Updates on the genotoxicity of TiO_2

Part 1: Data gaps and new data

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A weight of evidence review of the genotoxicity of titanium dioxide (TiO_2)

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ABSTRACT

Titanium dioxide is a ubiquitous white material found in a diverse range of products from foods to sunscreens, as a pigment and thickener, amongst other uses. Titanium dioxide has been considered no longer safe for use in foods (nano and microparticles of E171) by the European Food Safety Authority (EFSA) due to concerns over genotoxicity. There are however, conflicting opinions regarding the safety of Titanium dioxide. In an attempt to clarify the situation, a comprehensive weight of evidence (WoE) assessment of the genotoxicity of titanium di-

Results of Expert Panel review

- Structured weight of evidence (WoE) review of 192 datasets from relevant test systems and endpoints.
- Applied a “cut-off” based on robustness of study design and quality of data (i.e. datasets achieving overall weights of “Moderate” or higher) led to only 34 datasets that made the “cut”.

Study type	**No. of datasets reviewed**	**No. achieving moderate or higher weight after WoE assessment**
In vitro		
Bacterial reverse mutation (Ames test)	15	0
Mammalian cell gene mutation	16	2
MN or CA	62	12
In vivo		
Gene mutation	9	2
MN or CA	35	13
Comet	51	3
8-OHdG adducts	4	2
Totals	**192**	**34**

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Fig 1: Profile of results for *in vitro* studies

