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

Overview of the EFSA Guidance on Particle – Technical Requirements and proposed appraisal routes to establish the presence of small particles in food and feed applications

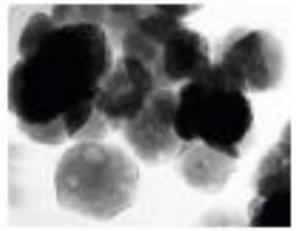
Maria Chiara Astuto

EFSA Methodology and Scientific Support Unit

Trusted science for safe food

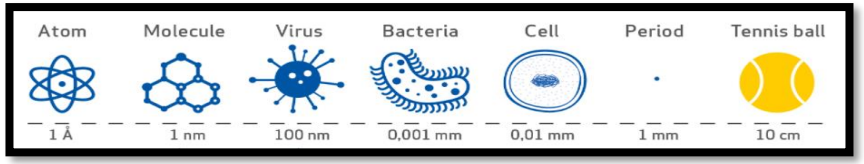
European Food Safety Authority

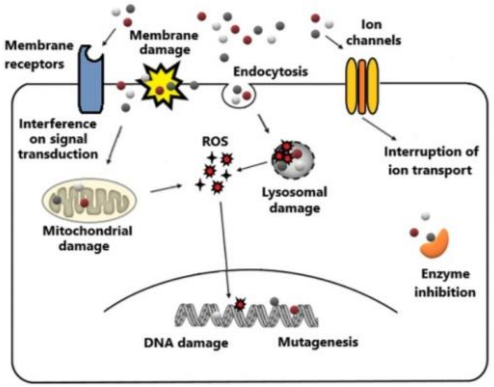
Nanoscale: why specific assessment is needed?



~1nm ~250nm

Size scale

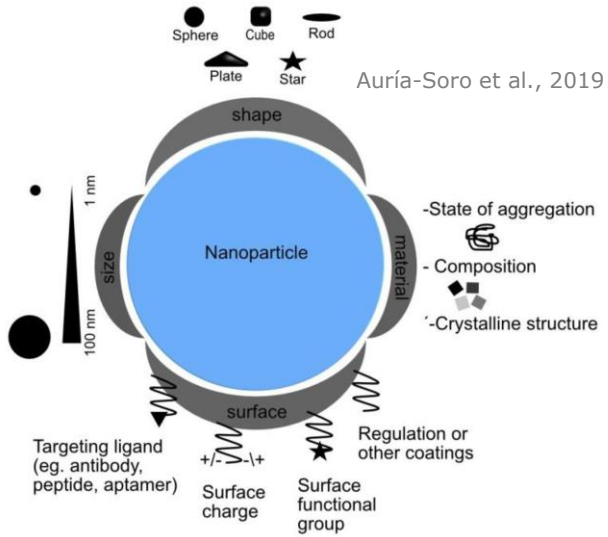




Brandelli, 2020

Nanoscale may lead to different biological responses compared to the corresponding non-nanoform

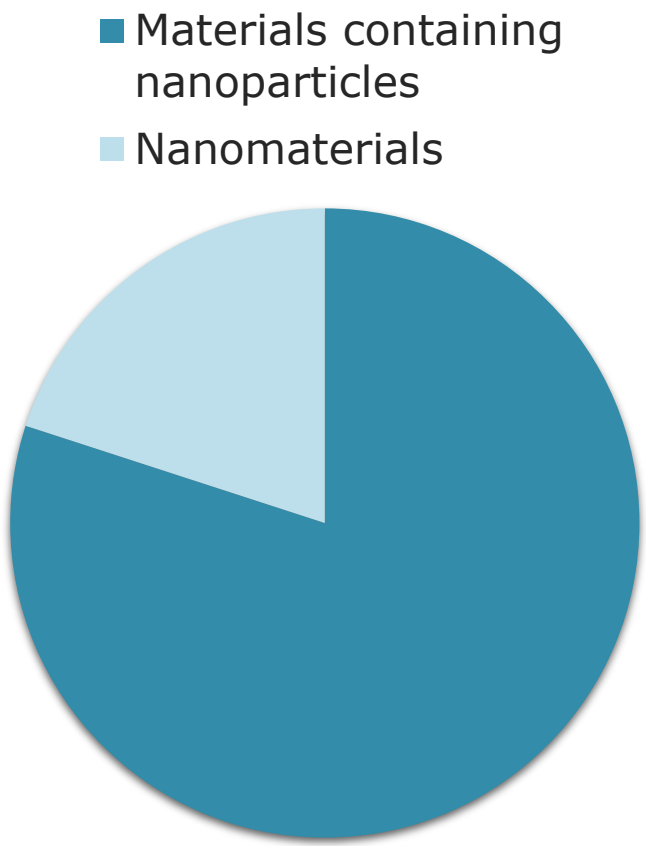
- Particle-driven Toxicokinetic (e.g. difference in ADME behaviour)
- Particle-driven Toxicodynamic (e.g. due to large surface area)



