Stakeholder workshop on small particles and nanoparticles in food, 30 March – 1 April 2022

Overview of the EFSA Guidance on Nano – Risk Assessment

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European Food Safety Authority

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, EU MSs, ECHA, DG SANTE** 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**;

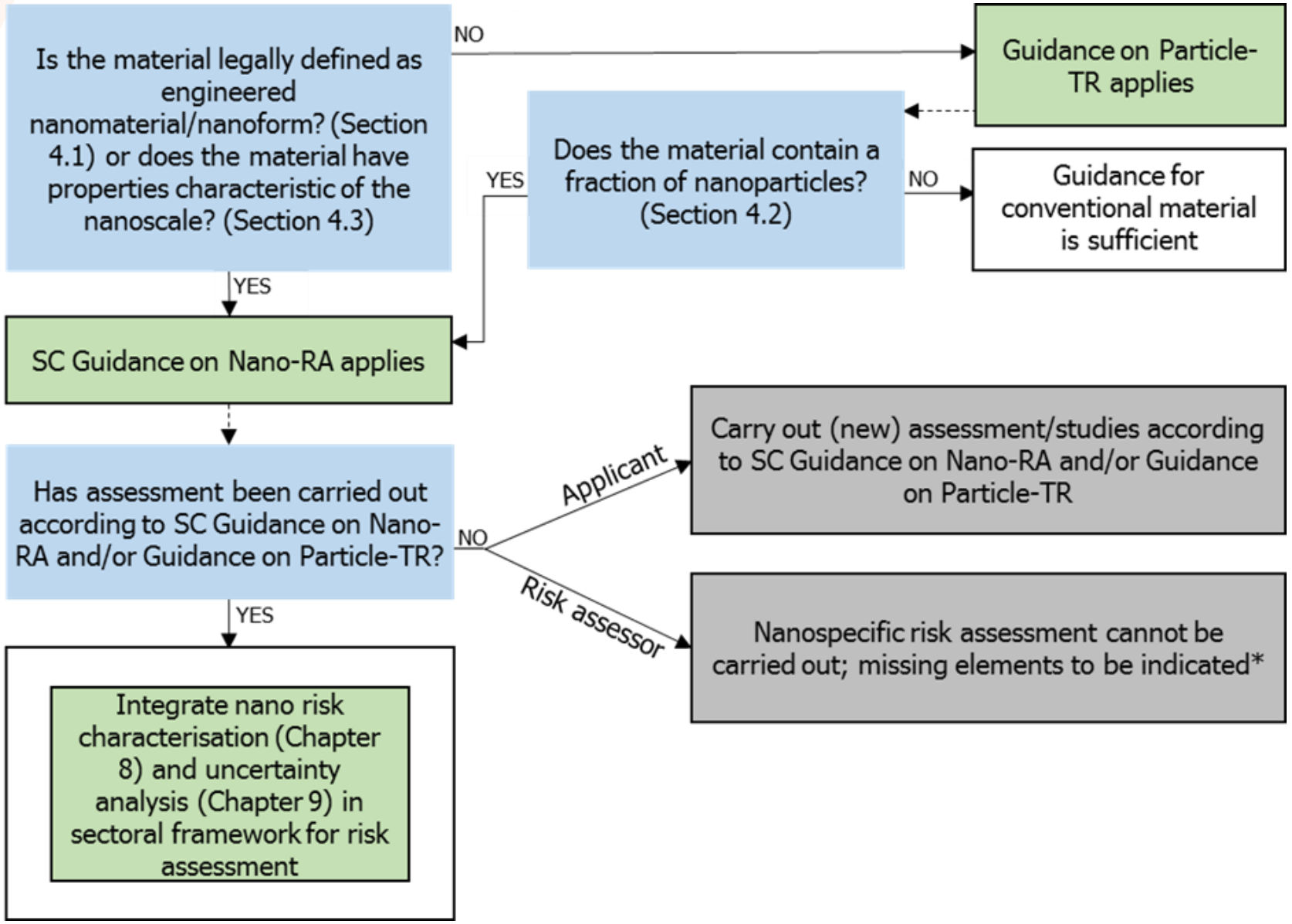
d) does **not meet the above-mentioned legal definitions (a, b, c) but consists of or contains a fraction of small particles** requiring assessment in the nanoscale, identified according to the Guidance on Particle-TR, setting out information requirements for applications in the regulated food and feed 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 (1)

