by the NanoGuidance.

- The Guidance proposes a structured pathway for carrying out safety assessment of nanomaterials and any other type of substance falling under the food law that might present hazards related to the nanoscale, independently from regulatory definitions, providing practical suggestions for the types of testing needed and the methods that can be used for this purpose.

- Whenever possible tiered approaches or circumstances under which data generation can be waived are suggested, e.g. in phys-chem characterisation, in exposure assessment, and in hazard identification and characterisation.
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