Auría-Soro et al., 2019

Trend and uses

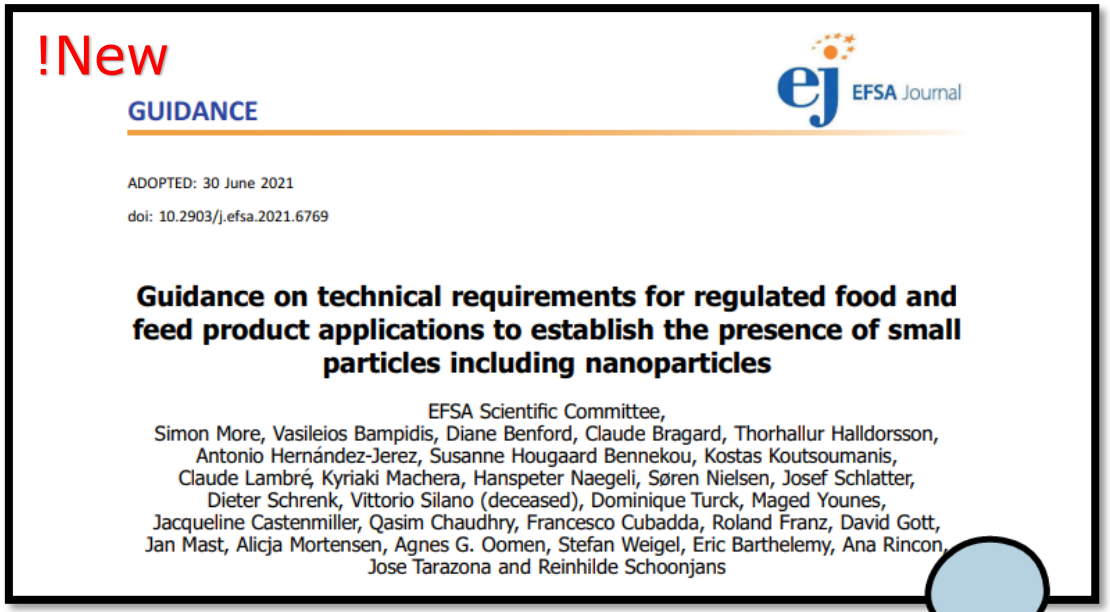
Examples of EFSA's applications requiring nanoscale considerations			
Novel foods	**Food additives and flavourings**	**Feed additives**	**Food contact materials**
			
Aim: To improve quality of food and increase nutrients bioavailability	**Aim:** To increase shelf-life and enhance colours or flavours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



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

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, Jose Tarazona and Reinhilde Schoonjans

Guidance on Nano - Risk Assessment (RA)

!Update

GUIDANCE

EFSA Journal

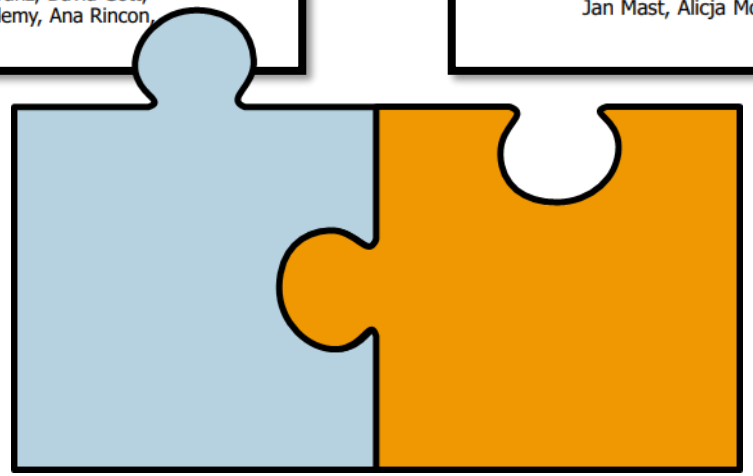
ADOPTED: 30 June 2021

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

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

DAY 1



DAY 2

Nano Guidances development

History

July 2018

Publication of EFSA Scientific Committee Guidance on Nanotechnologies

Sept 2019

End of the pilot phase and start of the update of the 2018 Scientific Committee Guidance on Nanotechnologies

June 2020

Draft Guidance on Particle - Technical Requirements endorsed for Public Consultation by the Scientific Committee

June-August 2021

Nano Guidances adopted by the Scientific Committee and published in the EFSA Journal

Sept 2019

European Commission mandate for the creation of a Guidance on Particle - Technical Requirements

Players involved

- ccWG on Nanotechnologies
- EFSA Panels and Units
- Nano Network (EU MSs)
- ECHA, JRC, DG SANTE
- EFSA Stakeholders

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

Background

- EFSA may receive two types of applications:

Materials that meet the definition of engineered nanomaterial as set out in the Novel Food Regulation (EU) 2015/2283 (which is also applicable to other EU legislation concerning regulated food products)

2018 Nano Guidance

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

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

Terms of Reference from the EC

- To develop a technical guidance setting out the information requirements for applications in the regulated food and feed product areas of conventional materials which do NOT meet the definition of engineered nanomaterial set out in the Novel Food Regulation (EU) 2015/22831, in order to:
  a. **demonstrate whether a portion or the whole of the material is in the nanoscale**;
  b. for those materials which have been determined to contain a fraction of small particles, including particles at the nanoscale, EFSA should provide the information requirements **demonstrating that the nanoscale fraction of the material was properly evaluated in the safety studies** (e.g. physicochemical criteria to help identifying these materials, technical and scientific information and related evidence requirements that the applicant needs to provide).

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.

Interpretation of Terms of Reference

- **This document guides the process to decide** whether or not the material, or a fraction of it, **does require specific assessment of properties at the nanoscale, providing ways for confirming that a conventional risk assessment** (i.e. prescribed by the sectoral guidances) **is sufficient**.
- Therefore, this Guidance should be considered as complementary to the sectoral guidances. If the Guidance on Particle - TR concludes that conventional risk assessment is NOT sufficient, the user is directed to the **Guidance on Nano – Risk Assessment**.

