PQRI Workshop:
TiO2 Use in Pharmaceuticals
Global Regulatory and Technical Challenges
June 13-14, 2023

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Critical considerations about the basis for EFSA's Opinion on TiO_2 Safety in Foods

Dr. David Lockley, Chair of TDMA Scientific Committee

Product Quality Research Institute (PQRI) Conference

13 June 2023

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The Titanium Dioxide Manufacturers Association (TDMA)

Cinkarna
Celje, Slovenia

Kronos
Langerbrugge, Belgium
Leverkusen, Germany (2 plants)
Nordernham, Germany
Fredrikstad, Norway

LB Group

Precheza
Prerov, Czech Republic

Tayca

The Chemours Company

Tronox LLC
Thann, France
Stallingborough, UK
Botlek, Holland

Venator
Uerdingen, Germany
Duisburg, Germany
Scarlino, Italy
Huelva, Spain
Greatham, UK

TiO_2 as a food additive

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- Legally separate from the carcinogenicity classification
- France initiated a ban on E 171 in food in advance of the opinion
- May 2021 – EFSA Opinion
  - Concern for genotoxicity could not be ruled out
  - TiO_2 / E171 no longer be considered as safe when used as a food additive
  - Did not say E 171 was unsafe
- Reversal of 4 previous opinions from 2016 that TiO_2 safe
- January 2022 - European Commission withdrew the approval for the use of E171 in food and animal feed
- Has many potential impacts beyond the European Union and food

efsa
European Food Safety Authority

Why could the concern for genotoxicity not be ruled out?

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- E171 and TiO_2 nanomaterials may accumulate
- Long elimination half-lives were estimated
- TiO_2 nanomaterials tested positive in MN and Comet assays
- Genotoxicity may occur through proposed but unproven mechanisms
  - Generation of reactive oxygen species (ROS)
  - Induction of chronic inflammatory response
  - Direct non-covalent binding to DNA
  - Binding to centromeres or other structures of cellular division
  - Data are insufficient to define threshold exposures below which genotoxicity will not occur

Concerns with EFSA's opinion of May 2021

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The safety status of E171 did not change – the scientific approach did

TDMA identified several issues with the opinion:

1. Test materials and methodology relied on not representative of TiO_2 in food
2. Exclusion of key components of the science dataset for E171 that show no adverse impacts
3. Inconsistent application of new EFSA nano guidance

Test materials not representative

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- E171 is a pigmentary material
  - Not a nanomaterial or nanoform
- Very few test materials used in the assessment met the specification criteria for E171
  - Manufacturing, particle size distribution …
- EFSA (2021) did not acknowledge factors previously noted by EFSA
  - Perform tests with representative in agricultural/food/feed chain and on the market and in compliance with specifications
  - Define physicochemical form of TiO_2 manufactured for foods, present in food matrices, used in toxicity tests, present in tissues (2016)
  - TiO_2 nanoparticle suspensions sonicated to minimize agglomeration may not occur in realistic environments (2018)

Test materials not representative II

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- EFSA (2021) did not justify grouping nanomaterials with E171
  - Used new nanoscale considerations (NSC) scoring scheme
  - Highest scores assigned to studies with TiO_2 nanomaterials prepared by dispersion / stabilization protocols developed for characterizing nanomaterials

- Conclusion
  - TiO_2 forms subjected to genotoxicity testing on which EFSA (2021) relied are not representative of E171 used in foods and present in the marketplace

Negligible absorption & accumulation

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- Rats
  - Systemic absorption of single or repeated oral doses of TiO_2 is negligible
  - At doses much greater than relevant human exposures
  - When extraordinary procedures are used to disperse and stabilize particles in the aqueous suspensions administered

- Human volunteers
  - No evidence of significant absorption, regardless of particle size distribution
  - Single doses just exceeded upper limits of intestinal exposures from diet in gelatin capsules

