

Titanium Dioxide - Statement on the safety of Titanium Dioxide (E171) as a Food Additive

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General information

31. Titanium dioxide (TiO₂) is an inorganic compound which exists in nature in different crystalline forms, the anatase and rutile being the two most important for its use as a food additive. Food grade TiO₂ (E171) is comprised of a mixture of both nano- and micro-sized particles.

32. The Chemical Abstracts Service (CAS) Registry number for TiO₂ is 13463-67-7 and the European Inventory of Existing Commercial Chemical Substances (EINECS) number is 236-675-5. The Colour Index (C.I.) number is 77891.

33. Food grade titanium dioxide (TiO₂) was an authorised Food Additive (E171) in the EU but from the 7th of August 2022 it is no longer permitted following the publication of Commission Regulation (EU) 2022/63, amending Annexes II and III to Regulation (EC) No 1333. Since August 2022, manufacturers in Northern Ireland have not been permitted to produce goods containing titanium dioxide according to EU legislation. It currently remains authorised in Great Britain. TiO₂ is identified by JECFA as INS 171 and is a food additive included in table 3 of the General Standard for Food Additives (GSFA).

34. The uses of TiO₂ as a food additive include:

- as a colour to make food more visually appealing,
- to give colour to food that would otherwise be colourless, and
- to restore the original appearance of food.

35. It is found in a wide variety of food items such as pastries, chocolate and sweets, sauces and chewing gum. It is also widely used in cosmetics and medicines (EFSA, 2016).

Physicochemical Characterisation of food grade TiO₂

36. The characterisation of TiO₂ has been described in detail by EFSA (2016) and in the Health Canada State of the Science Report (2022). This section provides a summary of the TiO₂ characterisation to provide background information for the better understanding of this statement on the safety of TiO₂ as a food additive.

37. TiO₂ is a white powder used as a pigment in various industries and is valued for its high refractive index. Specifically in food, the primary function of TiO₂ is as an opacifier and white pigment. To achieve this function, it is critical that food grade TiO₂ exists as an aggregate of smaller primary particles with a median particle size of 200 – 300 nm, which corresponds to roughly half the wavelength of visible light and provides optimal light scattering, thus producing the desired whitening effect (Winkler et al. 2018).

38. TiO₂ can also be in the form of engineered nano-TiO₂. One form of nano-TiO₂ that is generally sold for catalytic applications and is also widely used in toxicity studies is P25. Food-grade and P25 TiO₂ are engineered products, frequently synthesized from purified titanium precursors, and not milled from bulk scale minerals (Yang et al., 2014). Engineered nano-TiO₂ (e.g., P25, NM-101, NM-102, NM-103, NM-104 and NM-105, see Table 1 below) has 100% particles less than 100 nm in diameter, and is colourless and therefore unsuitable for use as a pigment/opacifier in food and pharmaceutical applications (Farrell and Magnuson, 2017)

39. Pure TiO₂ assembles in several crystal structures although only anatase, rutile or a mixture of the two are used in foods. Food- and pigment-grade TiO₂ are engineered products synthesized from purified Ti precursors. The anatase form can only be made using the sulfate process, whereas the rutile form can be made using both the sulfate and chloride processes (Ropers et al., 2017). The majority of food-grade TiO₂ is produced by the sulfate process in the anatase crystal structure. For many applications, including cosmetics and some therapeutic products, surface coatings are applied to TiO₂ particles that alter their physicochemical properties. Therefore, toxicity and ADME studies using these materials may not be relevant to TiO₂ used as a food additive, which

normally undergoes no surface treatment and is uncoated (EFSA, 2016). Due to differences in the manufacturing processes, food-grade TiO₂ differs in surface composition compared to other TiO₂ materials, such as TiO₂-NPs. The surface of food-grade TiO₂ particles is covered by superficial phosphate groups, while materials manufactured with alternative methods may have different surface functional groups (e.g., hydroxyl groups) (Dudefoi et al., 2017a; Yang et al., 2014). The surface properties of TiO₂ materials have a significant impact on their behaviour in various environments, including biological media. Therefore, TiO₂ materials with surface properties that differ from food-grade TiO₂ may not be relevant models when studying the fate of dietary TiO₂ (Dudefoi et al. 2017a).