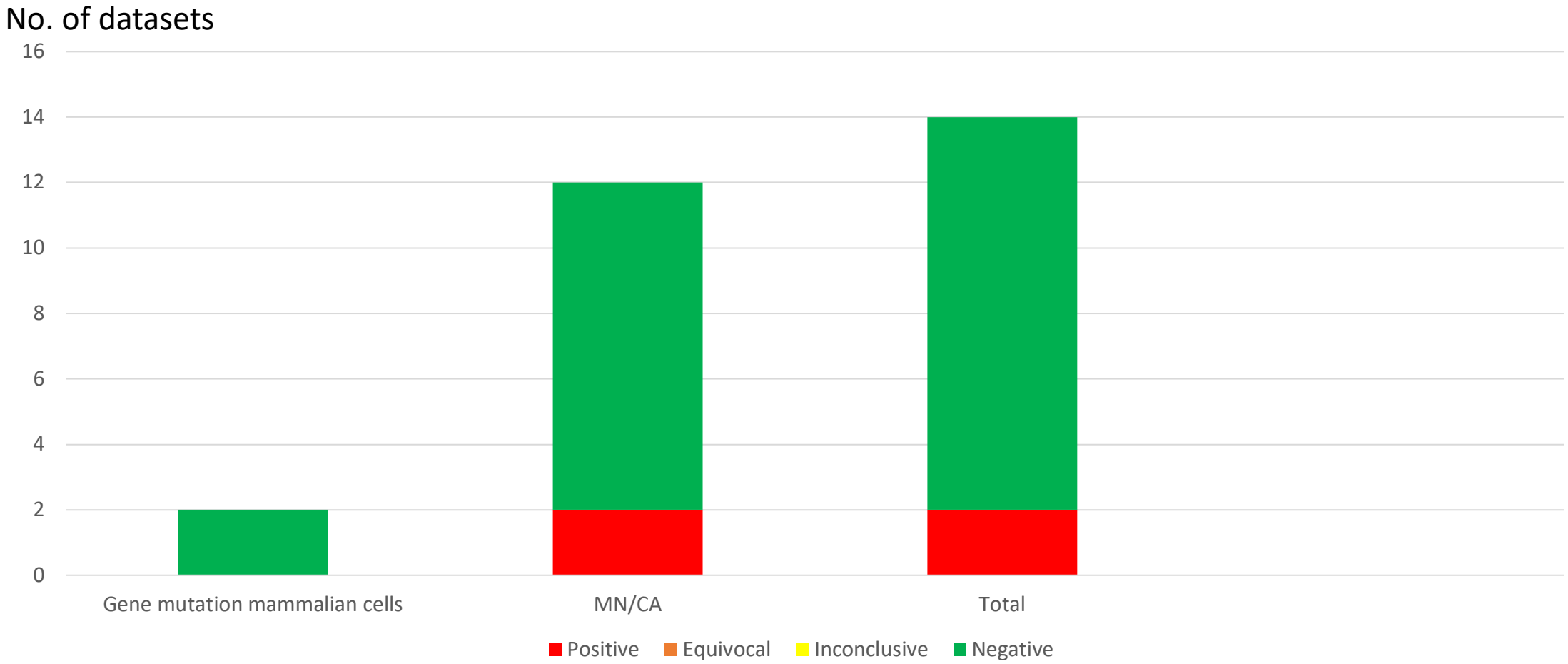
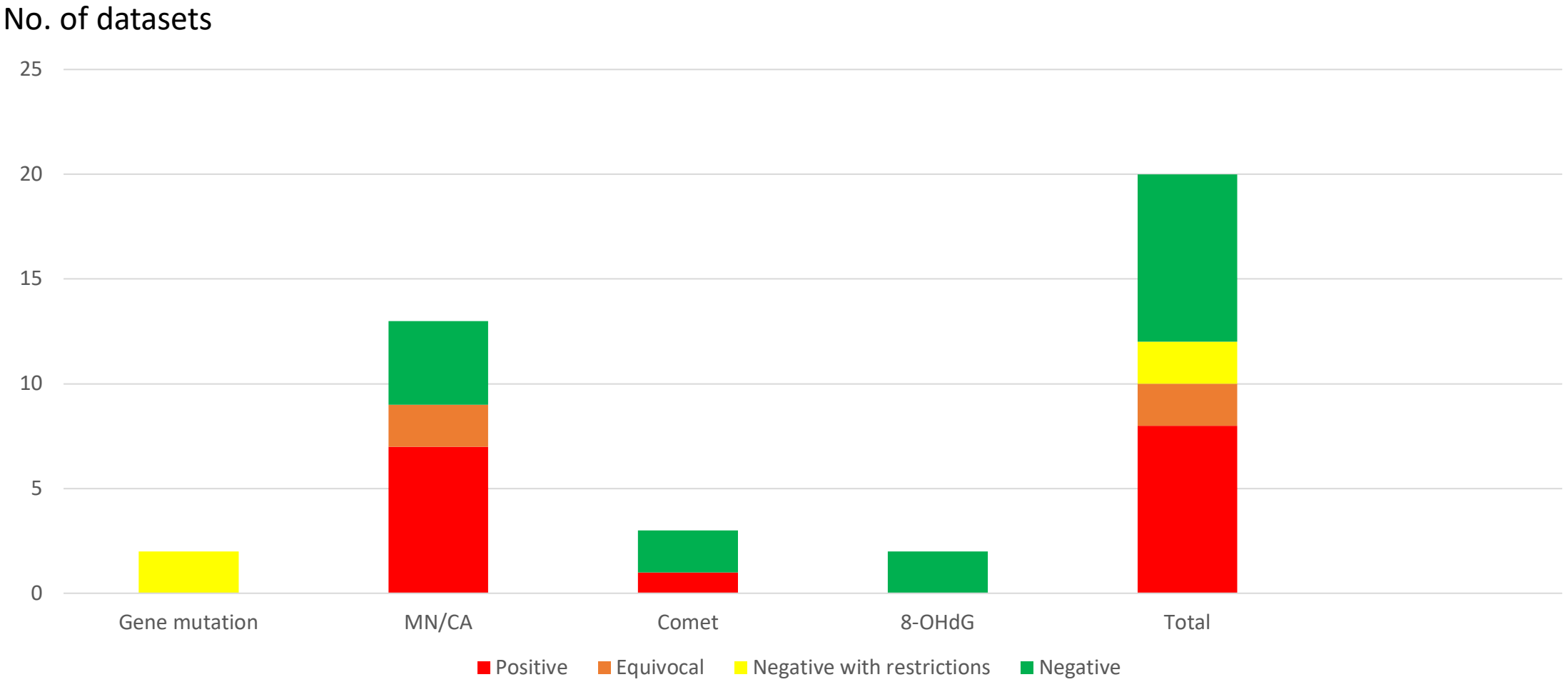