Nine Chapters

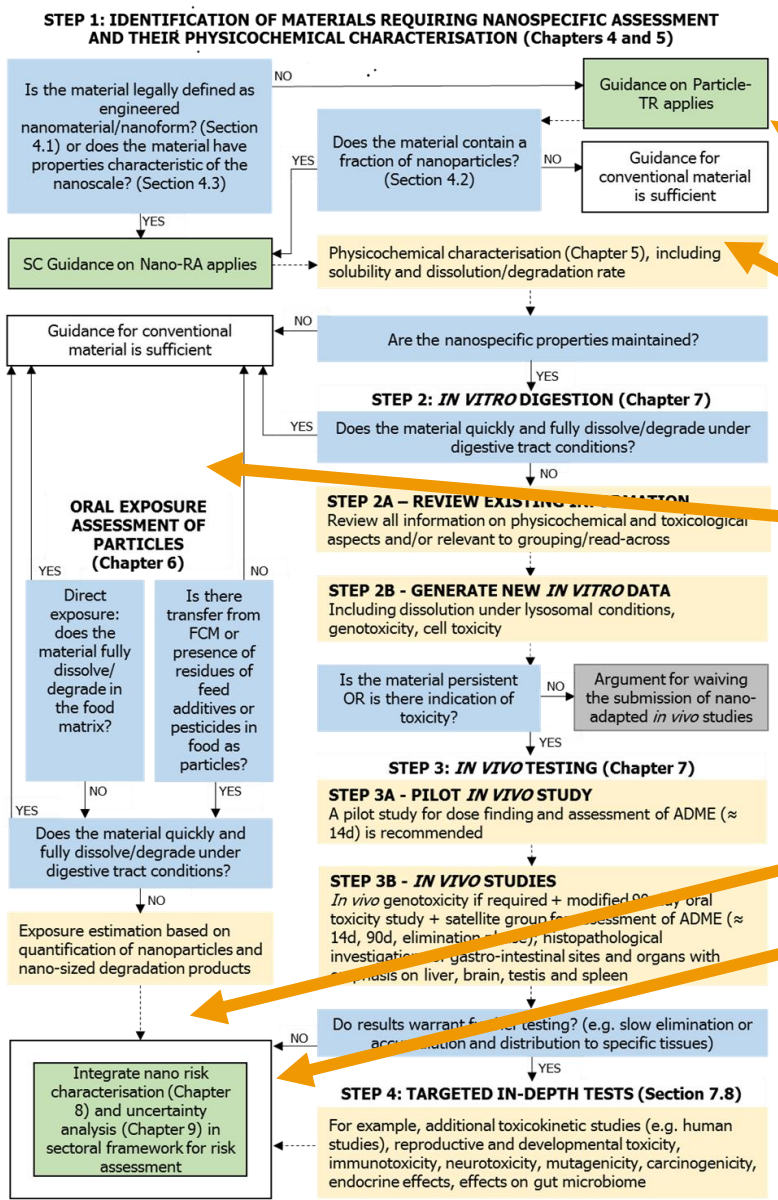
1. Introduction: Background, Terms of Reference, Scope, How to use the guidance
2. Data and methodologies
3. Risk assessment of nanomaterials: general outline
4. Materials to be assessed under this Guidance
5. Physicochemical characterisation of nanomaterial
6. Oral exposure assessment of nanomaterial
7. Hazard identification and hazard characterisation of nanomaterial
8. Risk characterisation of nanomaterial
9. Uncertainty analysis of nanomaterial risk assessment

References, Abbreviations, Glossary

Four 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

Guidance structure (2)