Negligible absorption & accumulation II

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- Human cadavers
  - Low systemic bioavailability under normal life conditions
  - Reflecting steady state levels (given advanced ages of subjects)
  - No pro-inflammatory or other adverse effects observed in “pigment cells” of Peyer’s patches
  - Cell monolayers in vitro: No penetration of epithelial lining of the GI tract
- Conclusion
  - No evidence that long-term exposure can lead to significant absorption or accumulation
  - Therefore, no systemic genotoxicity

Toxicokinetics – Oral bioavailability

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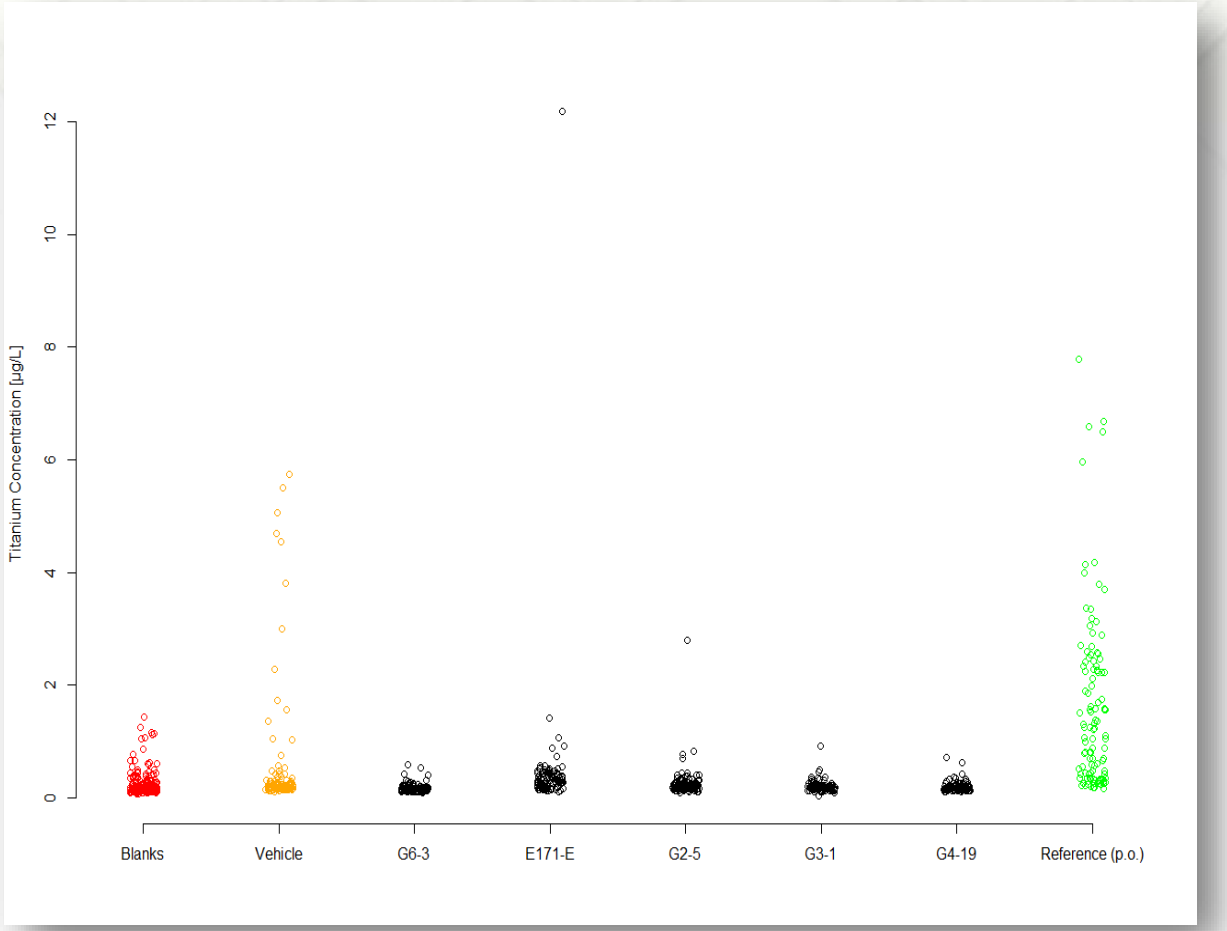
- Single oral gavage (1000 mg/Kg bw) of rats with 5 TiO_2 grades including E171
- Blood samples taken at regular intervals between t = 0 and 96 hours
- Blood levels of Ti remain in range of vehicle control/method blanks throughout, consistent with previous robust reports of very low systemic absorption of E171 from oral exposure
- Therefore, blood levels < 0.00075% of oral dose
- Work continues to find out just how low



