EFSA 2021 Nano Guidances: Guidance on Particle – Technical Requirements and Guidance on Nano – Risk Assessment

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EFSA Methodology and Scientific Support Unit

Trusted science for safe food

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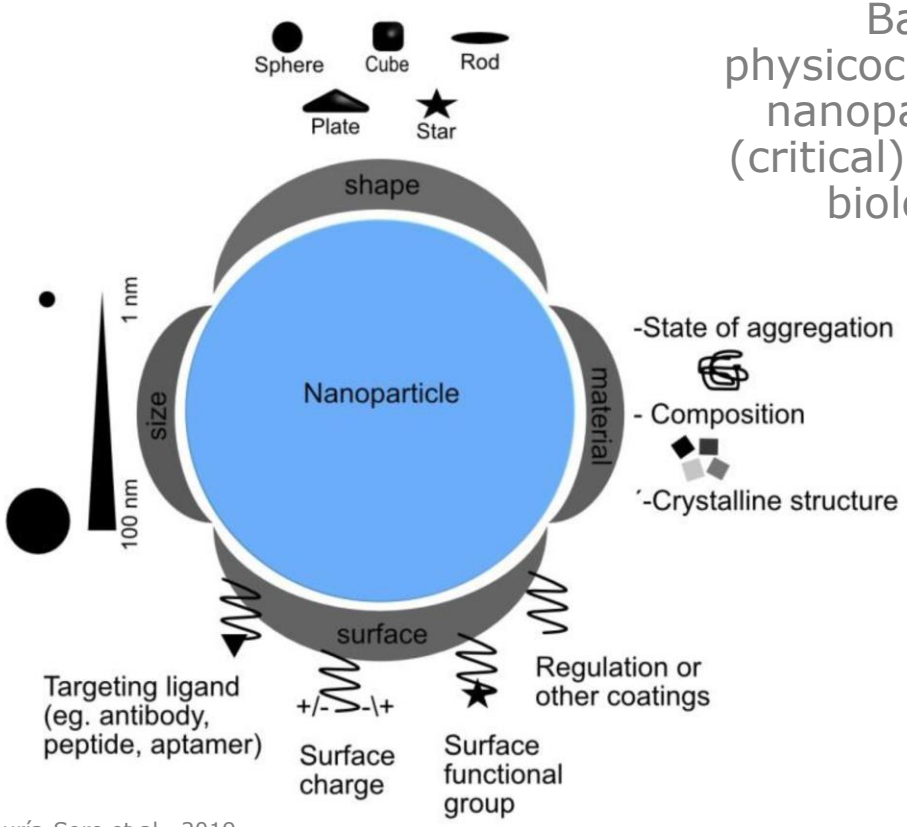
European Food Safety Authority

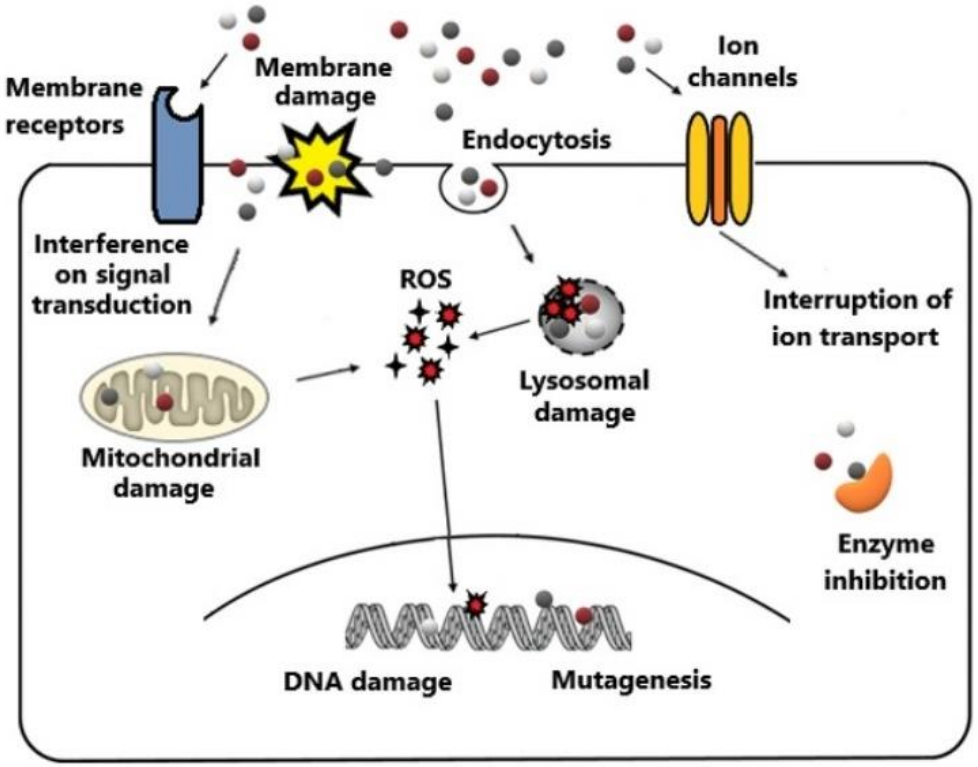
Nanoscale: why specific assessment is needed?

Nanotoxicology: particle-driven toxicokinetic & toxicodynamic



Auría-Soro et al., 2019

Based on their physicochemical properties, nanoparticles may have (critical) **interactions** with biological systems

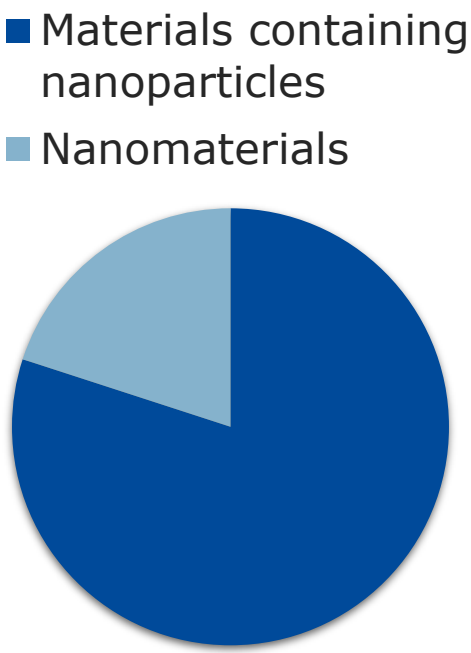


Brandelli, 2020

The mechanism of intestinal uptake is likely to be size dependent and the optimum size for uptake is tentatively around **50 nm**, with a potential uptake for particles up to **250 nm**

Trend and uses

Examples of EFSA's applications requiring nanoscale considerations			
Novel foods	**Food additives and flavourings**	**Feed additives**	**Food contact materials**
			
Aim: To improve quality of food and increase nutrients bioavailability	**Aim:** To increase shelf-life and enhance colours or flavours		**Aim:** To develop sustainable smart packaging and sensors to optimize and/or monitor product shelf-life



