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

Examples related to the assessment of existing safety studies

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Trusted science for safe food

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European Food Safety Authority

Evaluation of new forms of assessed substances

- The substance has a long history of uses in other formulations and various routes of exposure
- The form being assessed was new and met the definition of a nanomaterial
- This new form had been characterised but there was limited information on existing forms

- **Key questions to answer**
- Was enough known about historical uses to compare the materials and use these data
- Were these data relevant to the route of exposure
- Was there sufficient information on the characteristics of the older materials

Why many assessments were inconclusive?

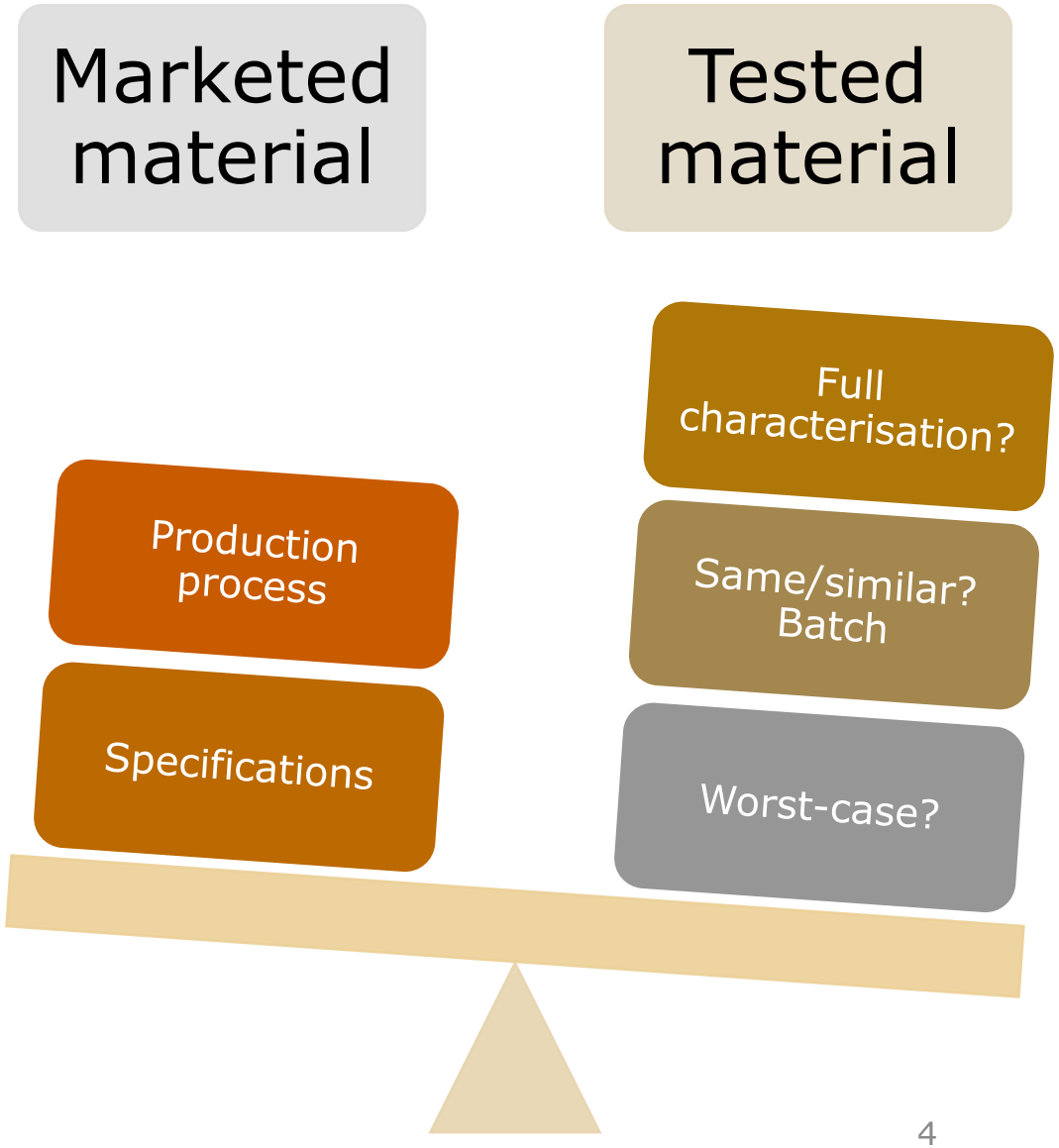
- We knew a lot about the consequences of historical exposures
- We knew lots about different routes of exposure
- We knew about the mechanisms of the effects
- We didn’t know the detailed characteristics of these old formulations – but we knew they were different to the new one
- Most of the data was from different routes – we knew it behaved differently to oral
- There were no data to demonstrate the new formulation could produce the key part of the mechanism