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

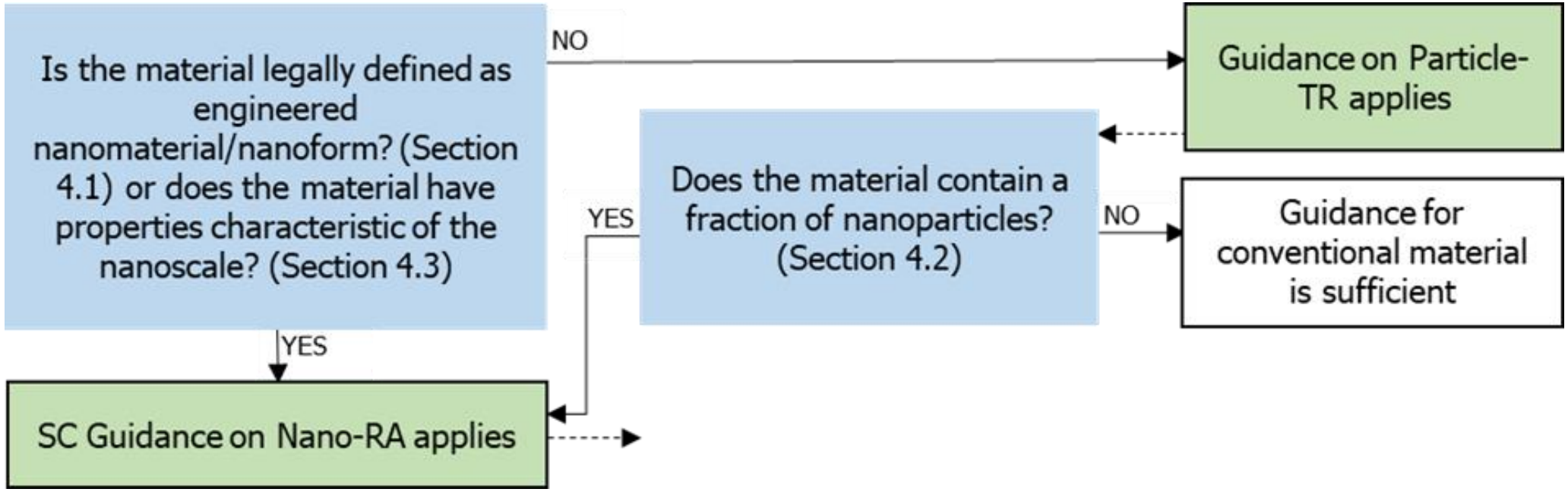


Figure 3: Step 1 includes the identification of materials requiring assessment according to the SC Guidance on Nano-RA (detail from Figure 2 of the Guidance on Nano – RA)

Chapter 5: Physicochemical characterisation of nanomaterial (1)

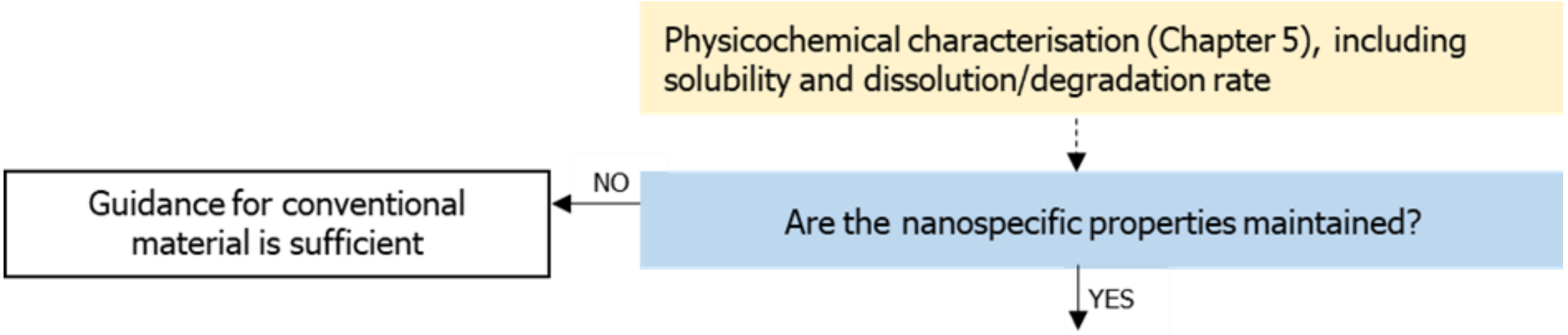


Figure 4: Step 1: Physicochemical characterisation (detail from Figure 2 of the Guidance on Nano – RA)

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.