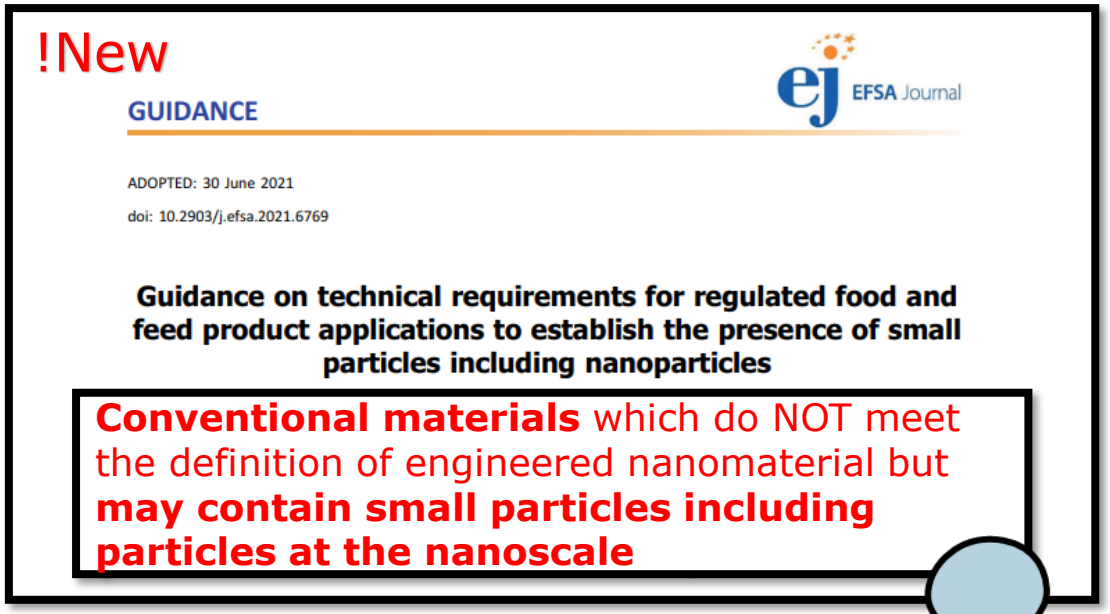
European nanomaterial market expected to grow

*"While the largest segment is currently the metal oxides market, growth is predicted to be driven mainly by **nanoclays**, **nanocellulose** and **carbon-based nanomaterials**". Helsinki, 7 November 2022*

2021 Nano Guidances overview

Guidance on Particle - Technical Requirements (TR)

!New


GUIDANCE

EFSA Journal

ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6769

Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles

Conventional materials which do NOT meet the definition of engineered nanomaterial but **may contain small particles including particles at the nanoscale**

Guidance on Nano - Risk Assessment (RA)

!Update


GUIDANCE

EFSA Journal

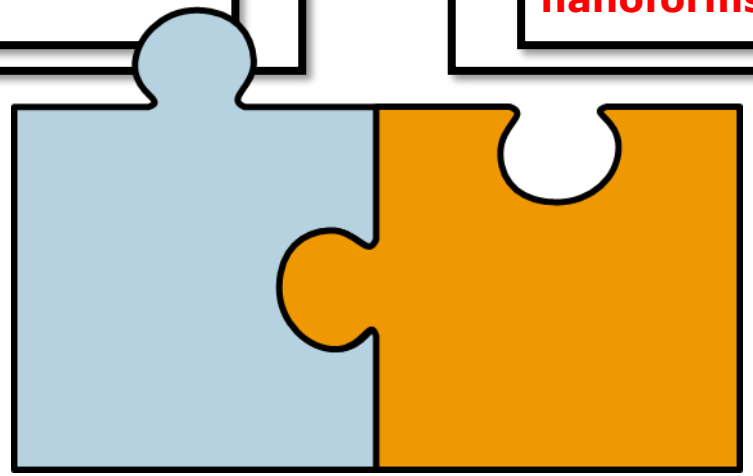
ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6768

Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health

Materials that meet the definition of **engineered nanomaterial**, **nanostructured materials** or **nanoforms**

Are 'nanoscale considerations' needed for the risk assessment?



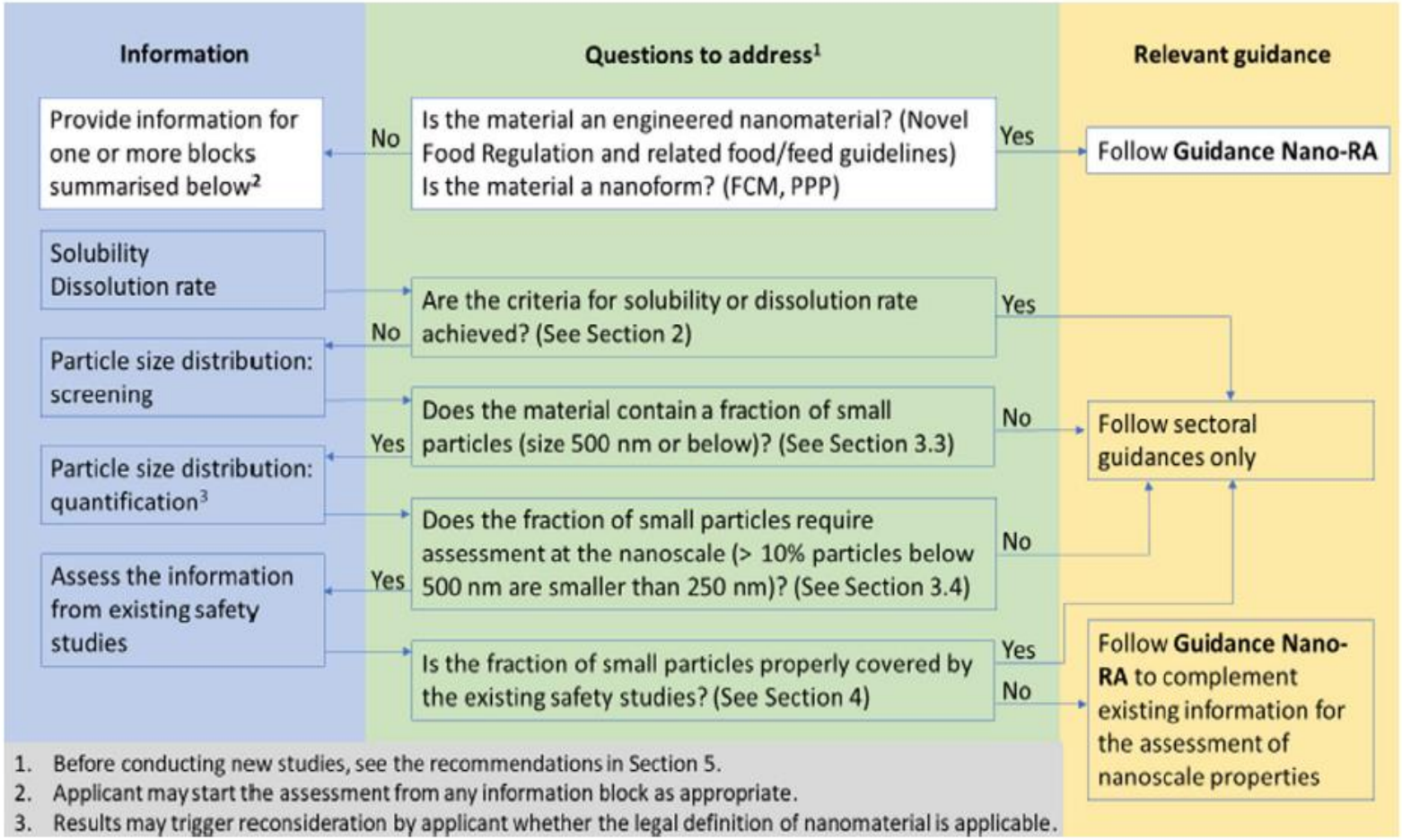
How to conduct a 'nanoscale' risk assessment?