40. Suspended TiO₂ particles tend to aggregate/agglomerate to form larger clusters. The term “aggregate” designates an assembly of particles held together by covalent or metallic bonds. Instead, “agglomerates” result from weak forces like van der Waals interactions, hydrogen bonding, electrostatic attractions or adhesion by surface tensions. Due to this aggregation and agglomeration, it is important not to equate the nanoparticle fraction measured by number with the same value by mass (Winkler et al., 2018).

41. However, despite having a mean particle size in the desired range (200 - 300 nm), primary particles in food-grade TiO₂ form a broad size distribution that invariably contains particles below 100 nm, i.e. nanomaterial. A number of studies have measured particle size in food grade TiO₂ or E171 and found a range of 17 - 36% by number of TiO₂ NPs with a diameter less than 100 nm (Verleysen et al. 2020 and 2021; Yang et al, 2014; Dudefoi et al. 2017; Weir et al., 2012). The percentage by number of particles with a diameter less than 30 nm is of the order of 1% or less (EFSA 2021a).

42. When calculating exposure to TiO₂ NPs the equivalent mass of NPs is more relevant than particle number. Several studies reviewed by Ropers et al estimated the mass (weight %) of nanoparticles present in E171 across a range from 0.31 to 12.5% (EFSA, 2016; Bachler et al., 2014; Rompleberg et al., 2016; and Dudefoi et al., 2017). This variation explains some differences in the estimated exposures to TiO₂ nanoparticles in the literature including between the study by Rompelberg et al. who used a value of 0.31% of NPs and the evaluation of EFSA who used a weight ratio of 3.2%. (Ropers et al., 2017).

43. Since E171 is a particulate material containing a fraction of nanoparticles, and toxicology studies mainly focus on the systemic absorption of this nanofraction after ingestion, EFSA (2016) noted the need for more data; for example, information related to the particle size distribution of the titanium

dioxide when used as a food additive. Moreover, it was noted that the EU specifications for E171 should also include full characterisation of the particle size, distribution of the materials as well as an indication on the percentage (in number and by mass) of the particles in the nanoscale, together with the information on the analytical methods/techniques used for detection and quantification of the nanosized particles. In this respect, the European Commission called in early 2017 for data addressing those EFSA recommendations. (Verleyen et al., 2020; Geiss et al., 2020).

Physicochemical Characterisation of nano grade TiO₂

44. The consensus within the risk assessment community on the need for standardised and validated dispersion methods for nanoparticles to ensure reliable and reproducible results to help enable the advancement of their risk assessment is well known. Validated approaches for the preparation of TiO₂ nanoparticles solutions either monodispersed or protein stabilised dispersions in relevant biological media, are regularly used for acute *in vitro* or *in vivo* toxicity assessment. Dispersions in biological media, that remain stable for at least 48 h (acute testing timeframe) under typical incubation conditions, are considered useful. The initial objective of most studies is to determine whether monomodal nanoscale dispersions, in the tested media, can be achieved by mixing aqueous nanoparticle stock with a biological media without prior stabilisation with addition of fetal bovine serum (FBS) or bovine serum albumin (BSA), or other dispersants more relevant to the test system. To achieve this mono-dispersion surface modifications of the NPs, during synthesis, is often undertaken. Most academic studies are based on assessment of dispersed particle solutions, to help answer specific questions, which may not fully represent specific products or properties of products on the market.