Chapter 5: Physicochemical characterisation of nanomaterial (2)

European Food Safety Authority

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 Description of the composition of the groups or coatings on the particle surface
Production process
Surface area
Appearance
Melting point
Boiling point
Density
Porosity
Dustiness
Formulation Formulation medium Dispersing agents (stabilisers) Auxiliaries Concentration of nanomaterial in dispersion

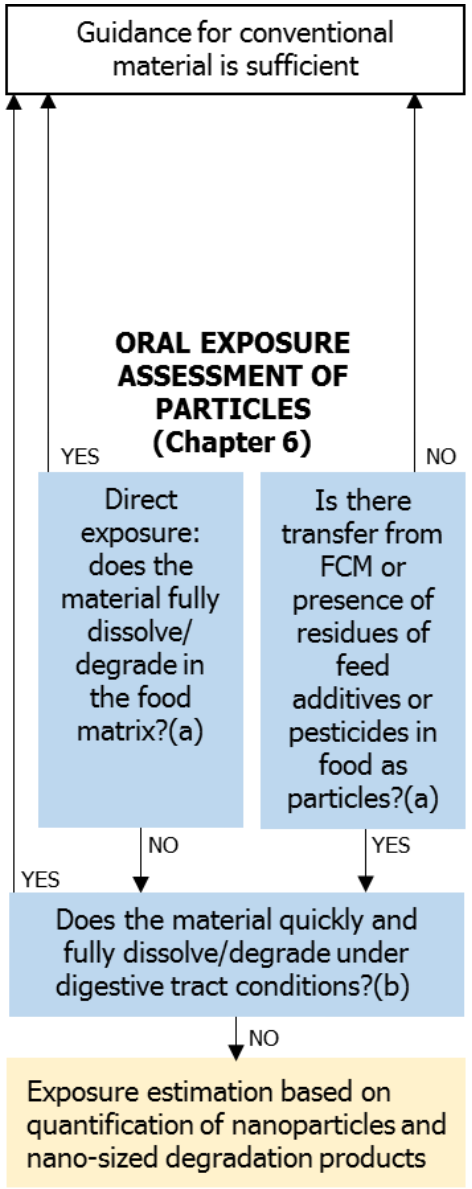
Table 1B: Information on the chemical components

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 (media dependent) 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
Reactivity when applicable

Chapter 6: Oral exposure assessment of nanomaterial



- If the NM is added to the food (direct exposure for humans/animals):
  - (a) NM (or degradation products) dissolution/degradation in **food/feed matrix**
  - (b) NM (or degradation products) dissolution/degradation in the **gastrointestinal tract**
- If it cannot be determined whether a NM is present in the food/feed matrix it should be assumed that nanomaterial is present.
- Specific considerations for:
  - FCM (transfer by migration or physical release)
  - Feed additives
  - Pesticides