Guidance on Particle - Technical Requirements

EFSA Scientific Committee, 2021. Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. *EFSA Journal* 2021;19(8):6769, 48 pp. https://doi.org/10.2903/j.efsa.2021.6769

Decision scheme Guidance on Particle - TR

Cost & Complexity



'Exit routes' of information requirements (complementing conventional risk assessment) designed to 'exclude' the need of nano-specific assessment according to Guidance on Nano - RA

Figure 1 of the Guidance on Particle - TR: Decision process for selecting the applicable guidance document(s) to be used for the risk assessment of the material regarding the assessment of small particles

Appraisal routes proposed

S.2	Solubility	**Aim**: demonstrate that consumers will not be exposed to small particles
S.2	Dissolution rate	
S.3	Screening particle size	**Aim**: demonstrate absence or quantity of small particles in properly dispersed samples
S.3	Quantification particle size	
S.4	Coverage by existing studies	**Aim**: demonstrate that the fraction of small particles is properly covered by existing safety studies

Each appraisal route and the underlying principles are extensively described in the dedicated Sections (S)

Appraisal routes proposed

S.2 Solubility

'Exit routes' for:
- Highly soluble materials of low concern
- Materials dissolved in the food or product

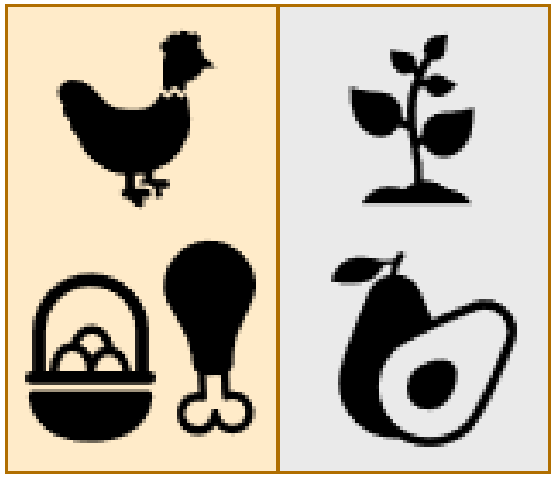
Parameters/ Options	Decision criteria[1]	Methodology	Comments
Solubility in water (Section 2.3.1)	Equal to or higher than 33.3 g/L	According to OECD TG 105 with specific considerations for small particles	For multi-constituent substances and mixtures, the decision criterion has to be fulfilled for each constituent/component
Solubility/ dissolution in the marketed product or in food (Section 2.3.4)	At the expected maximum levels: the substance is fully dissolved in an aqueous or a non-aqueous matrix; or residues in food are below the relevant solubility limit.	Solubility/dissolution tests of the substance in water, lipids or relevant simulants.	Results should confirm that under the intended use conditions (e.g. marketed product or food) the material or its residues in food will be solubilised in the products ingested by consumers

[1] Fulfilling the decision criteria for one of the parameters/options is sufficient for demonstrating that the assessment according to the sectoral guidance is sufficient

Specific provisions for:

- **FCM substances**
  [specific solubility limit of 60 mg/L]
  - 60 mg/L is a generic upper migration limit for FCM substances, if solubility is greater than 60 mg/L, will be in fully solubilised form and not as particles

- **Residues in food**
  [feed additives and pesticides]
  - Verifiable information that solubility of the residue is above the maximum levels ensures that consumers are only exposed to solubilised materials (not to particles)



Appraisal routes proposed

S.2 Dissolution rate

'Exit routes' for:

- Materials that will dissolve in the GIT after ingestion

Parameters/ Options	Decision criteria[1]	Methodology	Comments
Dissolution/ degradation rate in water (Section 2.3.2)	Half-life of 10 min or less corresponding to dissolved fraction equal to or higher than 88% in 30 min	Single concentration corresponding to exposure at the maximum use level in water	For multi-constituent substances and mixtures, the decision criterion has to be fulfilled for each constituent/component. If solubility is pH dependent, the criteria should be confirmed at pH=3 and/or pH=7*

[1] Fulfilling the decision criteria for one of the parameters/options is sufficient for demonstrating that the assessment according to the sectoral guidance is sufficient

A dissolution rate protocol is included in Section 2.3.2.

***A new Annex on 'Degradation/dissolution rate under acidic conditions' will be published soon**

Appraisal routes proposed

S.3 Screening particle size

'Exit routes' for:
- Absence of small particles (<500 nm)

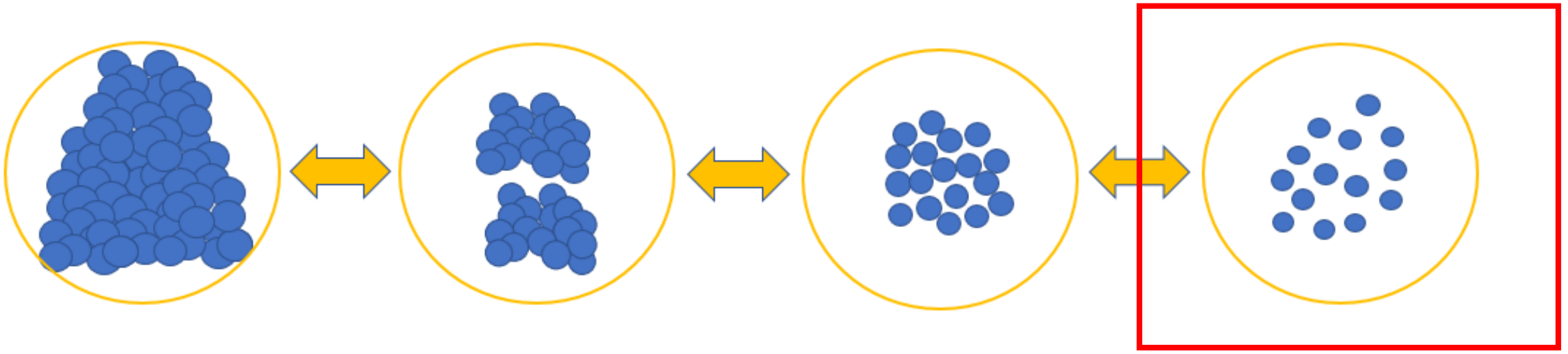
Parameters/ Options	Decision criteria[1]	Methodology	Comments
Particle size distribution of the material (Section 3.3)	Particles equal to or larger than 500 nm The detection capability of the method(s) used for this assessment should provide convincing evidence that the material contains less than 10% of particles (number-based) with at least one dimension smaller than 500 nm	The method selection should be justified, and detection capability should be reported, examples of possible methods are: - CLS - PTA - dEM - Filtration complemented with chemical analysis	Proper dispersion of the material should be ensured (Section 3.2)

[1] Fulfilling the decision criteria for one of the parameters/options is sufficient for demonstrating that the assessment according to the sectoral guidance is sufficient

Recommendations for ensuring proper dispersion are reported in Section 3.2

Agglomeration and consequences

- Due to their higher surface/volume ratio, nanoparticles have high tendency to stick together to form larger sized agglomerates via **weak forces*** (e.g. Van der Waals and electrostatic interactions). The agglomeration/de-agglomeration status is therefore a **dynamic process**, influenced by different physical and biological conditions.
- Therefore, **ensuring proper dispersion is key for the risk assessment of nanoparticles** as allows to test a **nano-sized worst-case scenario**.



*: Agglomeration ≠ Aggregation

Appraisal routes proposed

S.3 Quantification particle size

'Exit routes' for:
- Absence (or just a tail) of nanoparticles

Parameters/ Options	Decision criteria[1]	Methodology	Comments
Particle size distribution of fraction of small particles (Section 3.4)	Less than 10% of the particles (number-based) of the sub-500 nm fraction with at least one external dimension smaller than 250 nm	Quantitative EM or a different method with justification	Applies to the fraction of small particles of the full material (also for multi-constituent substances and mixtures) When the criterion is not met, this information is also required for assessing if the fraction of small particles is covered by the existing safety studies following the criteria described in Section 4

[1] Fulfilling the decision criteria for one of the parameters/options is sufficient for demonstrating that the assessment according to the sectoral guidance is sufficient

Appraisal routes proposed

S.4 Coverage by existing studies

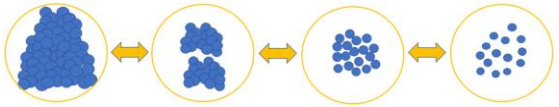
'Exit routes' for:

- Nanoparticles present but properly covered by existing safety studies

Parameters/ Options	Decision criteria[1]	Methodology	Comments
The studies address properly the potential hazards of the fraction of small particles (Sections 4.1. and 4.2)	The test material included the fraction of small particles AND The test design and level of dispersion/degree of agglomeration was sufficient for addressing the fraction of small particles	Characterisation of the test material, comparison with the marketed material, Specific consideration for genotoxicity and TK assessments, AND Demonstration of proper dispersion based on extraction of information from study protocol or additional information (Appendix II)	Specific considerations for existing studies see are detailed in Section 4. Before conducting new safety studies for materials containing a fraction of small particles, see the recommendations of the Guidance on Nano-RA.
The submitted risk assessment covers the fraction of small particles (Section 4.3)	The gaps observed in the safety studies are covered (or are of overall low relevance) and do not trigger additional concerns	The lines of evidence are combined in a weight of evidence approach	See examples under Table 4, Section 4.3