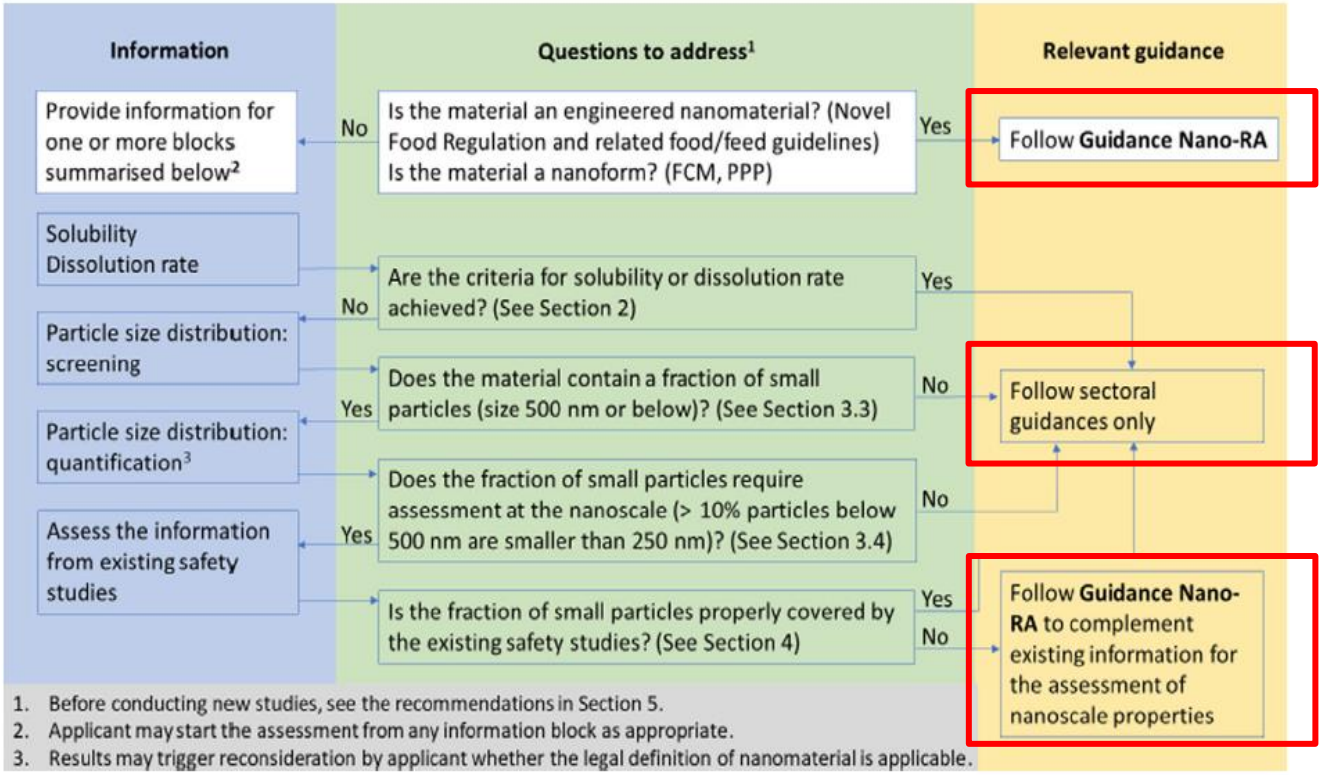


Figure 1: Decision process for selecting the applicable guidance document(s) to be used for the risk assessment of the material regarding the assessment of small particles

Interpretation of Terms of Reference

- The applicants may select the best appraisal route or combination of appraisal routes to justify:
  a. the **absence of a fraction of small particles**, or
  b. that **the material contains a fraction of small particles but that this fraction is covered by the conventional risk assessment and relevant sectoral guidance documents and does not require a separate assessment for the nanoscale.**
- The Guidance also provides information related to best practices for reporting and assessing existing studies, and recommendations for generating additional information.

