Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- Physicochemical Characterisation of nano grade TiO2

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Physicochemical Characterisation of nano grade TiO2

41. The consensus within the risk assessment community on the need for standardised and validated dispersion methods to ensure reliable and reproducible results to help enable the advancement of risk assessment research 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 FBS or 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.

42. OECD 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, http://www.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 (http://www.marina-fp7.eu/) and NANoREG (http://www.nanoreg.eu/). The NANOGENOTOX sample preparation protocol developed by CEA, INRS and 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.

43. 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). 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

44. 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 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

45. 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

46. Due to the differences in the characteristics of food grade TiO2 and engineered nano-TiO2 this assessment will focus 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 summarised in the text but details of the studies can be found in the study summary tables in Annex D

47. The majority of studies tend to use percentage of NPs by particle number rather than mass. All of the information provided in a study will be used in the text and included in the study summary table.

Absorption, Distribution, Metabolism and Excretion (ADME)

48. The EU Scientific Committee on Consumer Safety (SCCS) produced an opinion on TiO2 (nano form) coated with Cetyl Phosphate, Manganese Dioxide or Triethoxycaprylylsilane as UV filters in dermally applied cosmetics, confirming the safe use in cosmetics for products intended for application on skin (SCCS, 2017). Dermal absorption is not considered any further in this statement.

49. The COT reviewed a number of studies to assess the ADME of TiO2, which were also reviewed by EFSA, Health Canada and FSANZ. These studies are described below and a brief overview of the review and conclusions of EFSA, Health Canada, and FSANZ are included. The COT review and conclusions are presented at the end of each section.