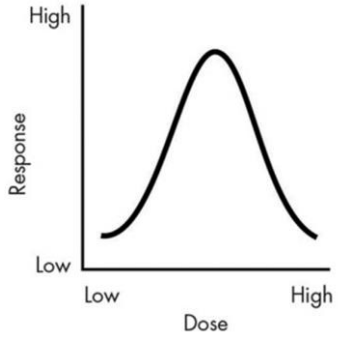
Critical elements to be considered when evaluating the coverage by (existing) toxicity studies

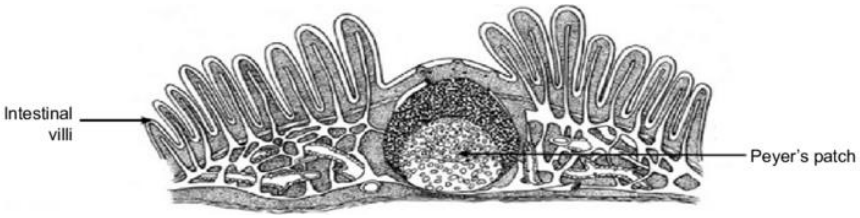
Particle toxicity

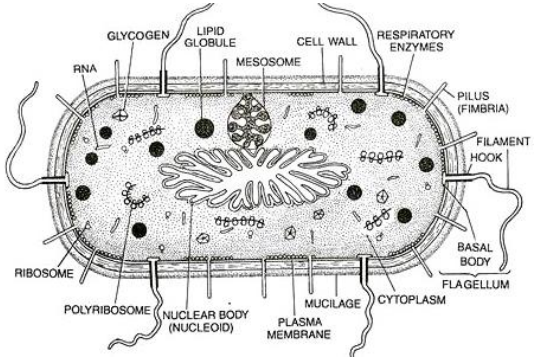


[exposure to particles = worst-case scenario]

- The **lack of a proper dispersion method** and **high doses** promote **agglomeration** resulting in disproportionality between internal dose and external dose
- Proper **duration** (e.g. 90d) + **examination of first site contact** (e.g. Peyer's patches and GIT epithelia) **with appropriate techniques** (e.g. ICP-MS) as fundamental requirement
- Complete genotoxicity test battery needed considering that **Ames test is not suitable** for the assessment of nanomaterials and nanoparticles and a **mammalian cell gene mutation test** (OECD TG 476 or 490) **should be preferred**







Guidance on Nano – Risk Assessment

EFSA Scientific Committee, 2021. Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health. EFSA Journal 2021;19(8):6768, 111 pp. https://doi.org/10.2903/j.efsa.2021.6768

Background

GUIDANCE

EFSA Journal

ENDORSED: 29 May 2018

doi: 10.2903/j.efsa.2018.5327

Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael John Jeger, Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Josef R. Schlatter, Vittorio Silano, Roland Solecki, Dominique Turck, Maged Younes, Qasim Chaudhry, Francesco Cubadda, David Gott, Agnes Oomen, Stefan Weigel, Melpo Karamitrou, Reinhilde Schoonjans and Alicja Mortensen

GUIDANCE

EFSA Journal

ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6768

Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health

EFSA Scientific Committee,
Simon More, Vasileios Bampidis, Diane Benford, Claude Bragard, Thorhallur Halldorsson, Antonio Hernández-Jerez, Susanne Hougaard Bennekou, Kostas Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Nielsen, Josef Schlatter, Dieter Schrenk, Vittorio Silano (deceased), Dominique Turck, Maged Younes, Jacqueline Castenmiller, Qasim Chaudhry, Francesco Cubadda, Roland Franz, David Gott, Jan Mast, Alicja Mortensen, Agnes G. Oomen, Stefan Weigel, Eric Barthelemy, Ana Rincon, José Tarazona and Reinhilde Schoonjans

2018

2021

Guidance update:

- **Original focus** maintained
- **Scientific** knowledge **updates**
- Improved "**usability**" gained by the experience with actual cases from the pilot phase
- Legal clarifications from DG SANCO on the applicability of the definitions under the **Novel Food** (Regulation (EU) 2015/2283) and **REACH Regulations** ((EU) 2018/1881, (EU) 2020/878)
- **JRC, ECHA, DG SANTE, EU MSs** cooperation and input
- EFSA complementary **Guidance on Particle - TR**

Scope and when to apply this Guidance

A full assessment is required if the applicant or the risk assessor concludes that the material:

a) meets the criteria of the definition of **engineered nanomaterials** of the Novel Food Regulation (EU) No 2015/2283;

b) is **a substance to be used to manufacture FCMs,** which is in **nanoform** in accordance with Article 9(2) of Commission Regulation (EU) 10/2011, or deliberately engineered to particle size which **exhibit functional physical and chemical properties** that significantly differ from those at a larger scale in accordance to Article 5(2)(c)(ii) of Commission Regulation (EC) No 450/2009;

c) is **an active substance in PPPs,** consisting of or containing **nanoforms** according to the provisions of Commission Regulations (EU) 2018/1881, and (EU) 2020/878, amending the Annexes I, II, III, VI, VII, VIII, IX, X, XI, and XII of the REACH Regulation to introduce nanospecific clarifications, or is a **PPP with co-formulants in nanoform**;

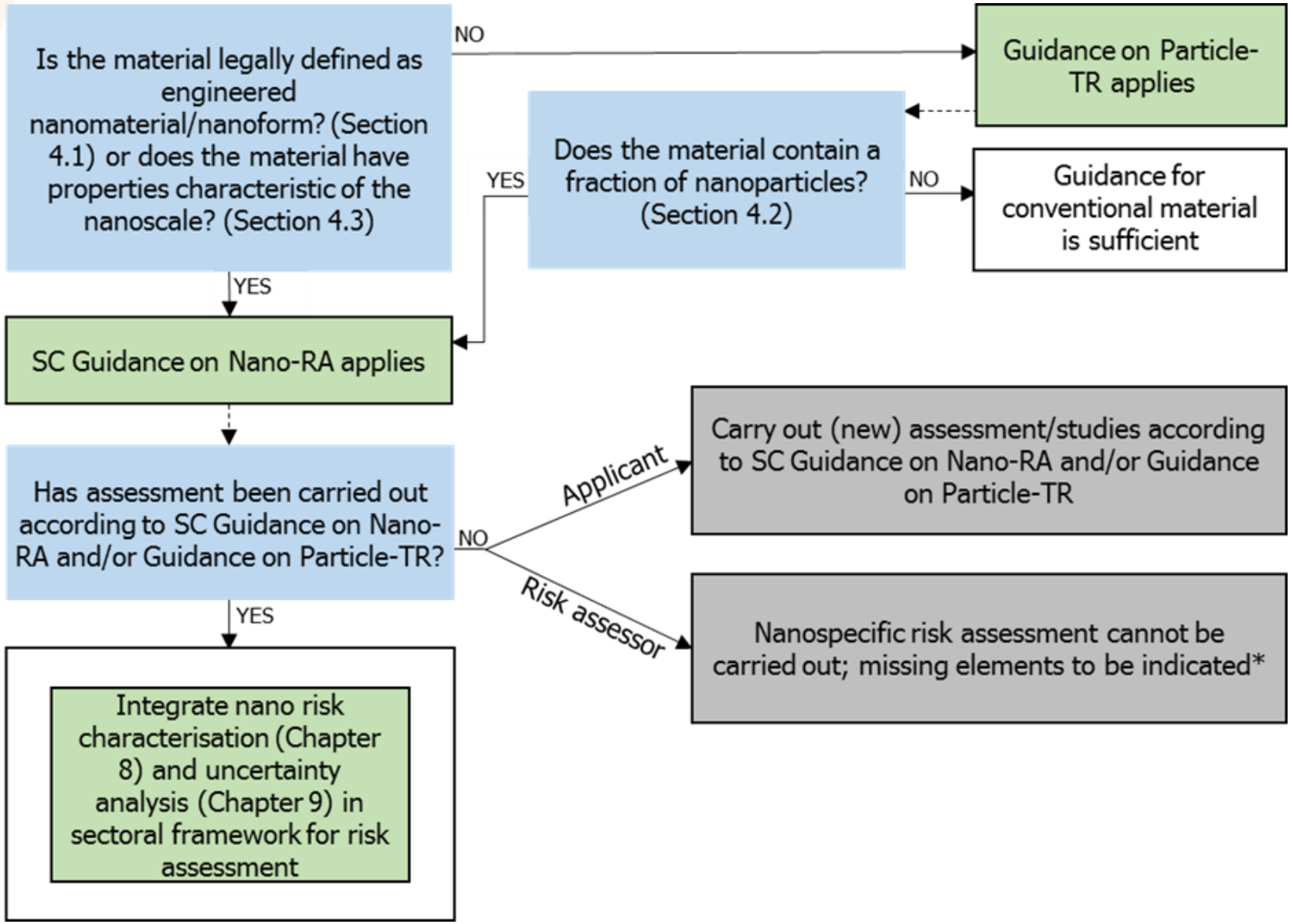
d) does **not meet the above-mentioned legal definitions (a, b, c) but consists of or contains a fraction of small particles** requiring assessment in the nanoscale, identified according to the Guidance on Particle-TR, setting out information requirements for applications in the regulated food and feed product areas, and establishing criteria for assessing the presence of a fraction of small particles;