Fig 2: Profile of results for *in vivo* studies



Comments on results

- No evidence of induction of gene mutations *in vitro*, although only 2 mammalian cell gene mutation studies achieved a final weight of “moderate”.
- Most *in vitro* tests for MN and CA were negative. Only 2 *in vitro* MN studies were positive or weakly positive
  - The concentrations at which these positive effects were seen induced oxidative damage, apoptosis and necrosis, although these changes were also seen in negative studies (secondary effects?)
- No evidence of induction of gene mutations *in vivo* from 2 TGR studies, although neither study fully complied with OECD guideline recommendations.
  - No *in vivo* Pig-a mutation studies met current best practices recommendations.
- Of the 13 *in vivo* MN/CA studies, 7 were considered positive
  - 2 of these scored Klimisch 3 in the ToxR tool and are therefore considered unreliable
- 5 of the 7 positive MN/CA studies used oral gavage or drinking water administration yet absorption via the oral route is very low (only 0.0006% of a single 1000 mg/kg oral dose of E171-E was found in the total blood compartment).
  - Bone marrow exposure would be negligible, and therefore the plausibility of these positive MN/CA results using oral dosing is questionable.
- By contrast, 3 of the 4 negative studies used IV dosing where exposure of the bone marrow would be assured.

Discussion

- In many published studies, endpoint evaluated is not relevant, study designs and/or the data are not reliable, or the results are questionable and too poor to support a robust assessment.
- Of the 34 relevant datasets, only 10 (29.4%) were positive. All (*in vitro* and *in vivo*) were from DNA or chromosomal damage studies, and it is accepted within many regulatory guidelines that such damage can be secondary to physiological stress.
- All positive findings were associated with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis, or combinations of these, so highly likely that the observed genotoxic effects of TiO_2, including those with nanoparticles, are secondary to physiological stress.
- There were no positive results from gene mutation studies, which is consistent with DNA/chromosomal damage being secondary to physiological stress, but very few robust studies.
- **Thus, the conclusions from the 34 robust datasets reviewed here, that achieved "moderate" or higher weight, did not support a direct DNA-damaging mechanism for TiO_2.**
- **However, carefully designed studies of apical endpoints (gene mutation, MN or CA), following OECD recommended methods, performed with well characterised preparations of TiO_2, would allow firmer conclusions to be reached.**

In vivo lung comet study

- In addition to the studies we would like to do (to enrich the database with quality data – will be presented by Carol Beevers and John Wills), a study was mandated by ECHA (REACH regulation)
- Initial phase uses intratracheal instillation (2 doses, 24 hrs apart) of 13 different grades of TiO_2 (representing ~600 different forms in use worldwide)
  - Includes E171 and P25
  - Most biologically active grades will then be tested by inhalation
- Sampling 2-6 hrs, 24 hrs & 28 days
- Top dose should induce some inflammation but not overload
  - Will be based on doses set for P25 which induces highest and most prolonged inflammation.
  - Additional measures for tissue toxicity, oxidative stress etc. will be included
- Will be discussed further by Carol Beevers

In vitro studies on cosmetic grades

- 2 grades, one with an inorganic coating (RM09) and one with an organic coating (RM11) were tested for induction of MN and *Hprt* mutations in V79 cells
- RM09 was only tested in the absence of S9
- RM11 was tested in the absence and presence of S9 in case the organic coating could be genotoxic
- Nano characterisation was performed by DLS
- The maximum concentration was 100 µg/mL as recommended by OECD
- All studies included treatments for 24 hrs to allow cellular uptake, which was confirmed using TEM
- For the MN assays 2000 binucleate cells/concentration scored
- For the *Hprt* assays 2-2.5 x 10^6 cells per concentration plated in 6-TG containing medium

Treatment schedules

- Both test articles dispersed using the recommendations of the Nanogenotox protocol.
  - Solvent was 0.05% w/v BSA water solution containing 0.5% ethanol
- For the MN assay with RM09:
  - 24 hrs –S9 followed by 20 hrs recovery in the presence of cytochalasin B
- For the MN assay with RM11:
  - 4 hrs – or +S9 followed by 20 hrs recovery in the presence of cytochalasin B
  - 24 hrs –S9 followed by 20 hrs recovery in the presence of cytochalasin B
- For the *Hprt* assay with RM09:
  - 24 hrs –S9 followed by 7 days expression time before plating in 6-TG medium
- For the *Hprt* assay with RM11:
  - 4 hrs – or +S9, or 24 hrs –S9, followed by 7 days expression time before plating in 6-TG medium

