Food additives consisting of particulate materials: challenges and way forward in safety assessment and analytical characterisation

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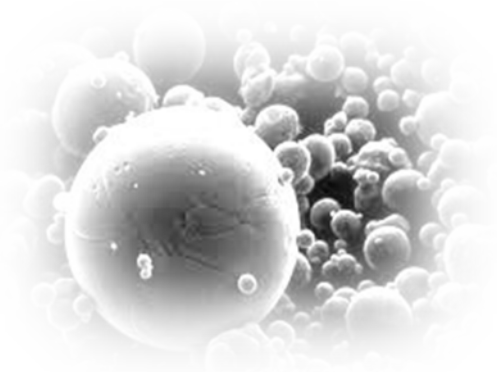

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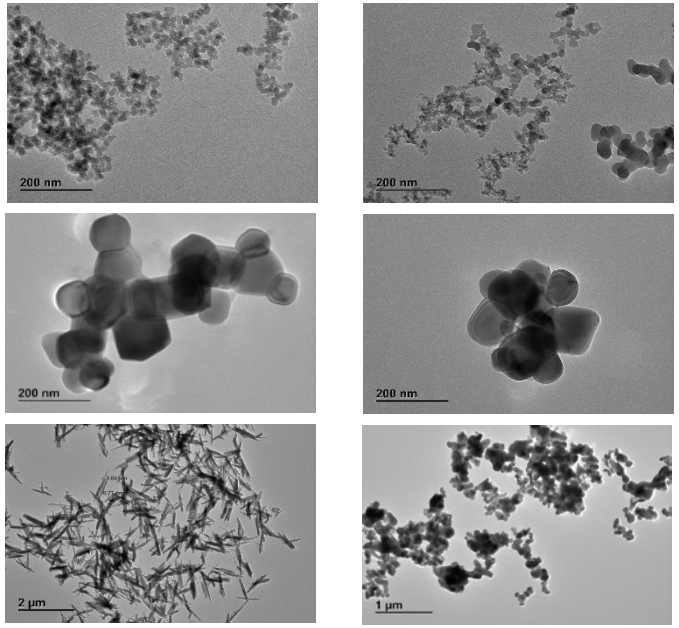
Food additives consisting of particulate materials

- **Several particulate food additives have been found to consist (or contain a substantial fraction) of nanoparticles which do not readily dissolve in the GIT and thus can reach the human intestine**
- **Examples:**
  - Synthetic amorphous silica (E 551)
  - Titanium dioxide (E 171)
  - Iron oxides/hydroxides (E 172)



Regulatory safety assessment of food-relevant particulate materials in the EU

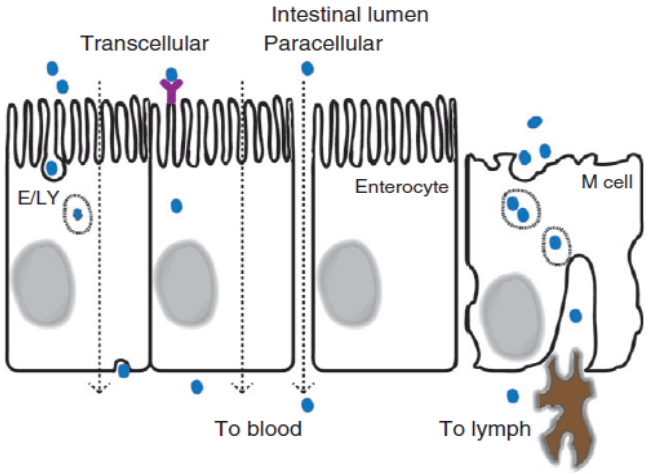
- It complements the traditional risk assessment performed according to the rules of the specific sector where the application lies and focuses exclusively on additional, nano-specific aspects
- First EFSA opinion on nanotechnology in 2009, first guidance on risk assessment in 2011, thorough update with more practical guidance in 2018 and finally in 2021
- In the last update of 2021, two interlinked guidance documents were produced: one on nanomaterials and the other on conventional materials with a fraction of small particles. The principles are the same, simplified screening options are offered for the conventional materials

Why safety assessment of particulate materials is different from conventional chemicals