Exposure methods not representative

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- In vivo genotoxicity tests of TiO_2 nanomaterials
  - Aqueous suspensions in water or solutions with dispersion stabilizers
  - High bolus doses administered by gavage

- Contrasts with more realistic human exposures to E171
  - Incremental, relatively low exposures, in foods consumed throughout the day
  - Interactions of TiO_2 particles with constituents, inhibiting absorption
    - Matrices of foods and beverages during manufacturing, delivery, storage
    - Chyme in the stomach and intestines
    - Physiological conditions and mixing in mouth and GI tract

Exposure methods not representative (II)

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- Digestive-system simulations demonstrate substantial agglomeration
- Rat study in vivo demonstrates lack of toxicity of E171 in diet at concentrations orders of magnitude greater than human exposures
- EFSA (2021) did not follow guidance for assessing nanomaterials:

> *"In specific cases, and especially when exposure occurs mainly through solid and liquid foods, additional groups with food or drinking water administration have to be included to determine whether hazards associated with the nanomaterial are observed under realistic exposure scenarios."*

- Conclusion
  - Oral exposure methods in studies EFSA (2021) relied on do not provide acceptable representation of the intended uses of E171

Changes in agglomerate size in simulated gastric passage (in vitro)

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- Agglomeration status of different TiO_2 grades tested in vitro
  (A) nano-Anatase (NM-1)
  (B) fumed TiO_2 anatase/rutile (NM-2)
  (C) food-grade Anatase

- Particle size after dispersion in 4 surrogate media:
  - Water
  - Saliva
  - gastric juice
  - duodenal fluid

- Small changes in size distribution in saliva
- Dramatic increase of hydrodynamic size in simulated gastric and duodenal fluid
- Presence of agglomerates/particles from 1 to 300 µm in gastric and duodenal fluids