Relevance of test material used in the study

Relevance of the tested material:

- Must be within the specifications
- Cover the worst-case conditions

- Three possible options
  - Full physical-chemical comparison
    - Size distribution, shape, crystallinity,…
    - Purity of tested material
  - Based on production process
    - Test material is within batch variability
  - "Read across" assessment
    - Test materials with different sizes, crystallinity,…



Selection method for oral administration

- Three main options
  - Gavage
  - Mixed with feed
  - Drinking water
- Consider worst-case of actual use conditions for exposure to particles and degree of agglomeration
  - E.g. release from surface or mouth process

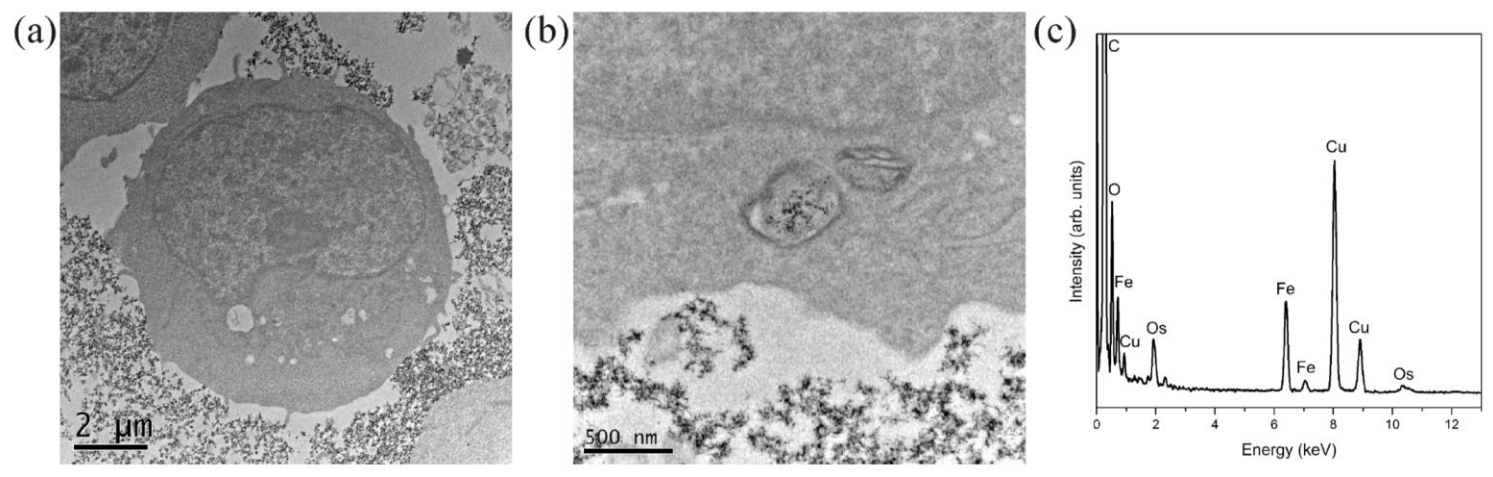
Consumer exposure patterns

Maximise exposure to nanoparticles

Relevance of *in vitro* studies

***In vitro* studies**

- Genotoxicity screening to be included in IATA approaches providing mechanistic information for nano-scale considerations and read-across
- Information on P-chem properties material used (as per general criteria)
- Nano-scale study design: good dispersion & stability in the media, SOP, …
- Measure cell internalisation, confirming presence as particles