- Particulate materials consisting of nanoparticles or containing a fraction of nanoparticles, due to the dual nature of particles (having both a physical and a chemical identity) may exhibit specific biokinetic behaviours and toxicological responses

- However this only happens for particles that reach the human intestine as such, i.e. do not dissolve/degrade during human digestion. *In chemico* (i.e. acellular *in vitro*) simulated digestion studies investigating the GIT fate are thus essential in regulatory risk assessment of particulate materials

- Another important concept is the existence of size thresholds for intestinal absorption: the overall evidence in that particles up to ~250 nm can be taken up and translocate across the intestinal barrier



Consequences for toxicological assessment

- The fact that **size thresholds** exist for **particle uptake** along with the **tendency of particles to form agglomerates when the particle concentration increases** has important consequences in toxicological testing, both *in vitro* and *in vivo*

- *Toxicological testing is normally performed at high concentrations (in vitro)/doses (in vivo) since identification of adverse effects and obtainment of a dose-response relationship are sought for*
- *Since the level of agglomeration is dependent on particle concentration, at such high concentrations/doses - in the absence of a proper dispersion protocol - particles tend to be heavily agglomerated and become too large to be taken up by cells, resulting in absence of internal exposure and toxicological responses*
- *This is why in nanotoxicological testing it is essential to properly disperse the particles. Maximal de-agglomeration is needed for in vitro testing. For in vivo testing, a degree of agglomeration which mirrors that of actual human exposure conditions at all the used doses is targeted. But how can the latter be determined?*

TOX TESTING

HIGH concentrations (in vitro) doses (in vivo)