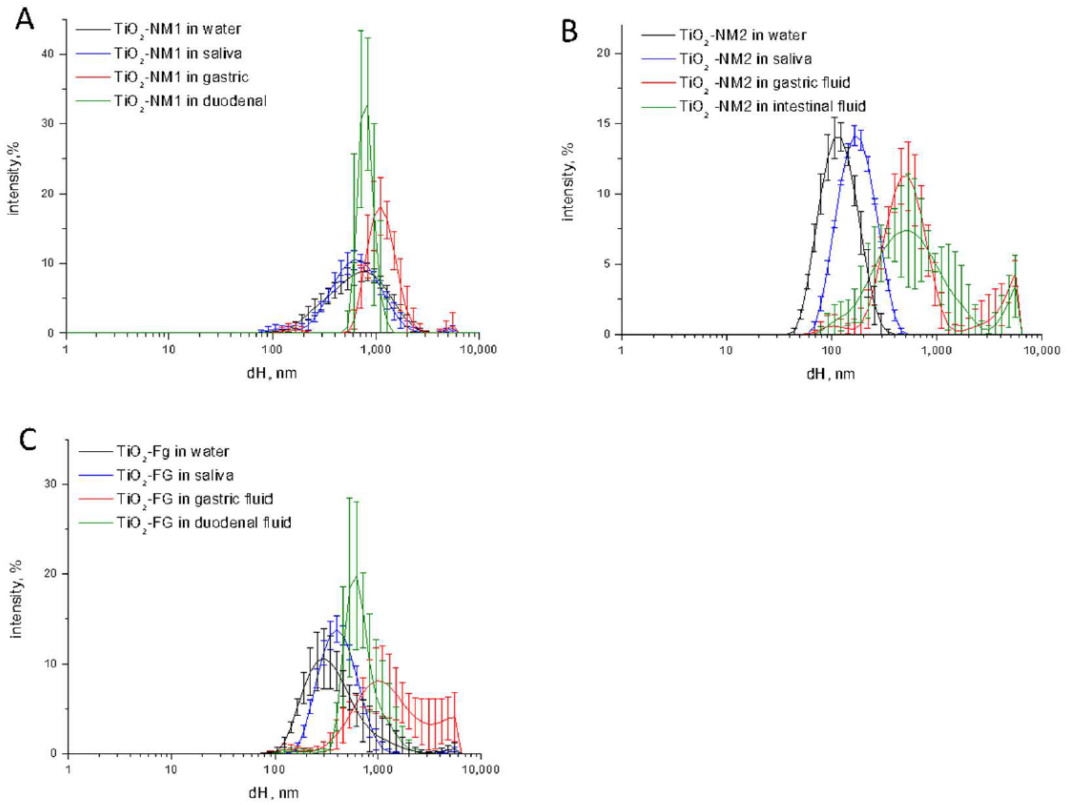

Figure 2. Size distribution monitored by DLS of the TiO_2 samples during the digestion cascade. (**A**) TiO_2-NM1; (**B**) TiO_2-NM2; (**C**) TiO_2-FG. Hydrodynamic diameters (d_H) distribution (% intensity) is expressed as mean value of 5 measurements in three independent experiments ±SD.

Marucco et al. (2020). Biotransformation of Food-Grade and Nanometric TiO_2 in the Oral–Gastro–Intestinal Tract: Driving Forces and Effect on the Toxicity toward Intestinal Epithelial Cells. *Nanomaterials*, *10*(11), 2132.

Studies dismissed or not considered

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- Chronic oral bioassays of pigmentary TiO_2 at doses orders of magnitude greater than relevant human exposures were negative
  - Lehman and Herget (1927) study of technical grade TiO_2 in Guinea pigs, rabbits and cats
  - NTP (1979) bioassay of pigmentary TiO_2 in rats and mice

- Pigmentary TiO_2 was negative for chromosome damage and aberrations in reliable in vivo tests
  - Shelby et al. (1993) MN induction tests in mice by daily i.p. injections
  - Shelby and Whitt (1995) MN test in mice by single i.p. injection
  - Bettini et al. (2017) Comet assay of E171 and nanomaterial in rats by daily gavage
  - Jensen et al. (2019) Comet assay of rats by weekly gavage

Validity of 1979 National Cancer Institute (NCI) Bioassay of TiO_2 for possible carcinogenicity