MNvit results for RM09

Treatment (µg/mL)	24 + 20 hrs -S9	
	% MN in binucleate cells	% reduction in CBPI
Deionised water	0.65	3.2
Solvent control*	0.85	-
1.1	0.50	0
3.5	0.55	0
10.7[P]	0.50	0
18.7[P]	0.75	0
57.1[P]	0.35	0
100[P]	0.30	0
Positive control**	**9.20**	0
Positive control***	**3.75**	5.1

* 0.05% w/v bovine serum albumin (BSA)-water solution containing 0.5% ethanol 10.0 % (v/v)

[P] = precipitation at end of treatment

** MMC

***Griseofulvin

Hprt results for RM09

Treatment (µg/mL)	24 hrs -S9 (Expt. 1)		24 hrs -S9 (Expt. 2)	
	Mutant frequency/10^6 cells	% relative adjusted cloning efficiency	Mutant frequency/10^6 cells	% relative adjusted cloning efficiency
Deionised water	10.6	100.0	14.6	100.0
Solvent control*	8.5	90.9	9.6	89.6
0.8	14.3	106.0	23.5	98.9
1.6	17.0	103.2	12.9	83.0
3.1	13.6	98.6	8.5	104.4
6.3	**20.6^{PS}**	96.5	11.7	101.9
12.5	12.7^{P}	82.2	11.5^{P}	99.0
25.0	**19.8^{PS}**	87.5	5.9^{P}	91.9
50.0	12.7^{P}	74.3	12.1^{P}	84.7
100	**26.5^{PS}**	73.9	10.4^{P}	108.7
Positive control**	**566.3**	80.1	**737.9**	74.7

* 0.05% w/v bovine serum albumin (BSA)-water solution containing 0.5% ethanol 10.0 % (v/v)

^{P} = precipitation at end of treatment

** 214 µg/mL EMS

^{S} = significant trend

MNvit results for RM11

Treatment (µg/mL)	4 + 20 hrs -S9		4 + 20 hrs +S9		24 + 20 hrs -S9	
	% MN in binucleate cells	% reduction in CBPI	% MN in binucleate cells	% reduction in CBPI	% MN in binucleate cells	% reduction in CBPI
Deionised water	1.60	0	1.05	0	0.45	0
Solvent control*	1.55	-	1.05	-	0.85	-
0.6	0.70	0	0.55	0.1	0.45	0
2.0[P]	0.75	0	0.90	0	0.40	0
6.1[P]	0.90	0	0.50	0	0.30	1.4
18.7[P]	0.95	0	0.70	0	0.25	0
57.1[P]	0.60	0	0.50	0	0.30	0
100.0[P]	0.45	0	0.65	0	0.65	0.8
Positive control**	**12.45**	18.7	**4.75**	11.3	**4.70**	6.4

* 0.05% w/v bovine serum albumin (BSA)-water solution containing 0.5% ethanol 10.0 % (v/v)

[P] = precipitation at end of treatment

** 0.3 µg/mL MMC 4 hrs -S9; 2.0 µg/mL CPA 4 hrs +S9; 7.0 µg/mL griseofulvin 24 hrs -S9

Hprt results for RM11

Treatment (µg/mL)	4 hrs -S9		4 hrs +S9		24 hrs -S9	
	Mutant frequency/10^6 cells	% relative adjusted cloning efficiency	Mutant frequency/10^6 cells	% relative adjusted cloning efficiency	Mutant frequency/10^6 cells	% relative adjusted cloning efficiency
Deionised water	14.4	100.0	14.9	100.0	10.1	100.0
Solvent control*	24.5	97.4	9.6	104.0	14.6	108.1
0.8	10.4	95.3	11.3	88.7	16.4	118.2
1.6	11.8	97.2	16.2	101.4	16.5	93.6
3.1	ND	-	ND	-	16.3	102.1
6.3^P	10.6	93.1	9.8	87.6	19.1	75.9
12.5^P	ND	-	ND	-	19.7	109.2
25.0^P	11.1	92.1	10.9	89.9	15.4	99.8
50.0^P	ND	-	ND	-	13.5	91.8
100^P	12.0	95.8	16.3	81.3	11.7	78.7
Positive control**	**130.6**	97.7	**58.0**	79.2	**269.8**	65.2

* 0.05% w/v bovine serum albumin (BSA)-water solution containing 0.5% ethanol 10.0 % (v/v)