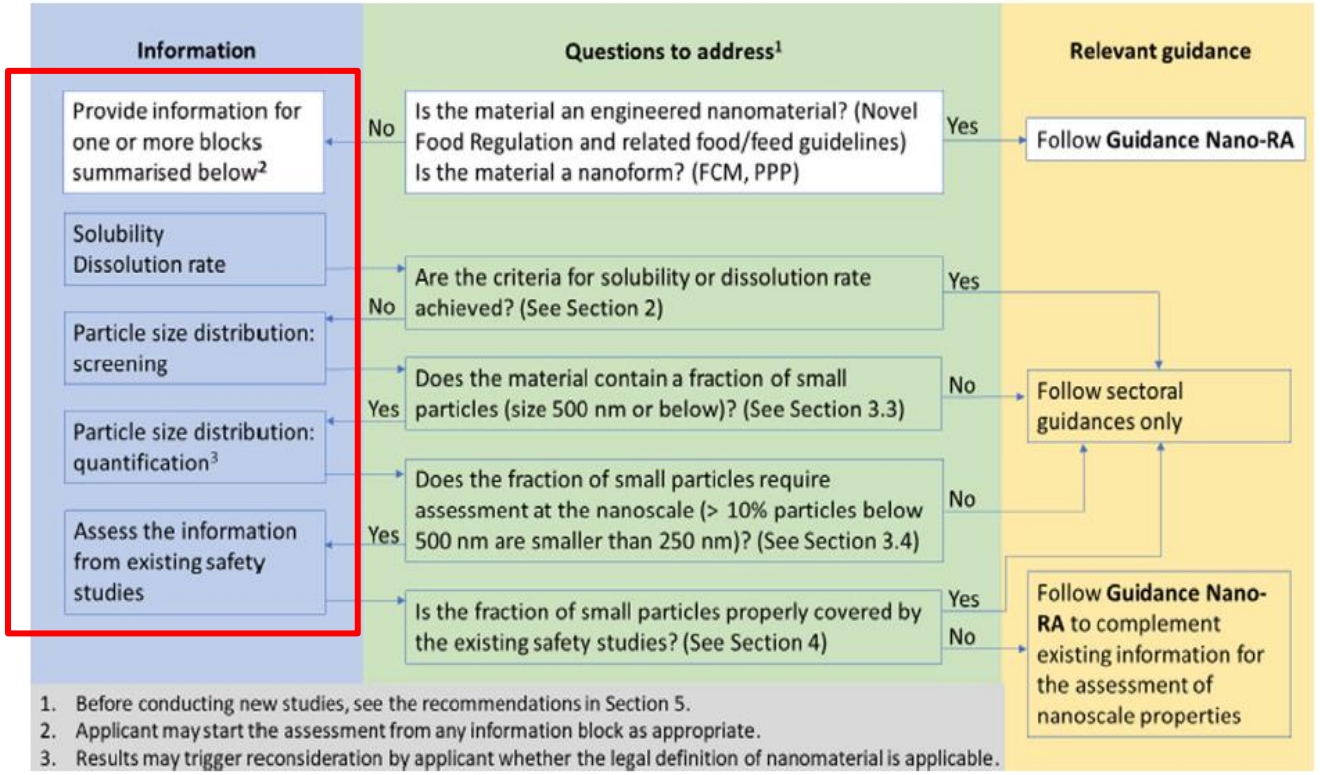
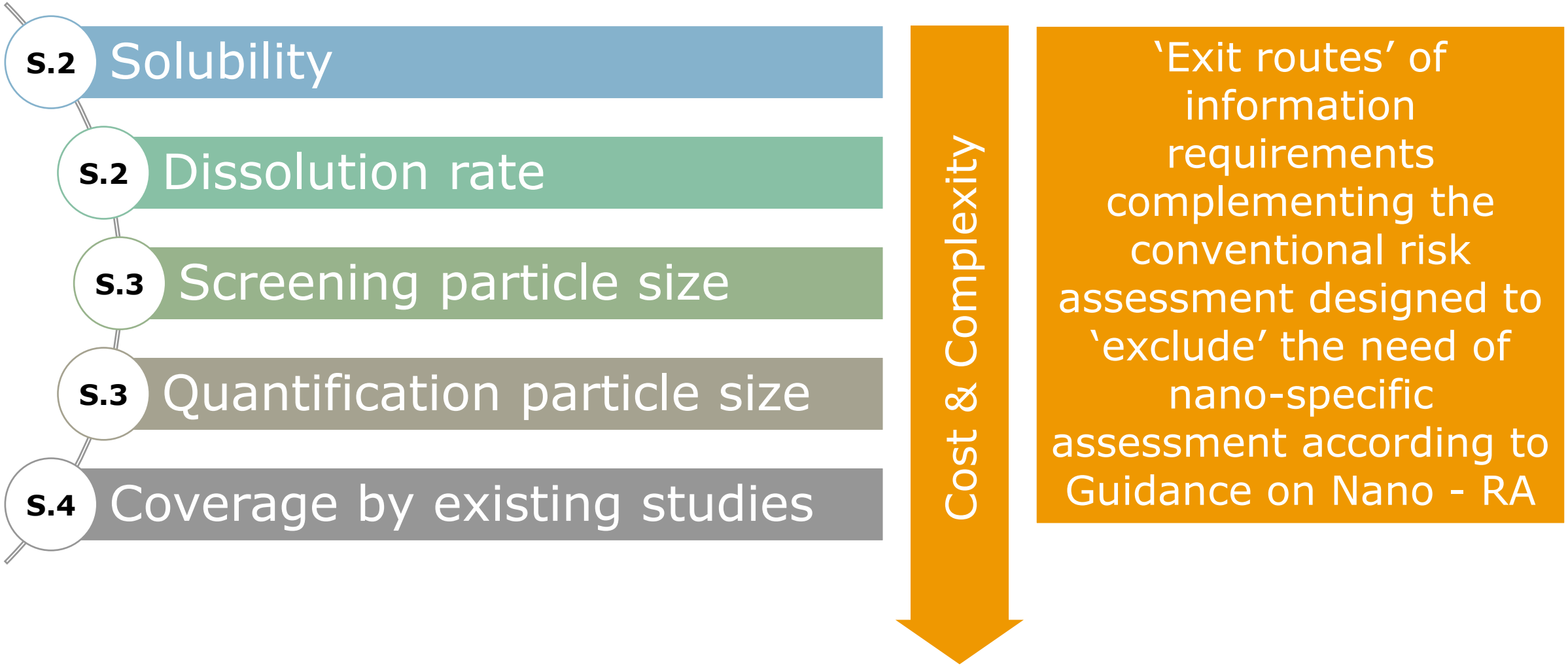


Figure 1: Decision process for selecting the applicable guidance document(s) to be used for the risk assessment of the material regarding the assessment of small particles