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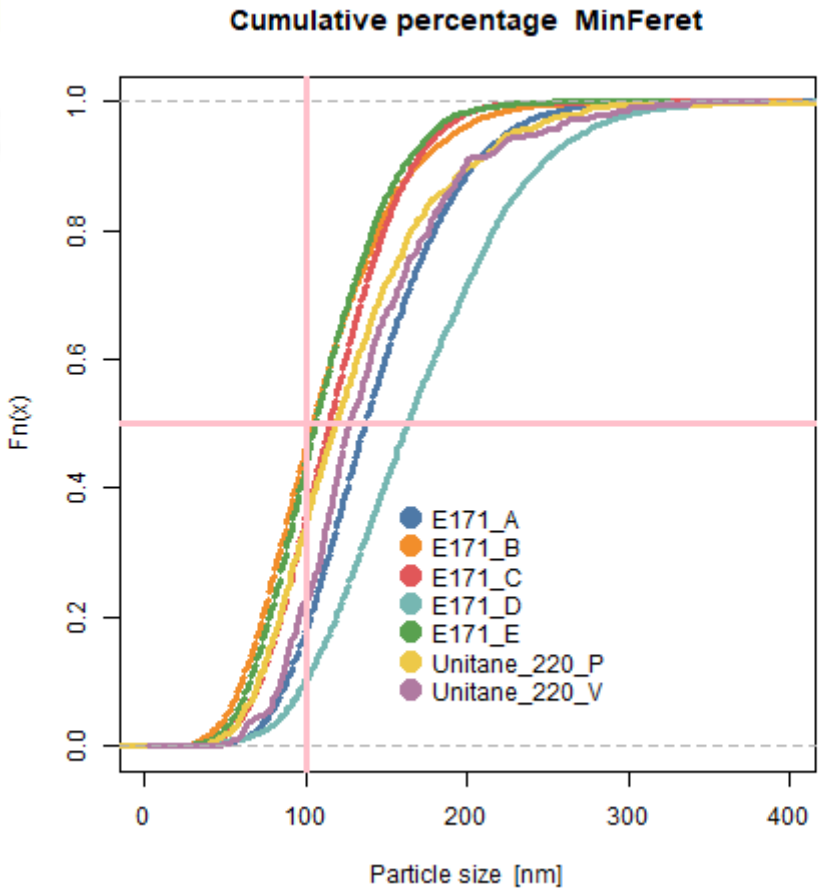
- Extensive NCI study performed in mice and rats with Unitane 0-220 TiO_2 concluded no cancer risk
- Highly relevant study, but Unitane 0-220 PSD not characterized
- TDMA analyzed PSD of 2 samples of Unitane grade
- PSD consistent to current E171 available in market i.e., uncoated, pigmentary, anatase
- Validating the relevance of this study



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Mechanisms of genotoxicity unlikely

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- Most plausible mechanisms of potential E171 genotoxicity have thresholds
  - Generation of reactive oxygen species (ROS)
  - Induction of chronic inflammation
- However, no evidence indicating
  - GI absorption or tissue accumulation sufficient to trigger these mechanisms after long-term exposures
  - ROS-induced stress responses in cells or inflammatory responses in tissues that appear to contain TiO_2 particles
- Proposed mechanisms involving the binding of TiO_2 particles to DNA or other elements of cell division
  - Are hypothetical at best and have no credible supportive evidence

Studies dismissed or not considered Conclusions

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- EFSA (2021) gave undue weight to results of in vitro genotoxicity studies that do not represent dietary intake of E171

- EFSA (2021) did not give appropriate weight to results of compelling in vivo genotoxicity studies