Confirmation of iron oxide NP in MCL-5 cells by TEM – EDX
Brown et al., 2014
Journal of Physics: Conference Series 522 (2014) 012058
doi:10.1088/1742-6596/522/1/012058

- Internalisation may depend on cells and cell conditions, select cells with internalisation potential, unless **no uptake** is confirmed by toxicokinetic assessment

Relevance of *in vivo* studies

***In vivo* studies**

- Info on P-chem properties material used (as per general criteria)
  - Studies with no or limited information on P-chem properties of material used in in vivo study are
    - not included in risk assessment or
    - are considered of low relevance
  - E.g. size distribution, purity

Relevance of in vivo studies

***In vivo* studies**

- Proper dispersion and stability of dispersion
  - Studies with no or limited information on dispersion in administration matrix are considered of low relevance.
    - Manner & matrix of administration
      - Gavage, in food, in drinking water => representative for human exposure?
      - Dispersion protocols
    - Information on dose levels
      - High dose => degree of agglomeration
    - Measurements on concentration and dispersion in administration matrix
      - E.g. DLS, EM

Relevance of in vivo studies

- Example of assessing dispersion and considerations for interpretation

Criteria for scoring	Considerations for interpreting the results hazard characterisation of small particles including nanoparticles:
Score 1: Verifiable evidence on degree of agglomeration and/or confirmation of exposure	Results relevant and reliable for assessing small particles, incl. nanoparticles.
Score 2: Incomplete but some evidence on dispersion considerations or on confirmation of exposure	Relevance of the results for assessing small particles, incl. nanoparticles may be affected by agglomeration.
Score 3: Lack of protocol or information, low doses tested	Relevance of the results for assessing small particles incl. nanoparticles cannot be verified
Score 4: Lack of protocol or information, only high doses tested	Results may still be informative for larger particles and large agglomerates, but not for the fraction of small particles including nanoparticles.

- Examination of internal exposure: Quantitative/qualitative analysis; reliability analysis

Implementation of verification principles on "adequate level of dispersion" in the guidance TR

At least one of the following principles:

- Dispersion covered by a verified Standard Operating Procedure (SOP) or a systematic approach (e.g., NANOGENOTOX, ENPRA, ISO, OECD),or
- Sonication with energy densities from 600 J/ml to 2500 J/ml sample volume AND stability for at least 30 min or through administration, or
- Confirmation of sufficient level of dispersion and of the stability of the dispersion (options include EM, DLS, zeta potential higher than 25mV or lower than -25mV in the dispersion media); or
- Effective dispersing agents or surfactants with a proper justification (and inclusion of solvent control); or
- If administration in the animal diet, information on the level of agglomeration in the stock suspension/powder used to mix with the feed and in the animal diet for each dose level, or
- Confirmation of cell/tissue exposure during execution of the test; including evidence that the particles correspond to the material

Relevance of *in vivo* studies

***In vivo* studies**

- Examination of internal exposure
  - Quantitative or qualitative analysis in tissues
  - Reliability analytical method

- Use of information on internal exposure
  - Verifies if (and to which extent) test material is absorbed from the gastrointestinal tract
  - Can provide insight in relationship dose level – exposure
  - Can provide insight in potential for accumulation (internal exposure for multiple time points needed)
  - Allows for better comparison between studies

Relevance of *in vivo* studies

***In vivo* studies**

- Control group
  - Background exposure
  - Internal exposure in control group to compare to the dose-groups
    - Analytical challenges
    - Element vs particle
- Duration of exposure/study
  - Nanomaterials may accumulate in time
  - Has steady state been reached during study?