Appraisal routes proposed



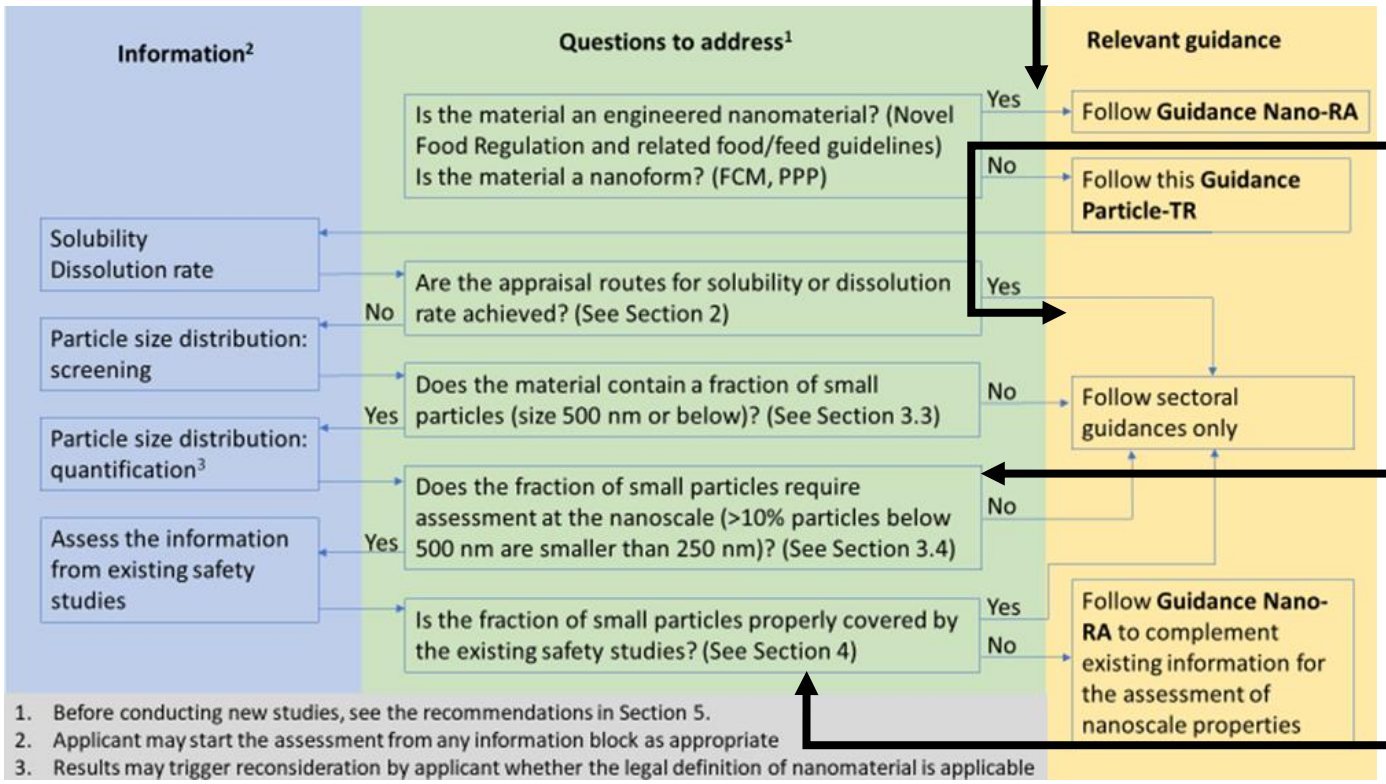
Each appraisal route and the underlying principles are extensively described in the dedicated Sections

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

Examples of applications and link with other Guidance documents



Zinc oxide nanoparticles

Guidance on Nano – RA

This **Guidance on Particle – TR**

Potassium chloride as Food additive

Solubility may confirm that guidance on food additives is sufficient

Magnesium silicate as Food additive

Generate information on particle size

Titanium dioxide as Food additive

Presence of a fraction of nanoparticles confirmed by the specifications

Appraisal routes proposed

S.2 Solubility

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

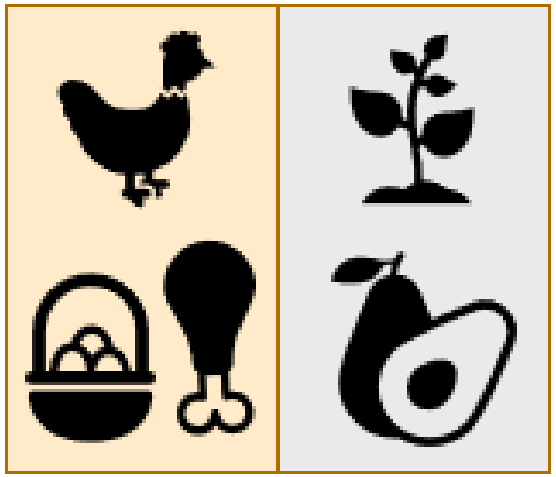
Parameters/ Options	Decision criteria[1]	Methodology	Comments
Solubility in water (Section 2.3.1)	Equal to or higher than 33.3 g/L	According to OECD TG 105 with specific considerations for small particles	For multi-constituent substances and mixtures, the decision criterion has to be fulfilled for each 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

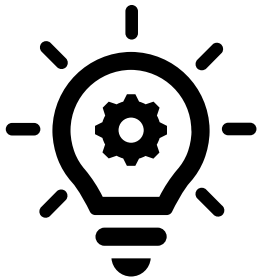
[1] Fulfilling the decision criteria for one of the parameters/options is sufficient for demonstrating that the assessment according to the sectoral guidance is sufficient

A dissolution rate protocol is included in Section 2.3.2.

Principles

Solubility:

- Value of **33.3 g/L** based on internationally agreed standard testing for chemicals (JECFA and EU/US Pharmacopeias). The material is expected to be fully solubilised (SCCS, 2019).



Dissolution rate:

- The dissolution rate threshold considers the time needed for particles to cross the mucus layer lining the intestinal epithelium and subsequent cellular uptake. If the particles dissolve within this time frame, there is no systemic exposure to particles.
  - The concentration to be tested is estimated based on the assumption that the amount ingested per day is diluted in the GIT up to a volume of 2L. Adaptation of the volume to 1L is needed when the process is expected to occur only, or mostly, at the acidic conditions of the stomach, as well as in case of assessments for infants and children.