Inconsistent application of new EFSA nano guidance

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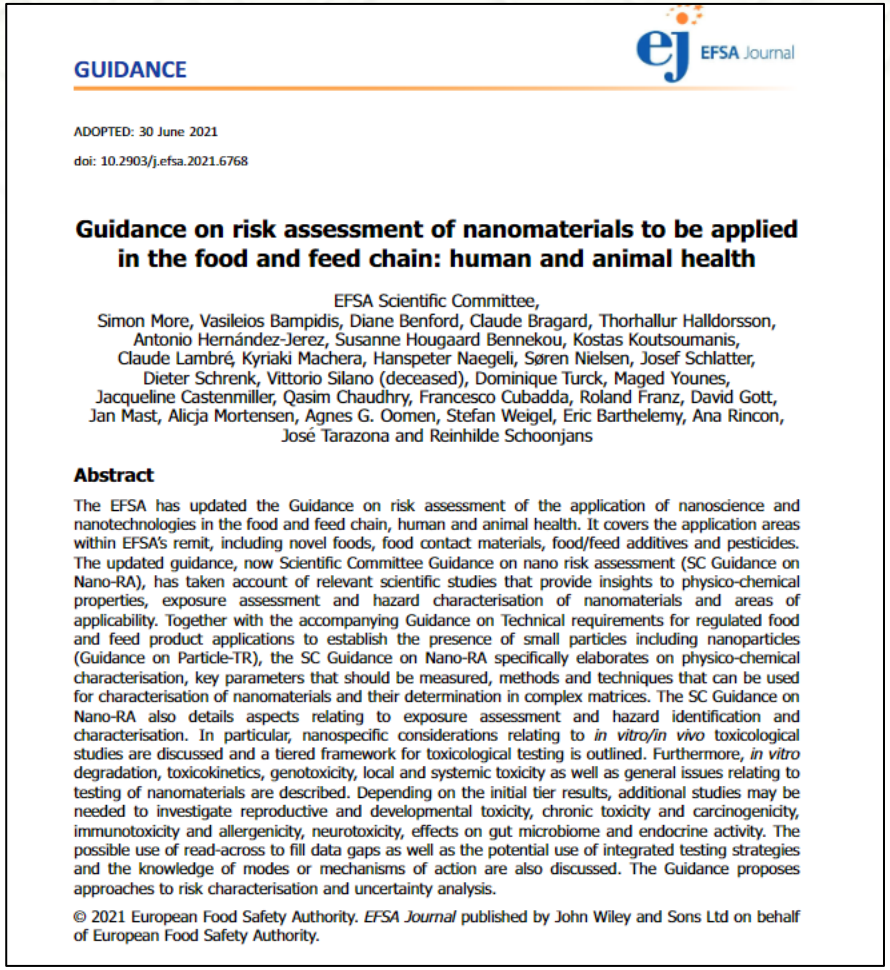
- E171 is a very specific form of TiO_2
  - Untreated/non-surface treated pigmentary non-nano grade with strict purity requirements
  - Does not meet the EU recommendation of a nanomaterial

- EFSA nano guidance is clearly related to different forms



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,
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Abstract

The EFSA has updated the Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain, human and animal health. It covers the application areas within EFSA's remit, including novel foods, food contact materials, food/feed additives and pesticides. The updated guidance, now Scientific Committee Guidance on nano risk assessment (SC Guidance on Nano-RA), has taken account of relevant scientific studies that provide insights to physico-chemical properties, exposure assessment and hazard characterisation of nanomaterials and areas of applicability. Together with the accompanying Guidance on Technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (Guidance on Particle-TR), the SC Guidance on Nano-RA specifically elaborates on physico-chemical characterisation, key parameters that should be measured, methods and techniques that can be used for characterisation of nanomaterials and their determination in complex matrices. The SC Guidance on Nano-RA also details aspects relating to exposure assessment and hazard identification and characterisation. In particular, nanospecific considerations relating to *in vitro/in vivo* toxicological studies are discussed and a tiered framework for toxicological testing is outlined. Furthermore, *in vitro* degradation, toxicokinetics, genotoxicity, local and systemic toxicity as well as general issues relating to testing of nanomaterials are described. Depending on the initial tier results, additional studies may be needed to investigate reproductive and developmental toxicity, chronic toxicity and carcinogenicity, immunotoxicity and allergenicity, neurotoxicity, effects on gut microbiome and endocrine activity. The possible use of read-across to fill data gaps as well as the potential use of integrated testing strategies and the knowledge of modes or mechanisms of action are also discussed. The Guidance proposes approaches to risk characterisation and uncertainty analysis.

EFSA nano guidance was applied to E171 in unusual circumstances

Application of the new EFSA nano guidance

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Read-across

- Read-across is foreseen in the EFSA guidance (page 65)
- EFSA applied a read-across approach to consider other forms of TiO_2
  - Often special industrial catalyst type nano grades not used in food used in the evaluation for genotoxicity

Nanoscale considerations (NSC)

- EFSA developed a comprehensive scoring for NSC in its 2021 opinion (Annex E)
- NSC are not specifically included in the EFSA Guidance and are inconsistent
  - Particularly as there is nothing E171 specific in the NSC
- EFSA focused on sonication – yet difficult to justify as no sonication occurs in the manufacture of food and TiO_2 agglomerated in the gut