45. The OECD Working Party on Manufactured Nanomaterials (WPMN) 'Guidance Manual for Sponsors of the OECD Sponsorship Programme for the Testing of Manufacture Nanomaterials' has collaborated with numerous international projects to develop an understanding of nanomaterials. The Joint Action, NANOGENOTOX, nanogenotox.eu, co-financed by the Executive Agency of the Directorate General for Health and Consumers of the European Commission and 11 EU member states was established to determine characterisation and testing of TiO₂, with a focus on reference materials produced by the Joint Research Centre laboratories. Examples of EU projects testing the materials from

the Repository are MARINA ([marina-fp](http://marina-fp.eu)) and NANoREG (nanoreg.eu). The NANOGENOTOX sample preparation protocol developed by Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Institut National de la Recherche Scientifique (INRS) and The National Research Centre for the Working Environment (NRCWE) and the final dispersion protocol is published on the project's web page. To highlight the interventions required to achieve dispersion “2.56 mg/mL of material sterile-filtered 0.05 % w/v BSA-ultrapure water are sonicated (probe sonicator) for 16 minutes, placed in an ice bath, at 400 W and 10 % amplitude while controlling that the sonication probe does not touch the walls of the scintillation vial. Use of different sonication conditions (power and amplitude) require different sonication times. The energy input should be calibrated to be in the order of 3,136 MJ/m³.” This highlights the upstream processes required prior to material assessment to investigate the possible relevance of the nano component in toxicology studies.

46. The physico-chemical characterisation of the titanium dioxide series from the JRC repository: NM-100, NM-101, NM-102, NM-103, NM-104 and NM-105 (NM-100 is included in the series as a bulk comparator) is summarised in Table 1. Each material is available as a 2,000 mg white powder kept in amber coloured vials under argon atmosphere. NM-103 and NM-104, have 2% of dimethicone as an external organic coating. The coatings of NM-103 and NM-104 may be unstable under in vitro test conditions. NM-105 is the purest form of the material with others showing trace background elements of C, O, K, Ca and Al.

Table 1. Properties and indicative content of TiO₂ for the JRC titanium dioxide series

NM code	Label name	Properties	Indicative content of TiO₂ (%Wt)
NM100	Titanium Dioxide	Bulk (non nano)	97.7
NM101	Titanium Dioxide	Anatase	98.1
NM102	Titanium Dioxide, Anatase	Anatase	99.6

NM103	Titanium Dioxide thermal, hydrophobic	Rutile	91.3
NM104	Titanium Dioxide thermal, hydrophilic	Rutile	92.7
NM105	Titanium Dioxide, Anatase-Rutile	Anatase-Rutile	99.8

47. Transmission electron microscopy (TEM) micrographs indicate that the TiO₂ NMs, detailed above, have a polydisperse particle size distribution; the average value of the primary particle size was estimated to be below 26 nm for NM-103, NM-104 and NM-105, below 10 nm for NM-102, and above 100 nm for NM-100; for NM-100 primary particle sizes ranging from 20 nm up to 300 nm were detected. The shape of the particles also varied with those for NM-103 and NM-104 given in Table 2.

Table 2. Particle characteristics of selected JRC titanium dioxide materials

	Material Sphericity	Shape Factor	General Morphology
NM-103	Low sphericity	Very angular to sub-angular	Angular, low sphericity
NM-104	Low sphericity	Angular to sub-rounded	Sub-angular, low sphericity

48. It should be noted that the variation of these test material physicochemical characteristics and the nature of their preparation, required for study, should be considered, when attempting potential read-across activities.

Physicochemical Characterisation considerations for this assessment

49. Due to the differences in the characteristics of food grade TiO₂ and engineered nano-TiO₂ this assessment has focussed on the data from studies using food grade TiO₂ or E171. Consideration has been given to studies that used

nano-TiO₂ or food-grade TiO₂ which was de-agglomerated prior to testing. However, as these are likely to provide limited information for the evidence base for food grade TiO₂ or E171 when tested as consumed, these are usually only summarised in the text, but details of the studies can be found in the study summary tables in Annex B.

50. The majority of studies tend to use percentage of NPs by particle number rather than mass.