HIGH agglomeration

LOW/NO internal exposure

HUMAN EXPOSURE

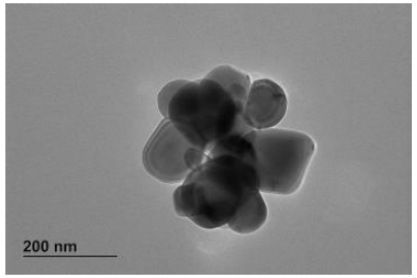
Lower doses

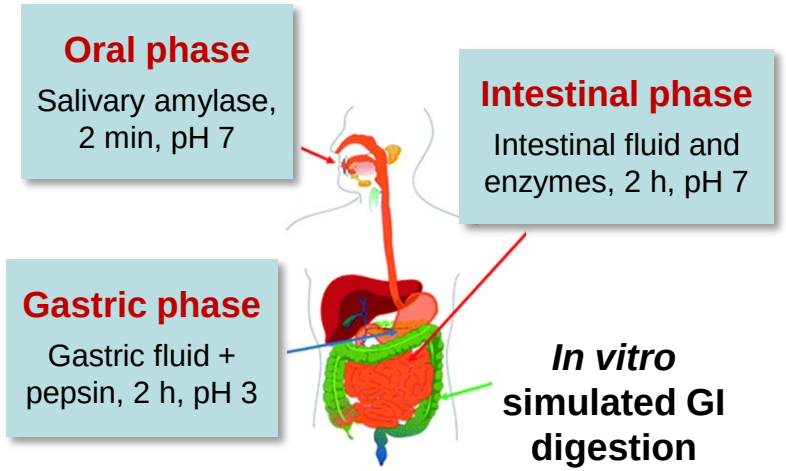
LOW agglomeration

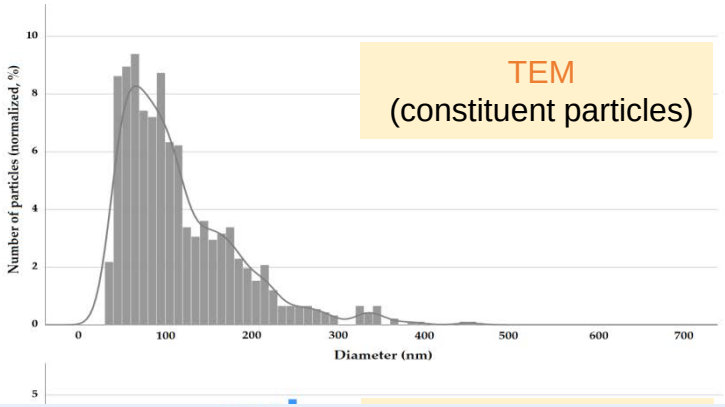
Higher internal exposure

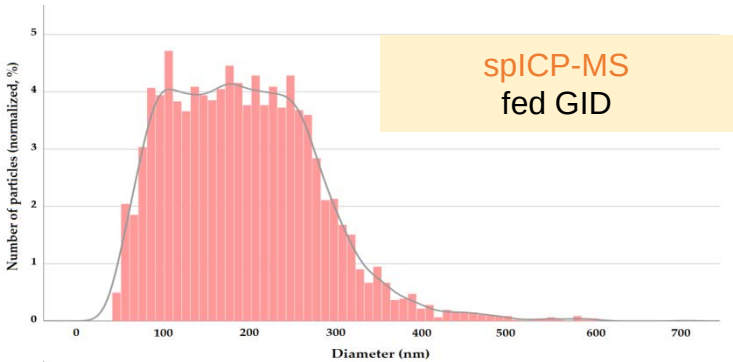
Simulated gastrointestinal digestion according to the EFSA Guidance: ISS-JRC study

- The GIT fate and agglomeration degree of a typical E 171 material was studied by applying the *in chemico* GID approach laid down in the EFSA guidance on nano-RA at the concentrations of estimated human exposure
- A standardized protocol developed by the international INFOGEST network was used for the simulation of GID in order to allow for the replication of experiments and ensure inter-comparability of the results
- E 171 GIT's fate was studied in fasted conditions (relevant to E 171 use in food supplements and oral medicines) and in fed conditions, using either a cereal-based model food or actual E 171-containing food samples
- A state-of-the-art multi-method approach for physicochemical characterization of both the pristine material (TEM-EDX, BET, ELS, CLS, DLS) and the particles in intestinal fluid (single particle ICP-MS and ultrafiltration coupled to ICP-MS) was used

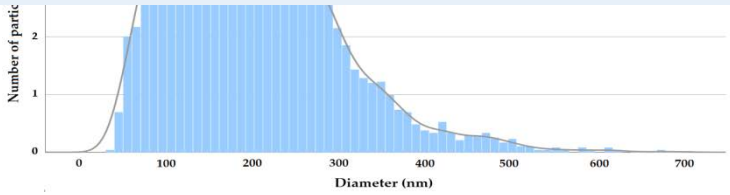


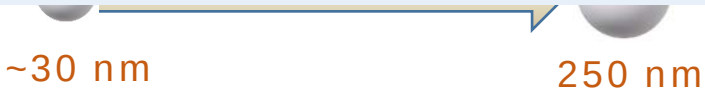






- These results indicate that humans are internally exposed to TiO_2 particles within a size range whereby they can be absorbed by the small intestine and cross it to reach the systemic circulation
- In addition, they indicate that for achieving a similar degree of dispersion in a water suspension of E 171 particles, as normally used in tox studies, acoustic energy via ultrasonication is needed even at a low E 171 concentration
- TiO_2 constituent particles were resistant to GI dissolution, and thus their stability in lysosomal fluid was investigated. The biopersistence of the material in lysosomal fluid highlighted its potential for bioaccumulation





≥70% of TiO_2 particles <250 nm

NANOPERSIT project in collaboration with the EC-JRC (Ispra)

Nanomaterials (2023) 13:1908

Article

Agglomeration Behavior and Fate of Food-Grade Titanium Dioxide in Human Gastrointestinal Digestion and in the Lysosomal Environment