Assessing local effects in GIT

The assessment of local effects is specifically relevant for nanoparticles

- Tendency to agglomerate, rather than disperse, may produce high local concentration

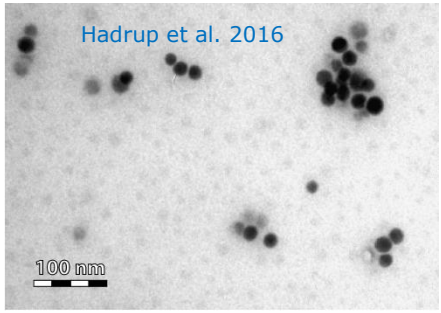
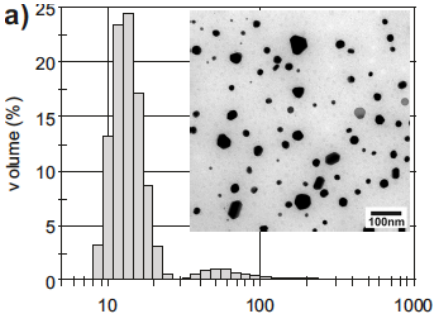


Figure 1 T Loeschner *et al. Particle and Fibre Toxicology* 2011, **8**:18



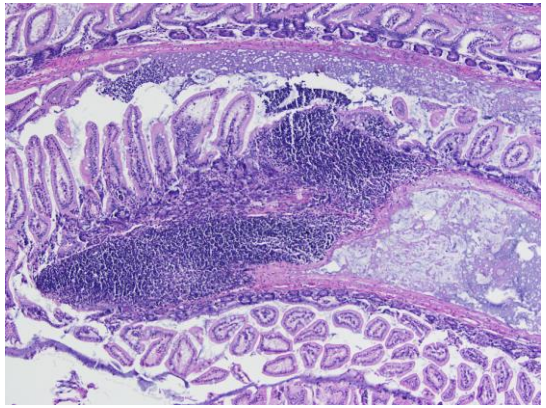
Loeschner *et al. Particle and Fibre Toxicology* 2011, **8**:18
http://www.particleandfibretoxicology.com/content/8/1/18

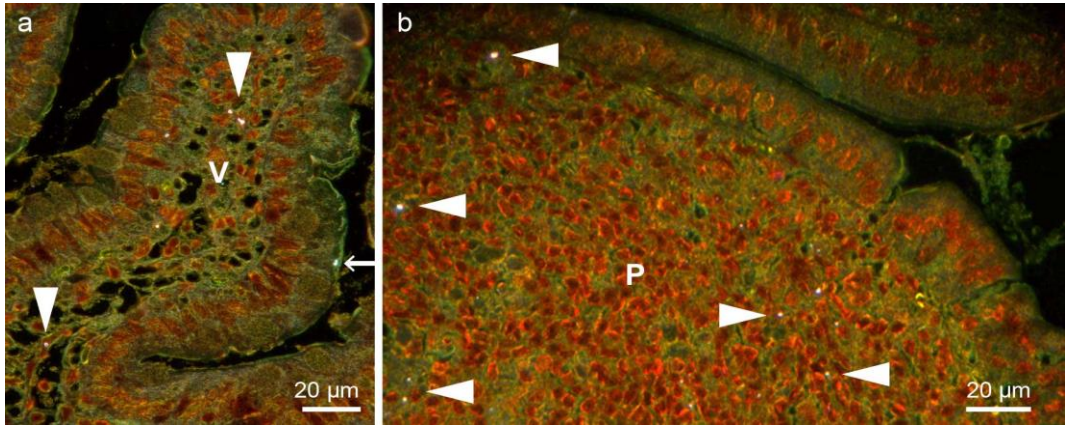
- Potentially different contact with GIT wall than dissolved chemicals
- Two mechanisms: Local release of chemicals & physical effects of presence of particles, including chronic inflammation

Figure 3 Autometallographic staining of ileum from a silver nanoparticle exposed rat. Optical micrographs of ileum section cuts showing, a) Overview and b) Detail with heavy staining in the tips of the intestinal villi (V) directly below the epithelium (E), within the lamina propria (LP), and in the submucosal region (SM).

Assessing local effects in GIT

- Local effects are relevant through external contact with the cell membrane of enterocytes and those in Peyer's patches and after cellular uptake by enterocytes, M cells, and cells in Peyer's patches (T-cells, B-cells, macrophages, dendritic cells)





Cytoviva enhanced darkfield hyperspectral system was used to detect particles

Particles in rat small intestine villus (V) and lymphoid tissue/Peyer's patch (P)

Photos by Trine Berthing, NFA, supported by EU project PATROLS and FFIKA from the Danish Government

Local effects in the GIT become the key element for assessing safety in case of no absorption