Appraisal routes proposed

S.3 Screening particle size

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

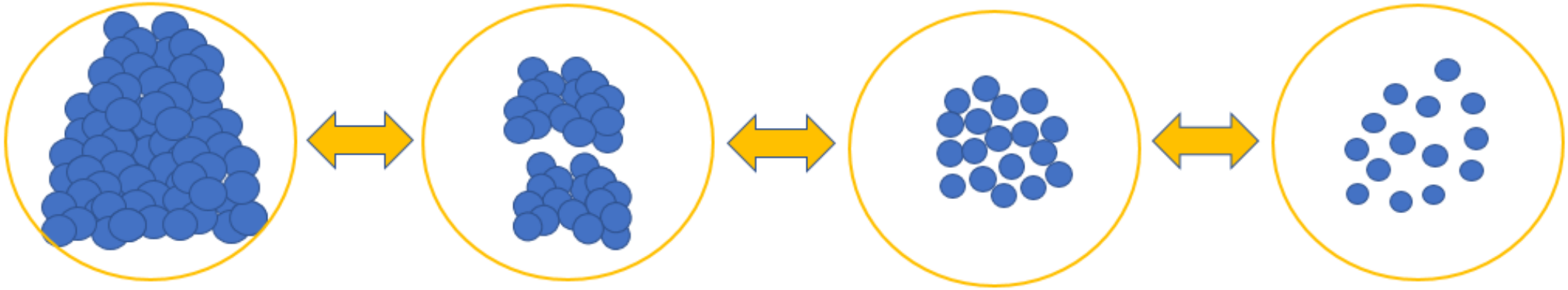
Parameters/ Options	Decision criteria[1]	Methodology	Comments
Particle size distribution of the material (Section 3.3)	Particles equal to or larger than 500 nm The detection capability of the method(s) used for this assessment should provide convincing evidence that the material contains less than 10% 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

Principles

Screening particle size:

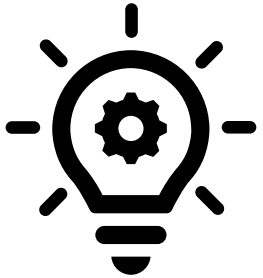
[10% number-based particle size smaller than 500 nm]

- Uptake from the GIT up to **250 nm**.
- **UF=2** to account for the limitations of available screening techniques (particle size range that can be detected, limits of quantification, conversion into number-based distributions,...)
- Assuming **normal size distribution of the full material** (worst-case scenario for conventional materials), 10% or less of the particles being smaller than 500nm implies that the fraction of nano-sized particles (1-100 nm) will be minimal, and uptake is negligible.

Quantification particle size:

[sub 500 nm fraction contains less than 10% particles (number-based) smaller than 250 nm]

- Uptake from the GIT up to **250 nm**.
- 10% for the sub-500nm fraction is a **technical threshold** based on the measurement uncertainty that can be achieved under typical conditions with the currently available EM methods



Appraisal routes proposed

S.4 Coverage by existing studies

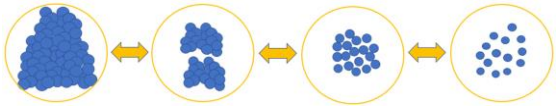
'Exit routes' for:

- Nanoparticles present but properly covered by existing safety studies

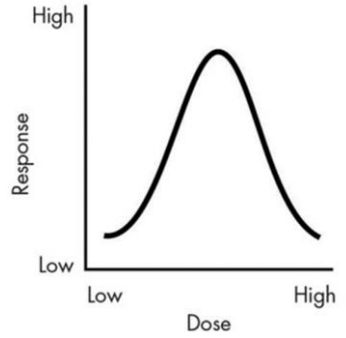
Parameters/ Options	Decision criteria[1]	Methodology	Comments
The studies address properly the potential hazards of the fraction of small particles (Sections 4.1. and 4.2)	The test material included the fraction of small particles AND The test design and level of dispersion/degree of agglomeration was 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

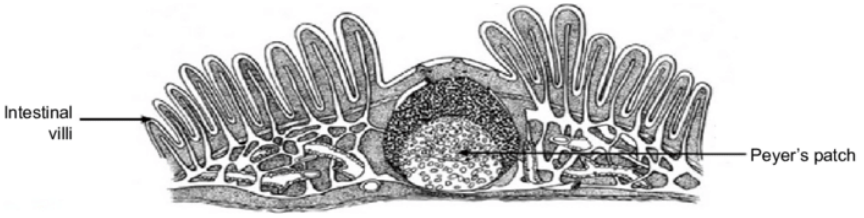
Particle toxicity

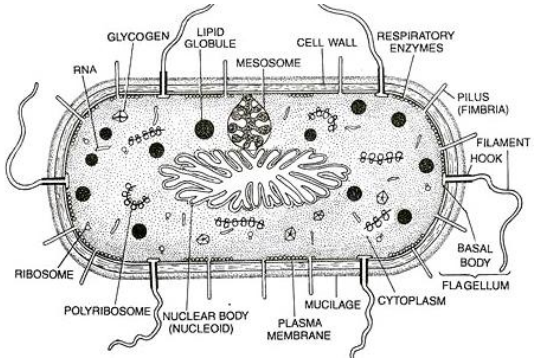


[exposure to particles = worst-case scenario]