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Abstract: In the present study, we addressed the knowledge gaps regarding the agglomeration behavior and fate of food-grade titanium dioxide (E 171) in human gastrointestinal digestion (GID). After thorough multi-technique physicochemical characterization including TEM, single-particle ICP-MS (spICP-MS), CLS, VSSA determination and ELS, the GI fate of E 171 was studied by applying the in vitro GID approach established for the regulatory risk assessment of nanomaterials in Europe, using a standardized international protocol. GI fate was investigated in fasted conditions, relevant to E 171 use in food supplements and medicines, and in fed conditions, with both a model food and E 171-containing food samples. TiO_2 constituent particles were resistant to GI dissolution, and thus, their stability in lysosomal fluid was investigated. The biopersistence of the material in lysosomal fluid highlighted its potential for bioaccumulation. For characterizing the agglomeration degree in the small intestinal phase, spICP-MS represented an ideal analytical tool to overcome the limitations of earlier studies. We demonstrated that, after simulated GID, in the small intestine, E 171 (at concentrations reflecting human exposure) is present with a dispersion degree similar to that obtained when dispersing the material in water by means of high-energy sonication (i.e., ≥70% of particles <250 nm).

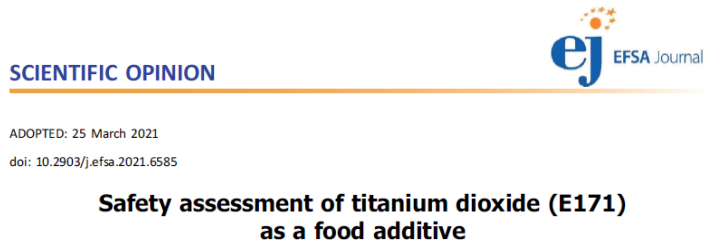
Keywords: titanium dioxide; E 171; fate; human gastrointestinal digestion; lysosomes; particle agglomeration; physicochemical characterization; single-particle ICP-MS; transmission electron microscopy; risk assessment

Citation: Ferraris, F.; Raggi, A.; Ponti, J.; Mehn, D.; Gilliland, D.; Savini, S.; Iacoponi, F.; Aureli, F.; Calzolai, L.; Cubadda, F. Agglomeration Behavior and Fate of Food-Grade Titanium Dioxide in Human Gastrointestinal Digestion and in the Lysosomal Environment. *Nanomaterials* **2023**, *13*, 1908. https://doi.org/10.3390/nano13131908

Challenges in analytical characterisation: the case of food-grade titania (E 171)

- Widely used food additive owing to the light-scattering effect of TiO_2 particles occurring in the particle size range of 200–300 nm (whitening properties)

- In 2021, EFSA completed a re-assessment of E 171 safety as a food additive which took into account the nanospecific considerations of the guidance on particle risk assessment and concluded that it could no longer be considered safe


SCIENTIFIC OPINION

EFSA Journal

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Safety assessment of titanium dioxide (E171) as a food additive

EFSA assessment (2021)

E 171 ban as food additive in the EU (2022)

- This led in 2022 to regulatory actions resulting in the ban of E 171 as additive in food and food supplements in the EU. However, testing compliance of food products poses significant challenges since TEM or SEM analysis coupled with elemental analysis (EDX) is not a feasible approach for official control laboratories

- As National Reference Laboratory for Nanomaterials in Food, we developed a viable analytical strategy for compliance testing

Article

An ICP-MS-Based Analytical Strategy for Assessing Compliance with the Ban of E 171 as a Food Additive on the EU Market

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Abstract: A method was developed for the determination of total titanium in food and food supplements by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted acid digestion of samples. Five food supplements, including one certified reference material, and 15 food products were used for method development. Key factors affecting the analytical results, such as the composition of the acid mixture for sample digestion and the bias from spectral interferences on the different titanium isotopes, were investigated. Resolution of interferences was achieved by ICP-MS/MS with ammonia adduct formation and viable conditions for control laboratories equipped with standard quadrupole instruments were identified. The method was successfully validated and enables rapid screening of samples subject to confirmatory analysis for the presence of TiO_2 particles. For the latter, single-particle ICP-MS (spICP-MS) analysis after chemical extraction of the particles was used. The two methods establish a viable analytical strategy for assessing the absence of titania particles in food products on the EU market following the E 171 ban as a food additive.