Chapter 6; Appendix C

Uncertainty analysis of high dissolution/ degradation rate

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

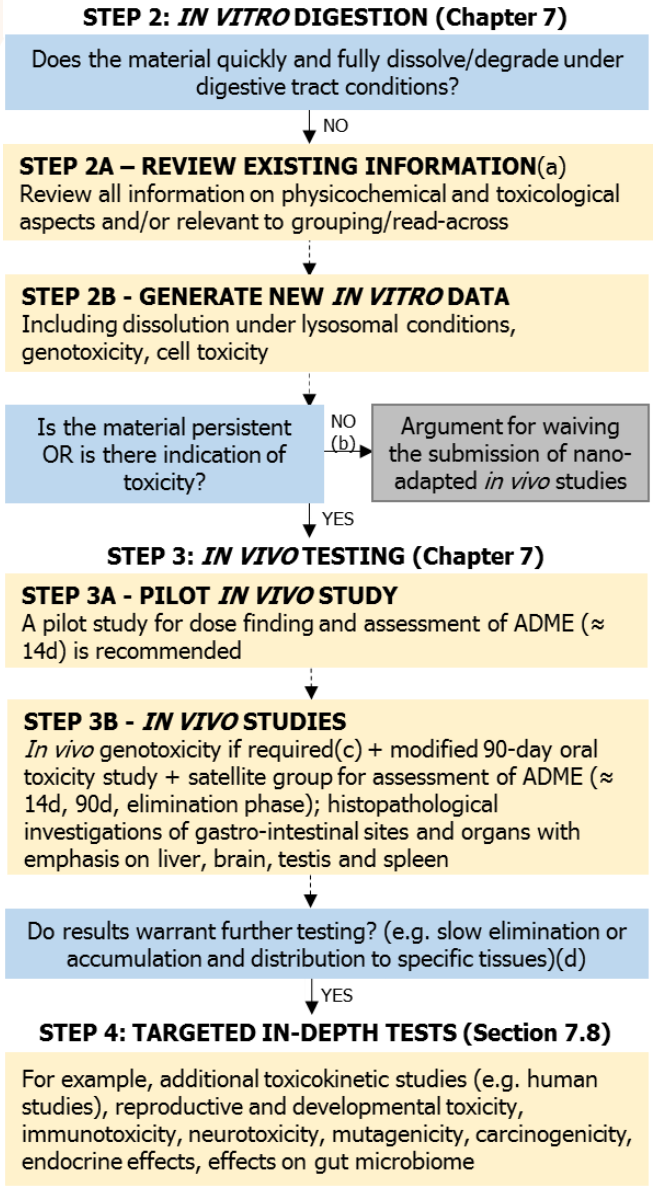
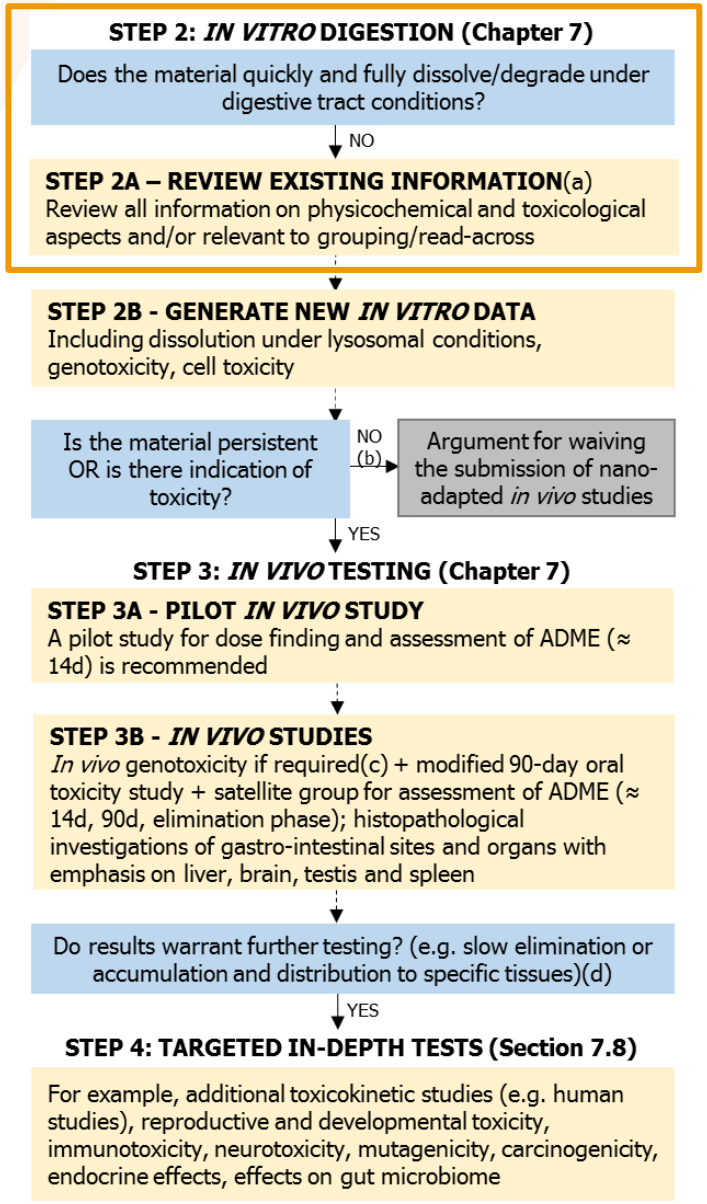