e) is a **nanostructured material** or a material, including **materials formulated in the form of nanocarriers** (see Appendix D.5), which could retain properties that are characteristic of the nanoscale, for example related to the large specific surface area of the materials or different toxicokinetic behaviour (i.e. significant changes in absorption, distribution and/or metabolism) as compared to its non-nanomaterial.

Audience:

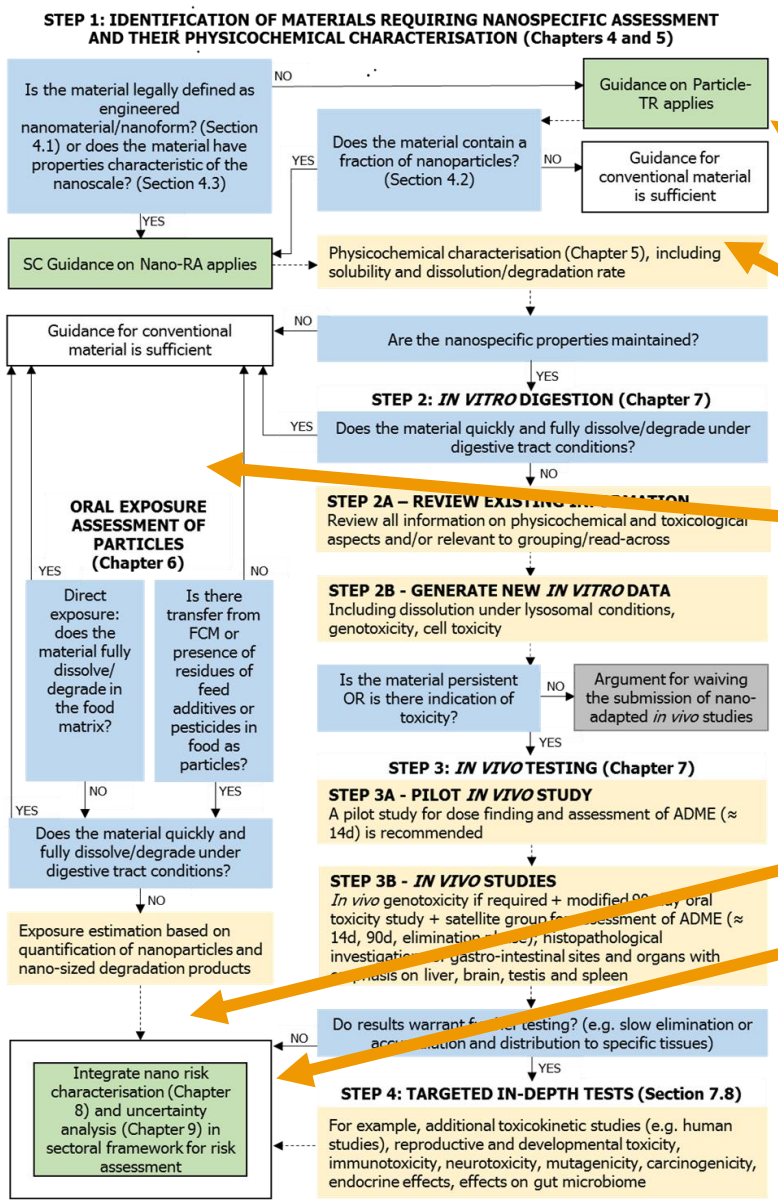
This Guidance should be considered by the **applicants** when preparing the application/dossier, and then by the **EFSA Panels and Units** when assessing the information submitted.

How to use this Guidance in relation to sectoral EFSA guidances



This Guidance is **complementary** to the EFSA Guidance documents on conventional materials

Guidance structure



Schematic outline for the implementation linking the Chapters

Chapter 4. Materials to be assessed under this Guidance

Chapter 5. Physicochemical characterisation of nanomaterial

Chapter 6. Oral exposure assessment of nanomaterial

Chapter 7. Hazard identification and hazard characterisation of nanomaterial

Chapter 8. Risk characterisation of nanomaterial

Chapter 9. Uncertainty analysis of nanomaterial risk assessment

Chapter 4: Materials to be assessed under this Guidance

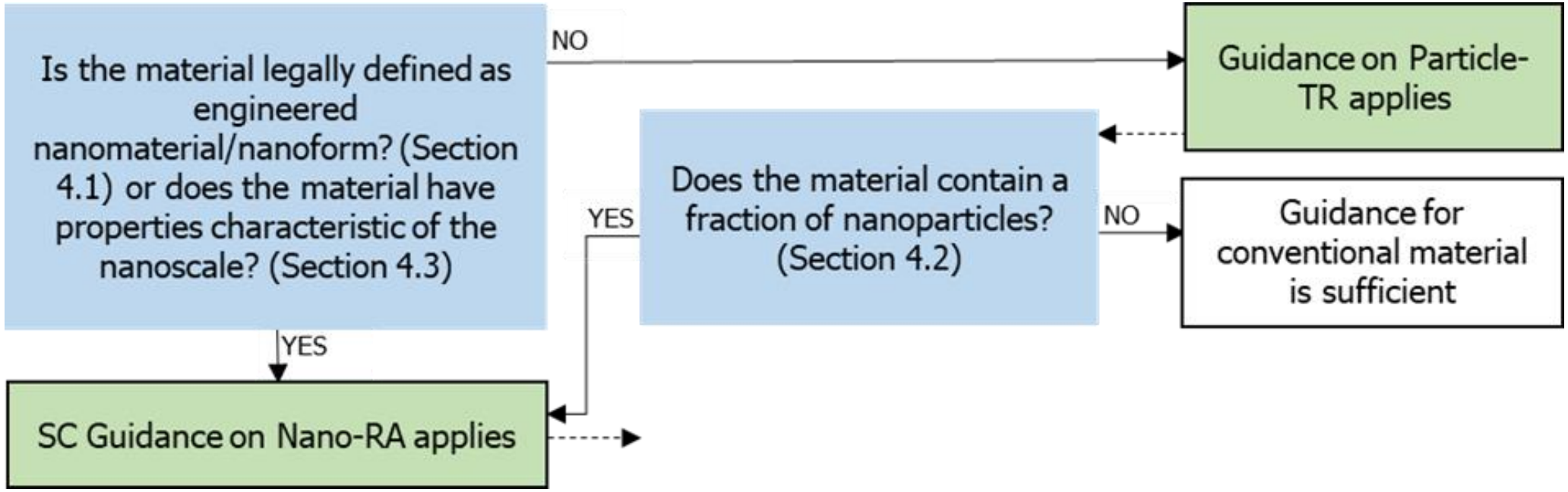


Figure 3: Step 1 includes the identification of materials requiring assessment according to the SC Guidance on Nano-RA (detail from Figure 2 of the Guidance on Nano – RA)