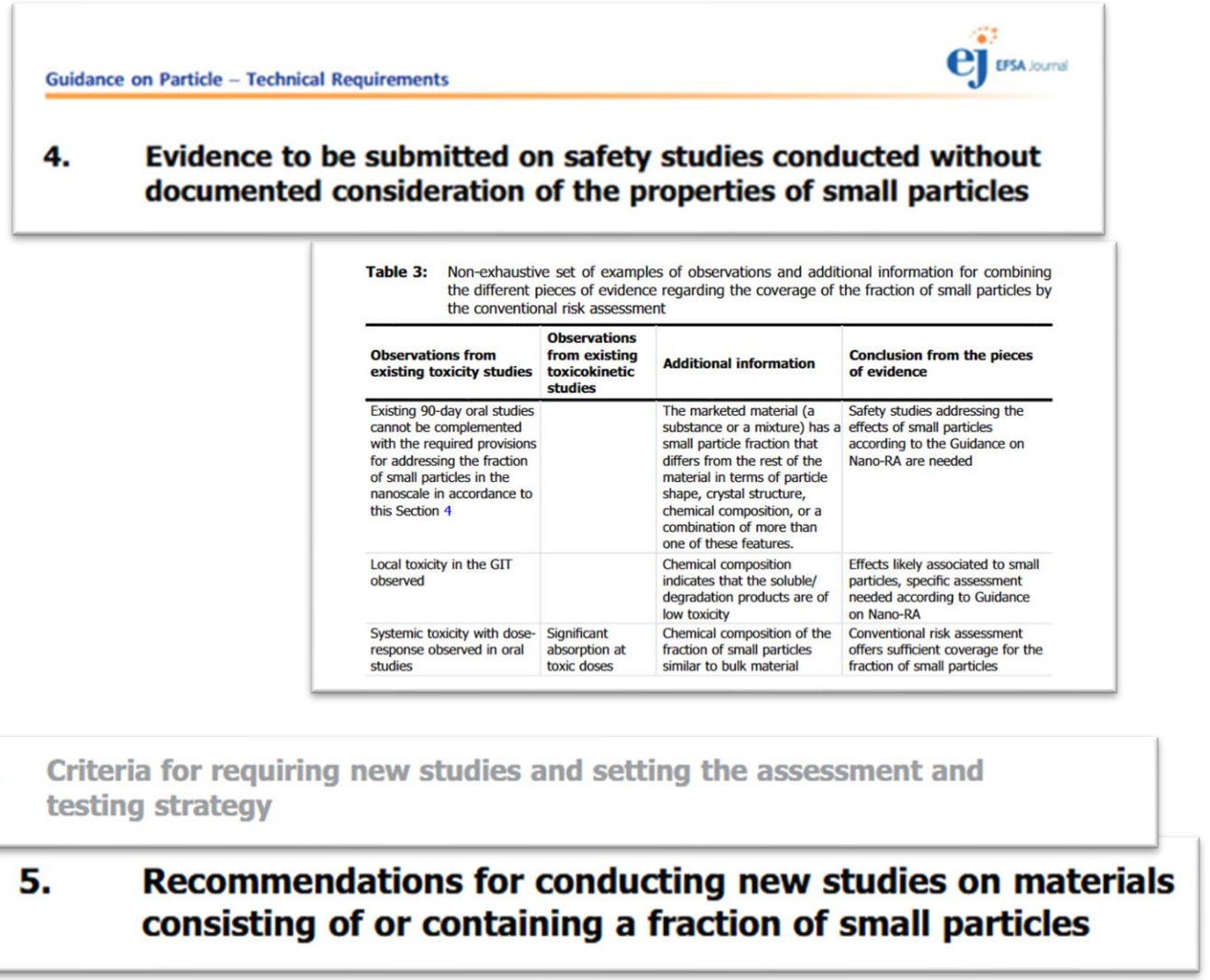
- The **lack of a proper dispersion method** and **high doses** promote **agglomeration** resulting in disproportionality between internal dose and external dose
- Proper **duration** (e.g. 90d) + **examination of first site contact** (e.g. Peyer's patches and GIT epithelia) **with appropriate techniques** (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**





Additional elements supporting applicants

- **Table 3:**
  Examples for combining the different lines of evidence and detecting data gaps regarding the coverage of the fraction of small particles by the conventional risk assessment
- **Section 4.3 and 5:**
  Recommendations on how to conduct new studies



Guidance on Particle – Technical Requirements

EFSA Journal

4. Evidence to be submitted on safety studies conducted without documented consideration of the properties of small particles

Table 3: Non-exhaustive set of examples of observations and additional information for combining the different pieces of evidence regarding the coverage of the fraction of small particles by the conventional risk assessment

Observations from existing toxicity studies	Observations from existing toxicokinetic studies	Additional information	Conclusion from the pieces of evidence
Existing 90-day oral studies cannot be complemented with the required provisions for addressing the fraction of small particles in the nanoscale in accordance to this Section 4		The marketed material (a substance or a mixture) has a small particle fraction that differs from the rest of the material in terms of particle shape, crystal structure, chemical composition, or a combination of more than one of these features.	Safety studies addressing the effects of small particles according to the Guidance on Nano-RA are needed
Local toxicity in the GIT observed		Chemical composition indicates that the soluble/ degradation products are of low toxicity	Effects likely associated to small particles, specific assessment needed according to Guidance on Nano-RA
Systemic toxicity with dose-response observed in oral studies	Significant absorption at toxic doses	Chemical composition of the fraction of small particles similar to bulk material	Conventional risk assessment offers sufficient coverage for the fraction of small particles