Figure 6: Steps in testing (detail from Figure 2 of the Guidance on Nano – RA)

Step-wise approach

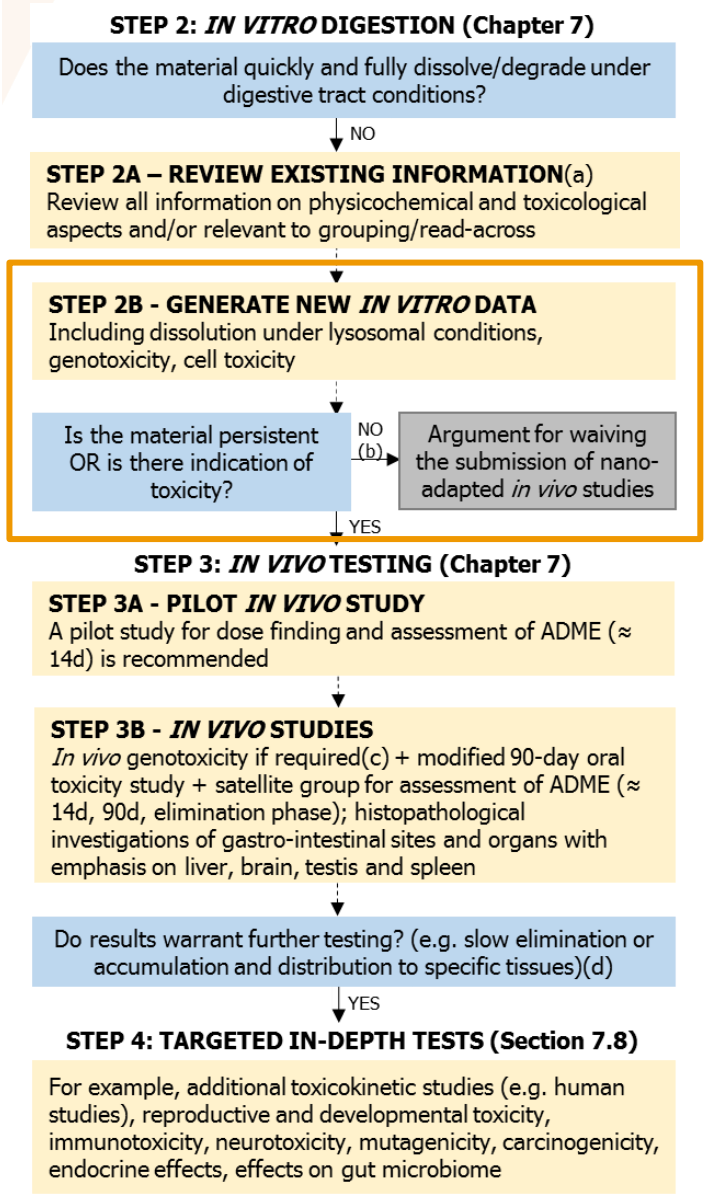


Step 2: degradation rate of the NM to a non-NM under representative conditions of the GIT using *in vitro* digestion models (fasted or fed, worst-case conditions)

- 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.