Chapter 5: Physicochemical characterisation of nanomaterial

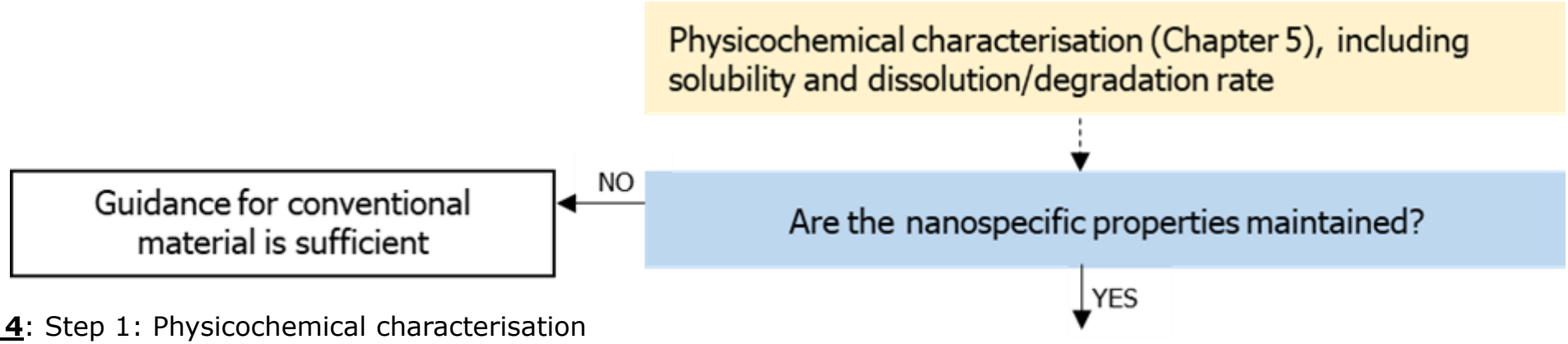


Figure 4: Step 1: Physicochemical characterisation (detail from Figure 2 of the Guidance on Nano – RA)

Detailed characterisation data must be provided for each nanomaterial in its pristine form (identity and relevant physicochemical properties)

Chapter 5; Appendix B

Overview of standard methods available at the time of issuing this Guidance.

Table 1A: Information to be provided on the overall material

Parameters
Name
Description
Intended use
Material composition and purity
Elemental composition Empirical formula of the complete material or relative amounts of elements
Constituent particle size Mean and median minimum external dimension with its number-based distribution
Particle shape Description of the shape, porosity, aspect ratio, EM image of the nanomaterial
Structure Description of the structure, including (relative) thickness of structural elements
Surface chemical composition

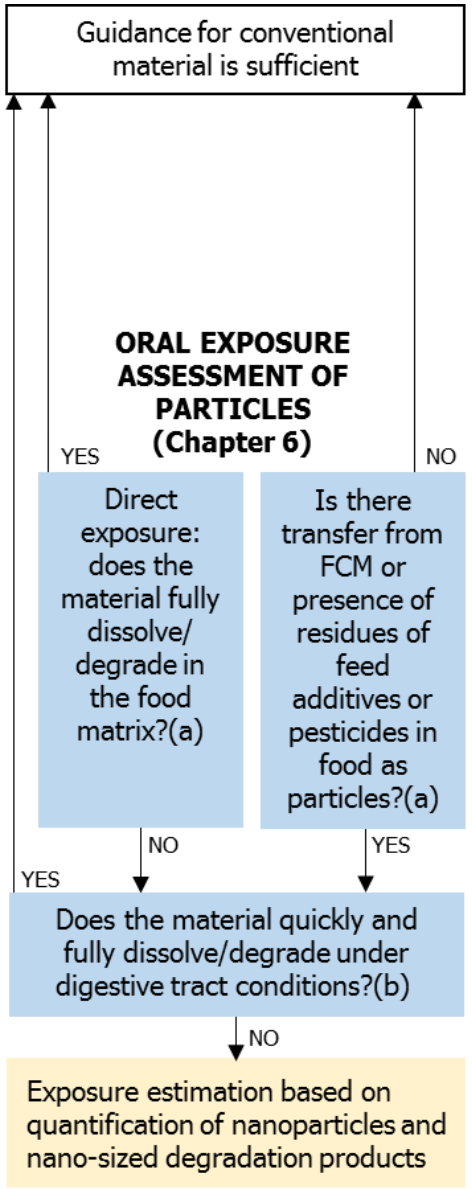
Table 1B: Information on the chemical components

Parameters (incl. specification ranges)
Component 1
Chemical Name
Trade name, common name, other names, synonyms
Registry numbers
Formula
Molecular mass or atomic mass
Elemental composition Empirical formula of this component
Crystal form Form and phase
Purity of the component
Production process component
Component 2
In case of multicomponent particles: Component 2, 3, etc

Table 1C: Extrinsic properties of the material as in the final product

Parameters (incl. specification ranges)
Stability
pH
Solubility (see glossary)
Dissolution/degradation rate
Dispersibility
Surface charge
Agglomeration and/or aggregation state and size Mean and median diameter graphical diagrams of size distribution

Chapter 6: Oral exposure assessment of nanomaterial



Main elements to be considered for nano-specific risk assessment:

- Exposure assessment should consider the presence of a nanomaterial (NM) (or nanosized degradation products) in **food/feed**, **food simulant** and/or ***in vitro* GIT conditions**.
- When a NM (or nanosized degradation products) dissolves under intended use conditions, risk assessment should be carried out according to the relevant sectoral guidance.
- Specific considerations are described for residues from FCM, pesticides and feed additives. It should be determined whether there is **transfer** and if the **exposure is to (nano)particles or solutes (ions, molecules)**.
- When it is not possible to determine the nanoparticles in complex matrices, it should be assumed as a **worst-case** that all NM added to a food/feed product is present and ingested as such.

Figure 5: Steps in oral exposure assessment (details from Figure 2 of the Guidance on Nano – RA)