^{P} = precipitation at end of treatment

** 300 µg/mL EMS 4 hrs -S9; 2.3 µg/mL DMBA 4 hrs +S9; 214 µg/mL EMS 24 hrs -S9

ND = cultures not continued since data from only 4 concentrations required

Updates on the genotoxicity of TiO_2

Part 2: Regulatory opinions, industrial approaches to alternatives to TiO_2, and new initiatives

Dr David Kirkland

Kirkland Consulting, Tadcaster, UK

Regulatory opinions

- **EFSA (2021)** – “a concern for genotoxicity could not be ruled out, and given the many uncertainties, the Panel concluded that E 171 can no longer be considered as safe when used as a food additive”.
- **Health Canada (2022)** - The Food Directorate of Health Canada “there is no immediate concern for the genotoxicity of the current form of TiO_2 added to food. However, due to the limited number of available studies with food-grade TiO_2 or test articles comparable to food-grade TiO_2, additional research is recommended, particularly well-conducted studies that adhere to modern OECD test guidelines for genotoxicity”.
- **Food Standards Australia New Zealand (FSANZ, 2022)** – “In vivo genotoxicity studies using dietary administration of food-grade TiO_2 are currently lacking. However, there is no evidence that food-grade TiO_2 administered by other modes (oral gavage, intraperitoneal injection) is genotoxic *in vivo*. In addition, no evidence of genotoxicity was found in *in vitro* studies with food-grade TiO_2. Additional GLP- and test guideline-compliant *in vivo* genotoxicity (e.g. mutagenicity and micronucleus) studies with food-grade TiO_2 would be valuable to confirm this conclusion.”

Regulatory opinions (unconfirmed)

- **UK Food Standards Agency (FSA)** - Committees on Mutagenicity and Toxicity draft conclusions are that the WoE does not support the conclusions drawn by EFSA, but have decided to launch their own review of the safety of titanium dioxide as a food additive.
- **US FDA (updated March 2023)** - Some of the genotoxicity tests included test materials not representative of the color additive, and some tests included administration routes not relevant to human dietary exposure. The available safety studies do not demonstrate safety concerns connected to the use of titanium dioxide as a color additive. The FDA continues to allow for the safe use of titanium dioxide as a color additive in foods generally according to the specifications and conditions, including that the quantity of titanium dioxide does not exceed 1% by weight of the food, found in FDA regulations at 21 CFR 73.575.
  - Also see Food Navigator, 12 Dec 2022

TIO_2 E171 IS A UBIQUITOUS EXCIPIENT IN MEDICINES GLOBALLY

- E171 (anatase) is used in medicinal products as an opacifier, in coatings, providing light protection to many active ingredients and formulations and as a white colourant to ensure smooth uniform appearance
  - Based on data from EMA, 66% of the »360,000 available ingested oral medicines in Europe contain titanium dioxide
    - https://www.ema.europa.eu/en/documents/other/annex-i-use-titanium-dioxide-excipient-human-medicines-industry-feedback-qwp-experts/ema-questions_en.pdf

- For more than 50 years titanium dioxide (TiO_2) has played a key role in the safety, quality, efficacy and compliance for the majority of medicines in Europe.

- TiO_2 meets the most stringent requirements governing the safety and quality of medicines, including those set by the European, Japanese and US pharmacopoeias.

- In the EU there is a legislative link between food additives and colourants in medicines. Under the Directive on Colouring Matters in Medicinal Products, pharmaceuticals must abide by the rules on colouring matters in the Regulation on food additives and the Regulation on Specifications for Additives for laying down the specific purity criteria.

PHARMACEUTICAL INDUSTRY DO NOT SUPPORT THE EFSA's CONCLUSIONS

- There is no evidence that TiO_2 E171 has mutagenic potential *in vitro/in vivo*
- Genotoxic effects observed: primary DNA damage (stand breaks) and chromosomal damage
- Several mode of actions inducing primary DNA lesion may exist, including
    - Formation of reactive (oxygen) species (induced directly, via inflammation or mitochondrial dysfunction)
    - Direct DNA interaction of TiO_2 but no proof for covalent binding of TiO_2 to DNA
    - However, these effects seem not to result in gene mutations
- Occurrence of primary DNA damage and clastogenicity in absence of mutation induction is not novel and has been identified for situations where primary DNA damage is efficiently repaired and does not result in tumour induction
- Carcinogenicity data considered in previous assessments were not considered in the recent EFSA assessment, but these are essential for informing the biological significance of *in vitro* and *in vivo* genotoxicity study results.
    - Recent re-valuation by Canadian Health Authorities considered the carcinogenicity study data as being relevant
- Informed benefit:risk assessment of TiO_2 in pharmaceuticals is key