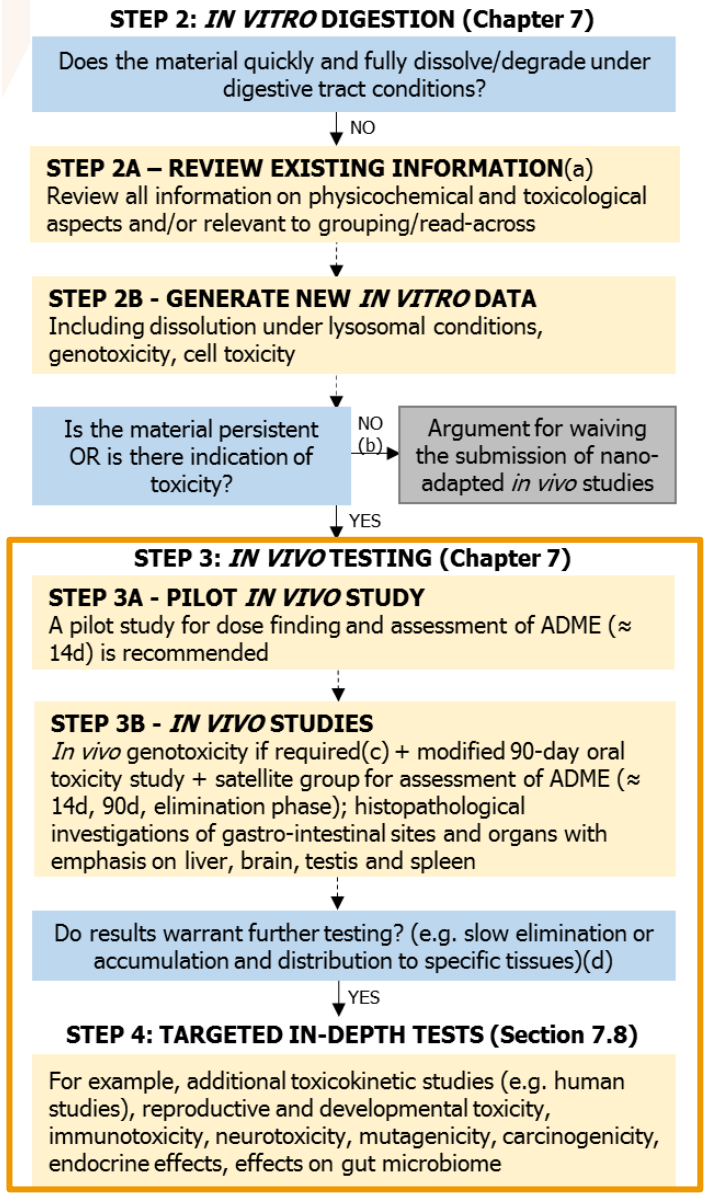
Step-wise approach



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.

Critical elements to be considered

- OECD Test Guidelines (TGs) and other testing protocols require specific **adaptations** for testing NMs (i.e. potential for aggregation/disaggregation, agglomeration/deagglomeration and stability in different media).
- **Ames test** (bacterial reverse mutation) is not considered suitable for NMs, a mammalian cell gene mutation test (OECD TG 476 or 490) should be used instead
- A justification on the selected doses/concentrations should be provided. Studies conducted at **high doses** (*in vitro* >100 µg/mL; in vivo >50 for liquid form or >100 mg/kg bw when incorporated in the food matrix) 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 (e.g. gavage).
- The **confirmation of exposure** in target tissues (*in vivo*) or in target cells (*in vitro*) **should be demonstrated** and if possible quantified with appropriate techniques;
- The reporting should be **supplemented with the detailed description of the nanospecific issues**.

Guidance structure

Appendices:

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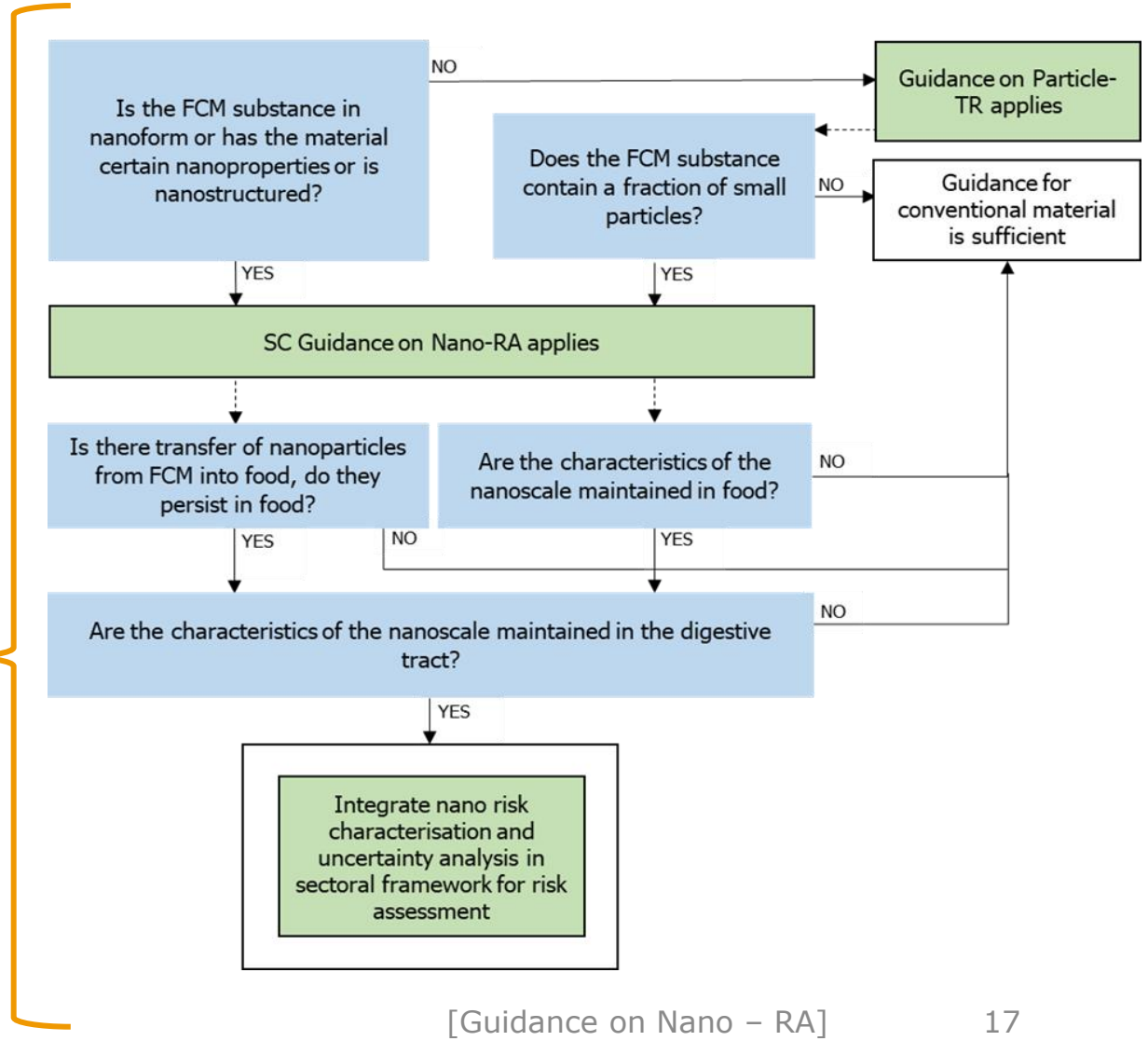

Figure D.1: Schematic outline and overview of workflow for the nanospecific risk assessment of FCM

Take home messages

OECD TGs require nano-adaptation when testing nanomaterials, nanostructured materials and materials containing a fraction of particles at the nanoscale

Nano-specific risk assessment should integrate the sectorial 'conventional' risk assessment: the Guidance on Nano - Risk Assessment is complementary to the EFSA Guidance Documents on conventional materials

When a conventional material contains a fraction of nanoparticles the risk assessment should consider the risk of both fractions: the combined information should cover the safety assessment of the full material

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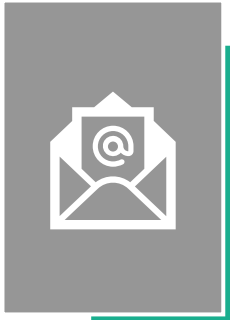

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