Chapter 7: Hazard identification and hazard characterisation of nanomaterial

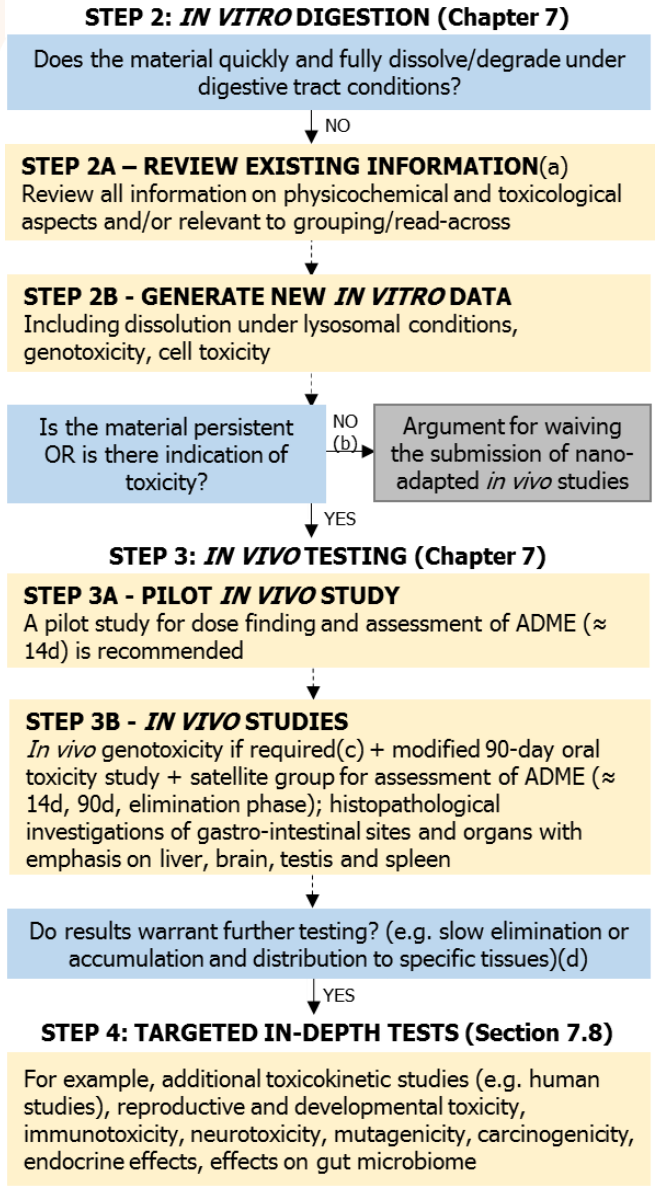
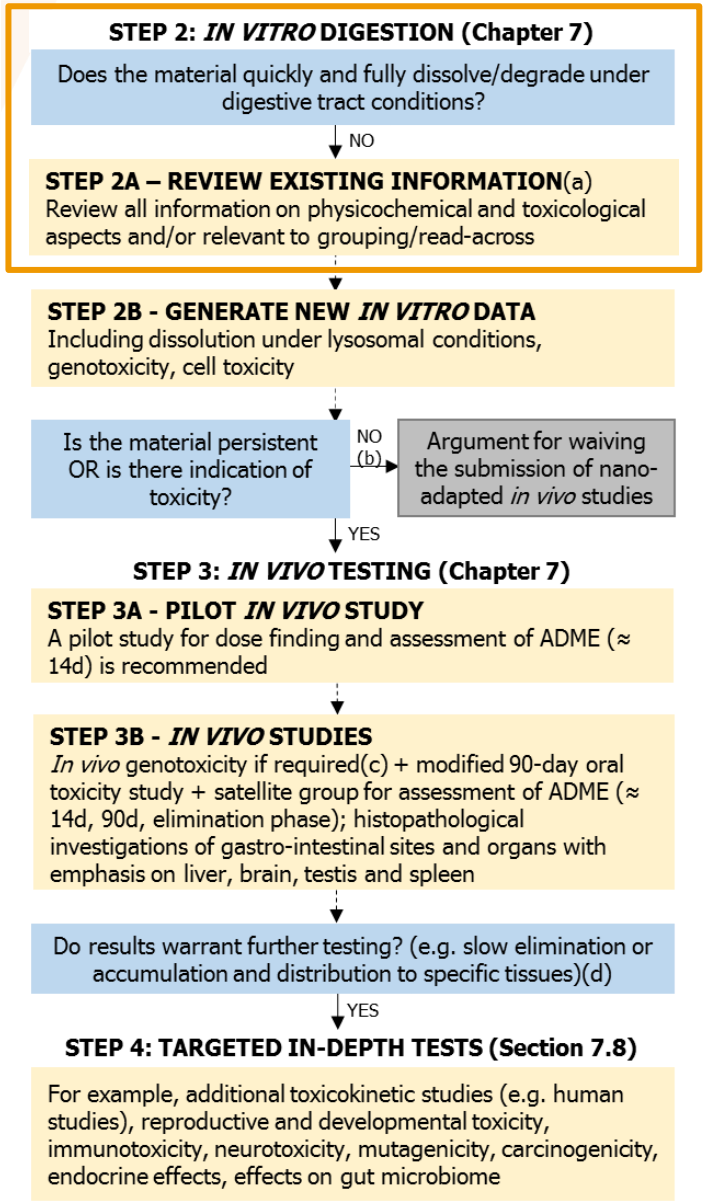


Figure 6: Steps in testing (detail from Figure 2 of the Guidance on Nano – RA)

Step-wise approach

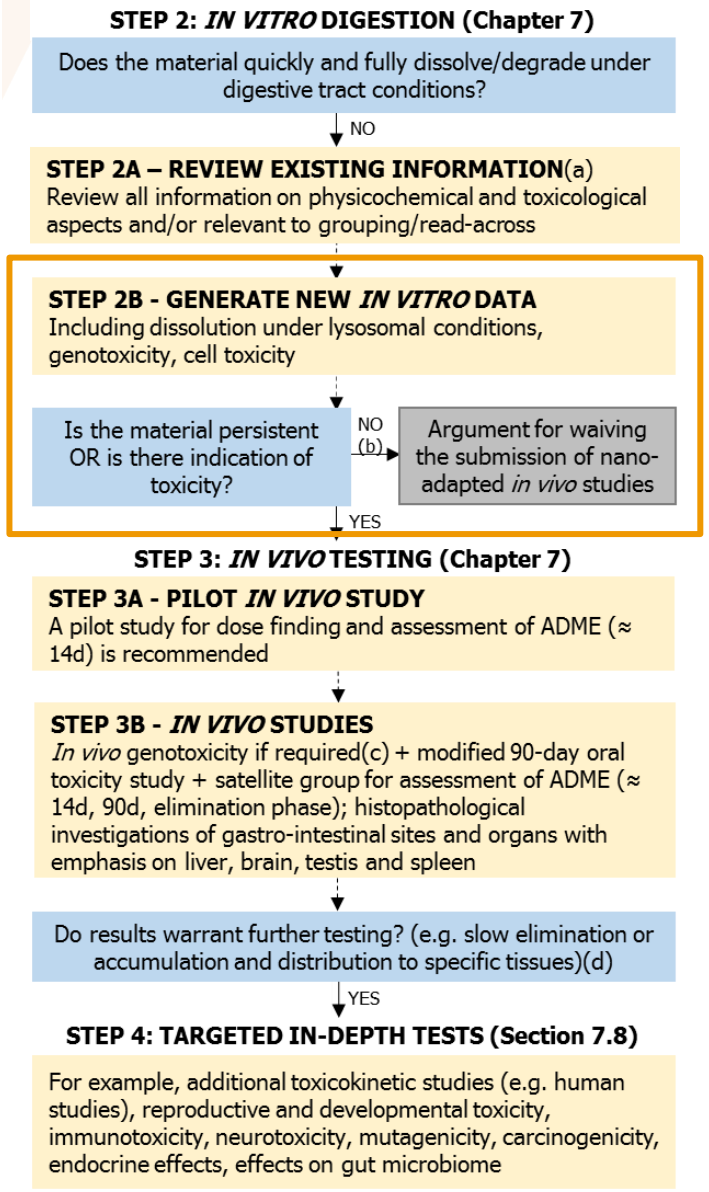


Step 2: degradation rate of the NM to a non-NM under representative conditions of the GIT using *in vitro* digestion models (fasted or fed, worst-case conditions)

- Yes? Quickly and fully dissolving NMs may be subjected to standard assessment.
- No? See below.

Step 2A: collection of available information and definition of a set of *in vitro* studies to identify hazards and the need of further testing.