Inconsistencies in the application of EFSA nano guidance to E171

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Conclusions

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- EFSA (2021) based evaluation of the genotoxicity endpoint
  - On test materials that are not representative of the food additive E171
  - Using oral exposure methods that are not representative of human exposures
  - Without considering critical differences in manufacturing processes, composition and other properties of nanomaterials compared with E171
  - Dismissing or not considering the most relevant studies, which demonstrate the lack of potential genotoxicity of E171 used as intended in food
- Therefore, the EFSA (2021) conclusion that “E171 can no longer be considered safe when used as a food additive”
  - Is not supported by the data reviewed by EFSA (2016, 2018, 2021)
  - Is mistaken and unwarranted

EFSA's position is not shared by Global Regulatory Authorities

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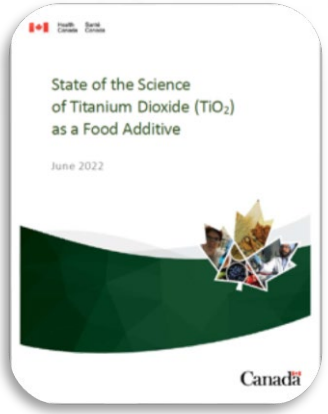
Several global regulatory authorities have carried out a critical review of the 2021 EFSA opinion on E171 considering available science relevant to food uses of TiO_2, incl. data generated after the EFSA opinion



Health Canada, June 2022

FOOD STANDARDS
Australia New Zealand

Food Standards Australia New Zealand, Sept 2022

Food Standards Agency

UK Food Standards Agency, July 2021

More details regarding the position of global regulators will be provided later by Dave Schoneker

JECFA

- The Joint FAO-WHO Expert Committee Report on Food Additives (JECFA) will review TiO_2
- Global food additive regulation coordinator
- November 2022 – Issued a call for data for TiO_2
- February 2023 – TDMA submitted a comprehensive dossier to JECFA (>200 pages)
- TDMA widely shared dossier with authorities
- JECFA debate TiO_2 in Q3 2023, opinion expected in 2024
- Will be key for food additives and important in other sectors such as food contact materials, cosmetics, toys and drugs

New study from Japan

- National Institute of Health Sciences, Japan
- Evaluating a nano TiO_2 in a 90-day rat study by gavage (6nm anatase)
- No adverse effects
- Japanese Ministry to start formal review of E171 in July 2023

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EU Cosmetics, toys, food contact and medicines

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Cosmetics

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- TiO_2 is widely used in cosmetics
  - Colorant in foundations, eye shadows and toothpaste
  - UV filter in suncreen
- July 2022 - European Commission issued a mandate to the Scientific Committee on Consumer Safety (SCCS) to reassess TiO_2 in cosmetics
- Result of the EFSA Opinion from 2021
- SCCS focus
  - Genotoxicity
  - Exposure via the inhalation and oral route
  - Lip care, lipstick, toothpaste, loose powder, hair spray

Cosmetics

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- Dermal/suncreens were excluded from the focus
  - Skin penetration of TiO_2 has been shown to be negligible
- Cosmetics Europe (CE) TiO_2 Consortium and TDMA have submitted extensive data to the SCCS by the deadline – 30 April 2023
- Included two new in vitro genotox studies on TiO_2 grades used in cosmetics
  - Both negative
  - Included a full evaluation of uptake
- Covers the uncertainties identified by EFSA
- Current deadline for SCCS – End June 2023

TiO_2 in toys

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- Currently being reviewed following the classification
  - Scientific Committee on Health, Environmental and Emerging Risks (SCHEER)
- Preliminary opinion was issued in May 2022
- Main concerned areas
  - Casting kit, chalk, powder paint, white colouring pencil
  - Finger paints, lip gloss/lipstick
- TDMA has provided detailed information on the safety to the SCHEER
  - Information from the legal case
  - Global developments