Keywords: titanium dioxide; E 171; ICP-MS; single-particle ICP-MS; spectral interferences

Citation: Ferraris, F.; Adelantado, C.; Raggi, A.; Savini, S.; Zougagh, M.; Ríos, Á.; Cubadda, F. An ICP-MS-Based Analytical Strategy for Assessing Compliance with the Ban of E 171 as a Food Additive on the EU Market. *Nanomaterials* **2023**, *13*, 2957. https://doi.org/10.3390/nano13222957

The method at glance

- The method was developed for food and food supplements and is divided in a screening analysis and a confirmatory analysis

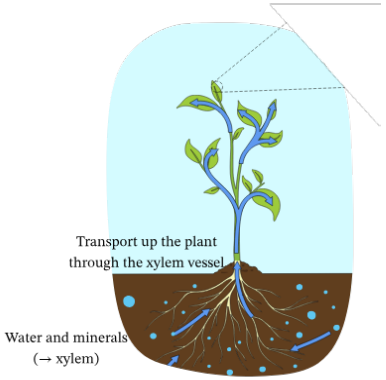
- The screening analysis consists in a total titanium determination after microwave-assisted acid digestion with concentrated HNO_3, H_2O_2 and HF:
  - ✓ If the result is >50 µg TiO_2 g^{-1}, the sample is positive (i.e. not compliant) and no confirmatory analysis is needed

- The confirmatory analysis is needed if the screening analysis values in the range 2-50 µg TiO_2 g^{-1} are found
  - ✓ It consists in a single-particle ICP-MS (spICP-MS) analysis after chemical extraction of the particles
  - ✓ In this analysis the particle size distribution is compared with that of a typical E 171 sample

The foundation of the method

- Ti concentrations in food are relatively low and uniform, which in the first place depend on the low mobility and availability of the element in agricultural systems

- Titanium is not an essential element for plants or animals. Titanium minerals are very resistant to weathering in soils and the titanium released precipitates as anatase; only upon dissolution, the soluble inorganic or organometallic species are sparingly taken up by edible plants



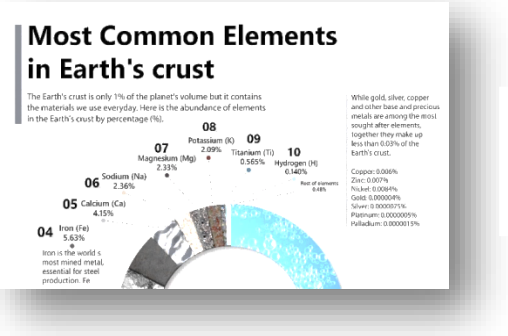
- Like most transition elements, root-absorbed Ti is largely accumulated in the roots with a small amount transported to shoots through the xylem stream

- Other than use of TiO_2 food additives, food processing is also not expected to markedly contribute to total Ti levels in products

Limitations of existing total Ti data in food

- Reliable analytical data on total Ti in food are limited. The element is rarely investigated and most of the historical data have limitations

- Ti is the 9th most abundant element in the earth's crust and the second most abundant transition metal after iron:
  - ✓ One general issue when dealing with background concentrations is positive contamination during sample preparation



- In recent years, ICP-MS has become the most widespread technique for elemental analysis, but all Ti isotopes are affected by severe spectral interferences arising from isobars and polyatomic ions

- Only data obtained by quadrupole mass spectrometers with reaction cells, 'triple-quadrupole' and high-resolution instruments can be considered generally reliable. Such data suggest that in most food, background Ti concentrations are generally <0.5 μg g^{-1} and can be as small as <0.05 μg g^{-1}

The screening component of the method: reliable total Ti analysis

- When E 171 is added to food, concentrations are often in the range of hundreds up to few thousands µg TiO_2 g^{-1}. However samples containg few tens µg TiO_2 g^{-1} have also been found

- Therefore, it is important to ensure accurate total Ti determination even at low Ti concentrations. This is achieved by:

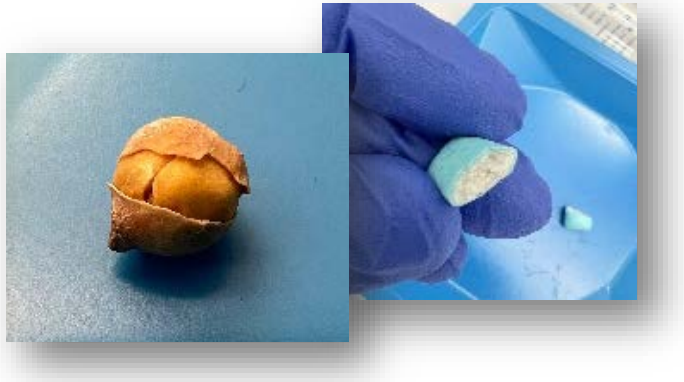
  - ✓ Using ultrapure grade reagents
  - ✓ At low Ti concentrations, the use of ICP-MS/MS with NH_3/He as reaction gas enabling interference-free determination via the ammonium cluster product ions at m/z 149 and 150, i.e., $Ti(NH_3)_6^+$ (47 → 149, 48 → 150). LOQs are ca. 0.08 µg Ti g^{-1} (0.06 µg TiO_2 g^{-1})
  - ✓ At higher Ti concentrations, using ^{49}Ti or ^{50}Ti with conventional quadrupole conditions (as interferences on these masses becomes negligible)
  - ✓ Using HF in the digestion mixture (mininal amounts are sufficient): without HF there is no dissolution of particles and the acid environment causes their precipitation as agglomerates leading to losses and poor total Ti recoveries

Confirmatory analysis by spICP-MS

- Alkaline extraction of particles with of 20% TMAH is performed (5 min probe sonication, followed by stirring overnight in a mechanical agitator)

- The particle size distribution fingerprint is then checked against a typical E 171 sample. The most robust parameter is the median diameter: a value within the range of compiled literature data positively identifies the sample

- As for total Ti analysis, since E 171 is normally used only in the external coatings of food, it is important to selectively analyse (in addition to the whole item) also the outer part only:
  - ✓ Being a forbidden substance, detection in the coating allow to conclude that the sample is not compliant

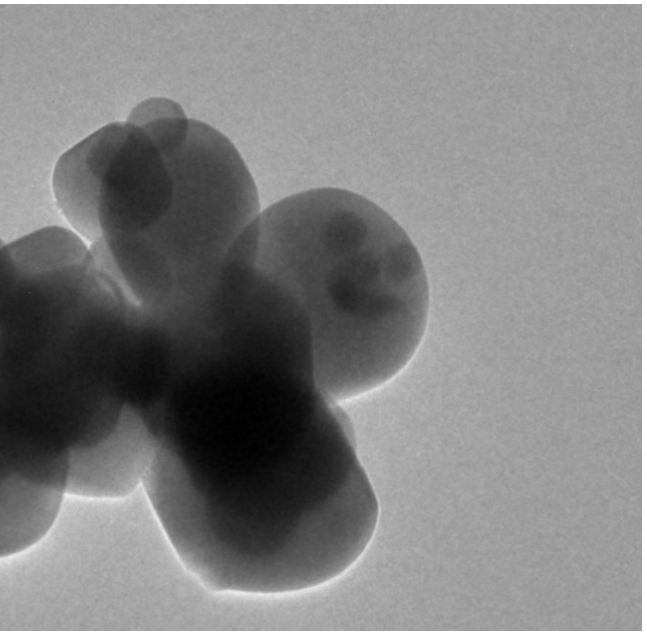


- As for total Ti, analysis of E 171-free samples of the same food item reduces uncertainty and is used at our NRL to build a database of background Ti concentrations

Conclusions

- **Safety assessment of particulate materials** is different from conventional chemicals and **specific requirements/ adaptations** have to be met
- The EFSA NanoGuidances propose a structured pathway for carrying out safety assessment of **nanomaterials** and **other types of substances falling under the food law that might present hazards related to the nanoscale**, independently from regulatory definitions
- **Dissolution rate in the small intestine** is key to assess if a nanospecific assessment is indeed needed. In chemico methods have been developed which enable to characterise the **fate on particulate materials in the human GIT**
- A pragmatic **method** has been validated for **checking compliance with the E 171 ban in food on the EU market**
- It includes a **screening analysis** via total Ti determination by ICP-MS and a **confirmatory analysis** by spICP-MS
- In this method, **ICP-MS/MS** is needed for reliabe determination of **low Ti concentrations**, otherwise **conventional ICP-MS** is sufficient

Questions?



THANK YOU FOR YOUR ATTENTION