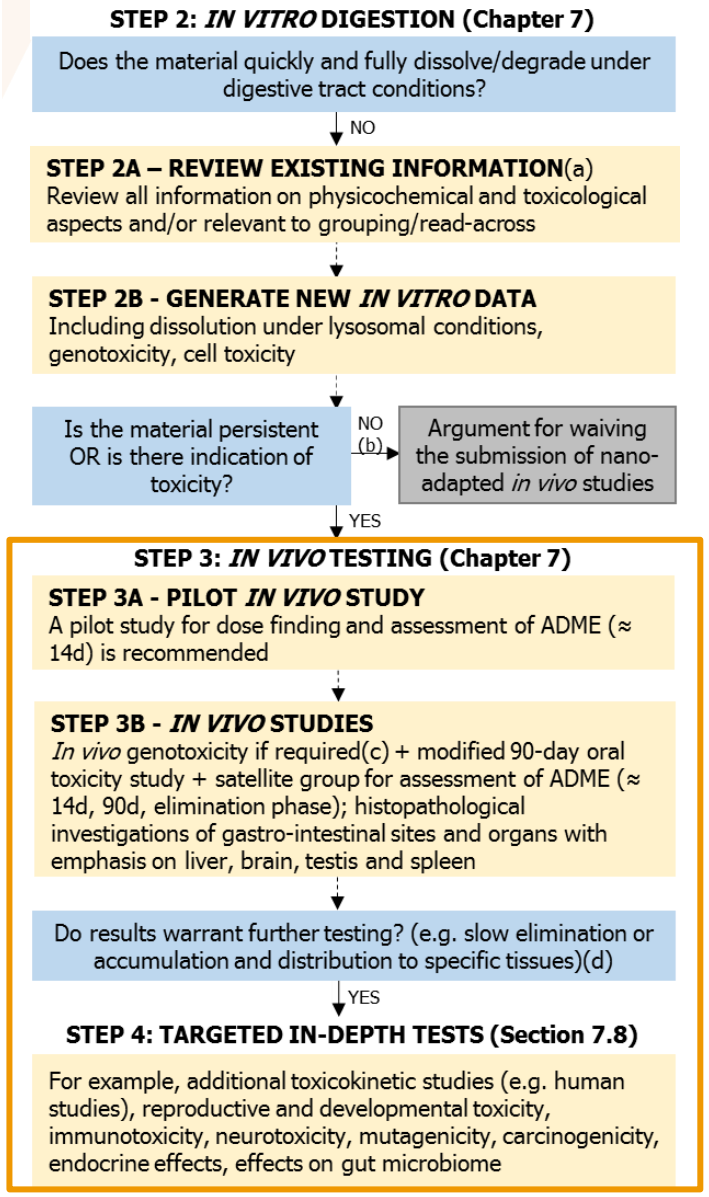
Step-wise approach



Step 2B: new *in vitro* data.

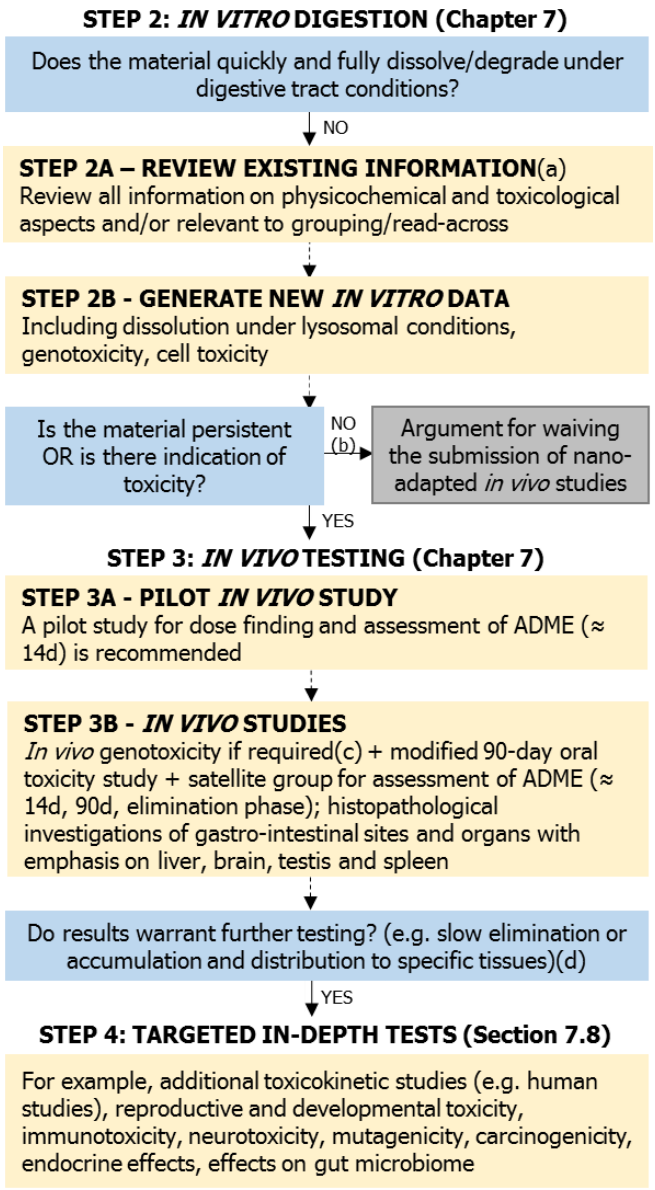
- Genotoxic testing:
  - follows the general indications of the EFSA genotoxicity testing strategy (EFSA SC, 2011) considering that Ames test is not suitable for the assessment of nanomaterials and nanoparticles and a mammalian cell gene mutation test (OECD TG 476 or 490) should be preferred
  - should always include an assessment of cellular uptake and a suitable battery of *in vitro* tests (critical endpoints: gene mutation, structural and numerical chromosome aberrations).
  - follow-up with *in vivo* study in case at least one of the *in vitro* tests indicates genotoxicity activity.
- Dissolution under lysosomal conditions
- Cellular toxicity

Step-wise approach



- **Step 3**: nano-adapted *in vivo* testing.
- **Step 3A**: pilot *in vivo* study (14-day) for dose-finding and assessment of absorption, tissue distribution, accumulation and excretion (ADME).
- **Step 3B**: toxicity test (90-day) covering local effects in the GIT and organs investigated by histopathology (liver, spleen, brain and gonads). Potential identification of NM with immunological, proliferative, neurotoxic, reproductive organ effects or endocrine-mediated effects.
- **Step 4**: further targeted in depth investigation.

Chapter 7: Hazard identification and hazard characterisation of nanomaterial



Main elements to be considered for nano-specific risk assessment:

- OECD TGs and other protocols require specific **adaptations** for testing NMs (i.e. ensure good dispersion & stability in the media);
- The **testing strategy for genotoxicity** should be designed considering that tests in **bacterial systems** are not suitable for NMs
- A justification on the selected doses/concentrations should be provided. Studies conducted at **high doses** without further information on dispersion and stability or confirmation of cellular/tissue exposure **are insufficient for hazard assessment of NMs**;
- When possible, an **experimental group** exposed to the corresponding non-NM should be included in both *in vitro* and *in vivo* studies;
- **Evidence on cellular uptake** (*in vitro*) and/or **exposure in target tissues** (*in vivo*) **should be provided** and, if possible, quantified with appropriate techniques;
- The Guidance provides options for integrating NAMs and existing information into IATAs, with one example for nutrients
- The reporting should be **supplemented with the detailed description of the nanospecific issues**.

Figure 6: Steps in hazard assessment
(details from Figure 2 of the Guidance on Nano – RA)

Guidance structure

Appendices:

- Appendix A. Demonstration fact sheet for component 2
- Appendix B. Characterisation techniques
- Appendix C. Uncertainty analysis of high dissolution/degradation rate
- **Appendix D. Additional information on specific regulated products**
  - **D.1 Feed additives**
  - D.2 Pesticides
  - D.3 Substances used in Food Contact Materials (FCM)
  - D.4 Nanofibres
  - D.5 Nanocarriers
  - D.6 Fertilisers

Nano-specific risk assessment for feed additives

- Safety to users
- Safety to consumers
- Safety to target animals

Take home messages

All materials should be assessed for the possible presence of small or nano particles according to the Guidance on Particle – Technical Requirements

If information suggests the need for nano-specific assessment, the applicant should follow the provisions described by the Guidance on Nano – Risk Assessment

Both assessments should integrate the sectorial 'conventional' risk assessment: the Nano Guidances are complementary to EFSA sectoral Guidance Documents

Thank you for your attention!

Questions?

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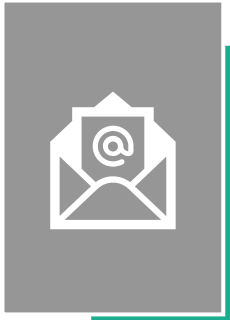

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