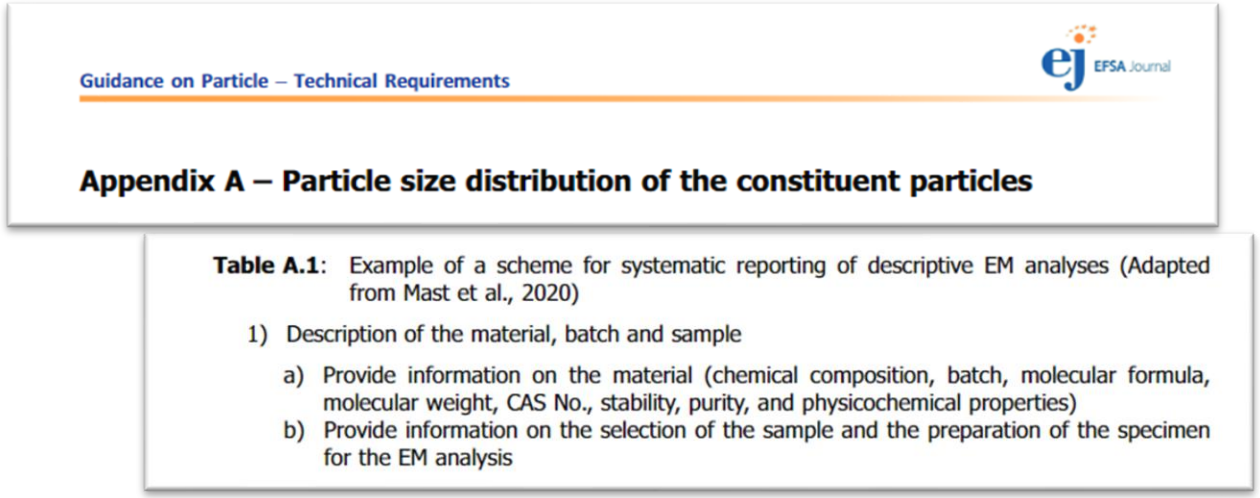
4.3. Criteria for requiring new studies and setting the assessment and testing strategy

5. Recommendations for conducting new studies on materials consisting of or containing a fraction of small particles

Additional elements supporting applicants

- **Appendix A:**
  How to report the characteristics of the fraction of small particles

- **Appendix B:**
  How to report the information from existing safety studies



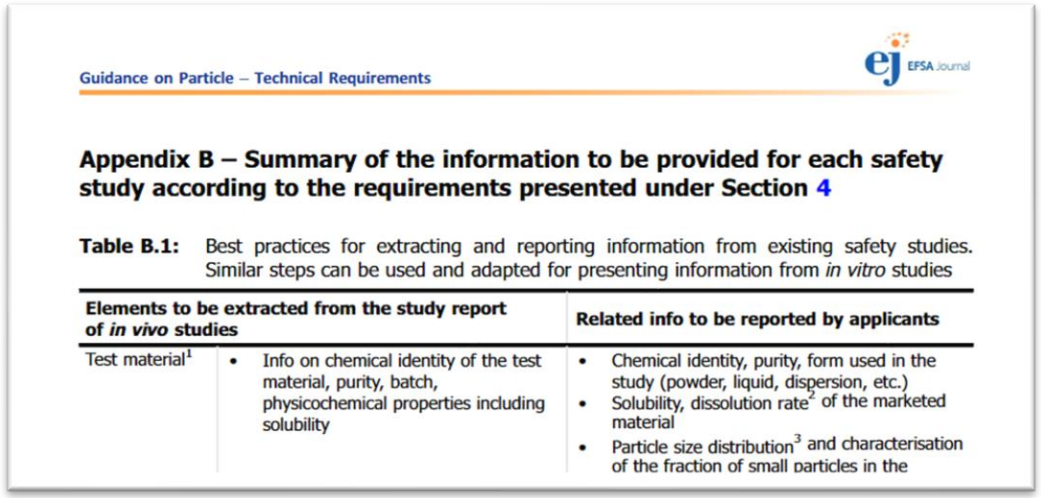
Guidance on Particle – Technical Requirements

EFSA Journal

Appendix A – Particle size distribution of the constituent particles

Table A.1: Example of a scheme for systematic reporting of descriptive EM analyses (Adapted from Mast et al., 2020)

1) Description of the material, batch and sample
   a) Provide information on the material (chemical composition, batch, molecular formula, molecular weight, CAS No., stability, purity, and physicochemical properties)
   b) Provide information on the selection of the sample and the preparation of the specimen for the EM analysis



Guidance on Particle – Technical Requirements

EFSA Journal

Appendix B – Summary of the information to be provided for each safety study according to the requirements presented under Section 4

Table B.1: Best practices for extracting and reporting information from existing safety studies. Similar steps can be used and adapted for presenting information from *in vitro* studies

Elements to be extracted from the study report of *in vivo* studies		Related info to be reported by applicants
Test material[1]	• Info on chemical identity of the test material, purity, batch, physicochemical properties including solubility	• Chemical identity, purity, form used in the study (powder, liquid, dispersion, etc.) • Solubility, dissolution rate[2] of the marketed material • Particle size distribution[3] and characterisation of the fraction of small particles in the

Take home messages

Besides the sector specific risk assessment, all 'conventional' materials should be assessed for the possible presence of small or nano particles according to the Guidance on Particle – Technical Requirements

The application dossier should properly report the information requirements described by the Guidance on Particle – Technical Requirements to exclude the need of nano-specific assessment

If the information provided are insufficient to exclude the need for nano-specific risk assessment, the applicant should follow the provisions described by the Guidance on Nano – Risk Assessment

Stay connected

European Food Safety Authority

Subscribe to

efsa.europa.eu/en/news/newsletters
efsa.europa.eu/en/rss

Receive job alerts

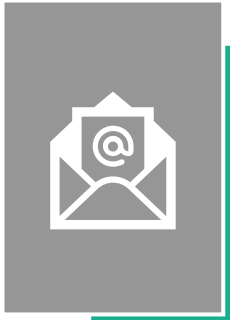
careers.efsa.europa.eu – job alerts

Follow us on Twitter

@efsa_eu
@plants_efsa
@methods_efsa
@animals_efsa

Follow us Linked in

Linkedin.com/company/efsa



Contact us

efsa.europa.eu/en/contact/askefsa