TiO_2 and food contact

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- TiO_2 is widely used in food contacts materials
  - Plastics, paper and ceramics
- In 2021 - European Commission (EC) took the position that there was no reason to investigate TiO_2 in food contact
- June 2022 - EC proposed to add a new migration limit
- EC stopped progressing this after judgment for legal case on classification

Medicines

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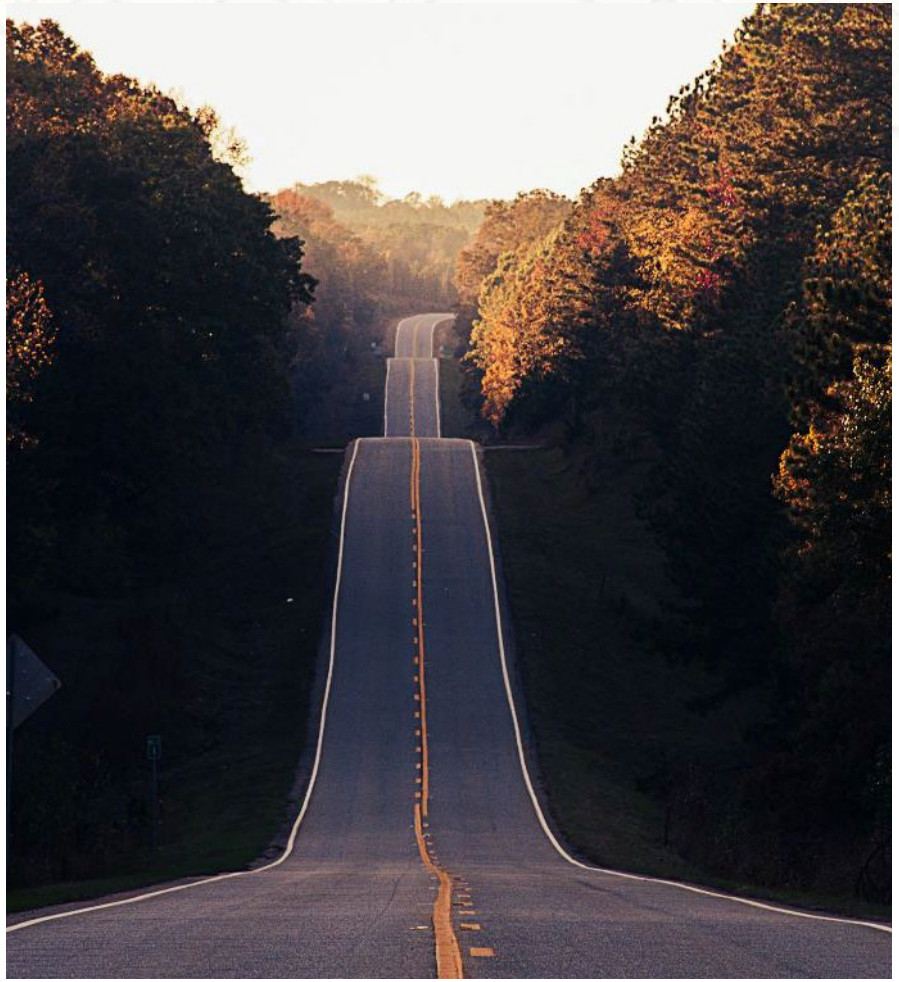
- Medicines safety package is extensive including long history of safe patient use
- EMA request a technical and safety progress report from pharmaceutical industry / stakeholders in replacing TiO_2 by November 2023 for review by 10 March 2025
- TDMA have submitted updates to the EMA about scientific developments
- TDMA aim to have made material progress in genotoxicity studies to support an EMA review supportive of continued use of TiO_2
- Combined with positive outcomes from other regions

Conclusions

- Growing consensus among key international regulatory authorities about the safety of E171
- TDMA making progress in addressing the novel approach taken in the EFSA opinion
- TDMA will keep engaging with relevant stakeholders to address concerns and ensure relevant science on E171 safety is considered



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Thanks for your attention!

Contact: tdma@cefic.be

More information on tdma.info

Questions

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