SUMMARY OF

REPOR

- Due to its multiple functionalities TiO_2 is ex
  in medicines.
- TiO_2 is used very frequently in oral solid
  forms. TiO_2 is also present in dosage forms
- It is present in many essential and life-savir
- To date, no single material has been iden
  safety and quality properties that are uniq
  - Separating out the different functionalities
    serves more than one function is difficult o
- The feasibility of replacing TiO_2 cannot
  medicinal product will need an individual
- Possible alternatives identified so far have

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

8 September 2021
EMA/504010/2021
European Medicines Agency

Final feedback from European Medicine Agency (EMA) to the EU Commission request to evaluate the impact of the removal of titanium dioxide from the list of authorised food additives on medicinal products

Executive Summary

- Titanium dioxide (TiO_2) is extensively used as an opacifier and colourant in medicines due to its multiple functionalities[1].
- TiO2 is used very frequently in oral solid dosage forms (e.g. tablets, soft capsules, hard capsules, granules/powders for oral solution and oral suspensions), in oral semi-solid dosage forms (e.g. oral paste, oral gel). It is present in many essential medicines for human including antidiabetics, antibiotics and others and several veterinary medicinal products. TiO2 is also present in dosage forms administered via routes other than oral, e.g., products for cutaneous, inhalation (capsule shells), oromucosal, sublingual, transdermal and vaginal use.
- To date, no single material has been identified that provides the same combination of properties that are unique to TiO_2 (e.g. opacity, enhancing contrast, inertness, protection from UV light and the finish/smoothness of the resulting product). Separating out the different functionalities of TiO_2 for those medicinal products in which it serves more than one function is difficult or might not be possible at all.
- Possible alternatives identified so far include calcium carbonate, talc and starch. A number of disadvantages have been identified with these alternatives (e.g. inability to obtain sufficiently thin films, supply chain issues, mined materials with associated elemental impurity risk).
- The feasibility of replacing TiO_2 cannot be confirmed at this stage. Each affected medicinal product will need an individual review and assessment, which will require investigation of alternatives, product reformulation, generation of new data related to manufacture, dissolution and stability etc. and potentially new clinical data (e.g. generation of

[1] Approximately 91,000 human medicinal products and 800 veterinary medicinal products contain TiO_2 in the EU according to EU Trade Associations (see Annex I and II)

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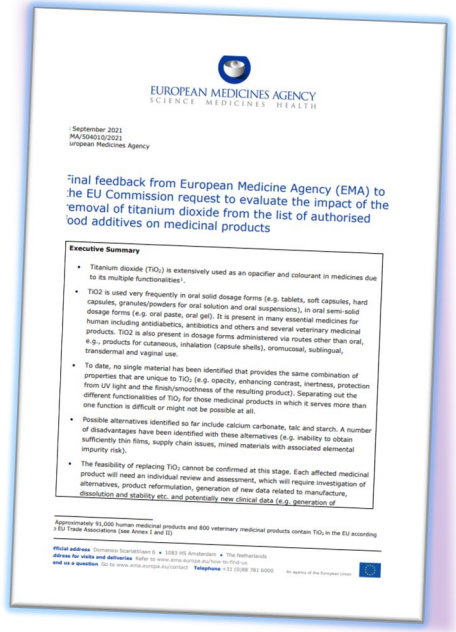
SUMMARY OF THE EMA ASSESSMENT 2/2

REPORT PUBLISHED OCTOBER 2021

- If TiO_2 would be banned in Europe, they would be the only region globally to ban TiO_2 as excipient in medicines, which would require industry to develop in a time consuming process new formulations to meet quality and safety.
- An acceptable transition period for phasing-out TiO_2 is currently difficult to envisage or estimate in particularconsidering the scale of the use of this excipient, the time and costs involved in the reformulation and the volume of products impacted.
- Replacing TiO_2 in medicines will almost certainly cause significant medicines shortages and discontinuations/withdrawals of medicines from the EU/EEA market with major implications for patients and animals. Particular concerns arise in relation to certain vulnerable classes/types of products such as paediatric medicines, orphan medicines, low sales volume products…



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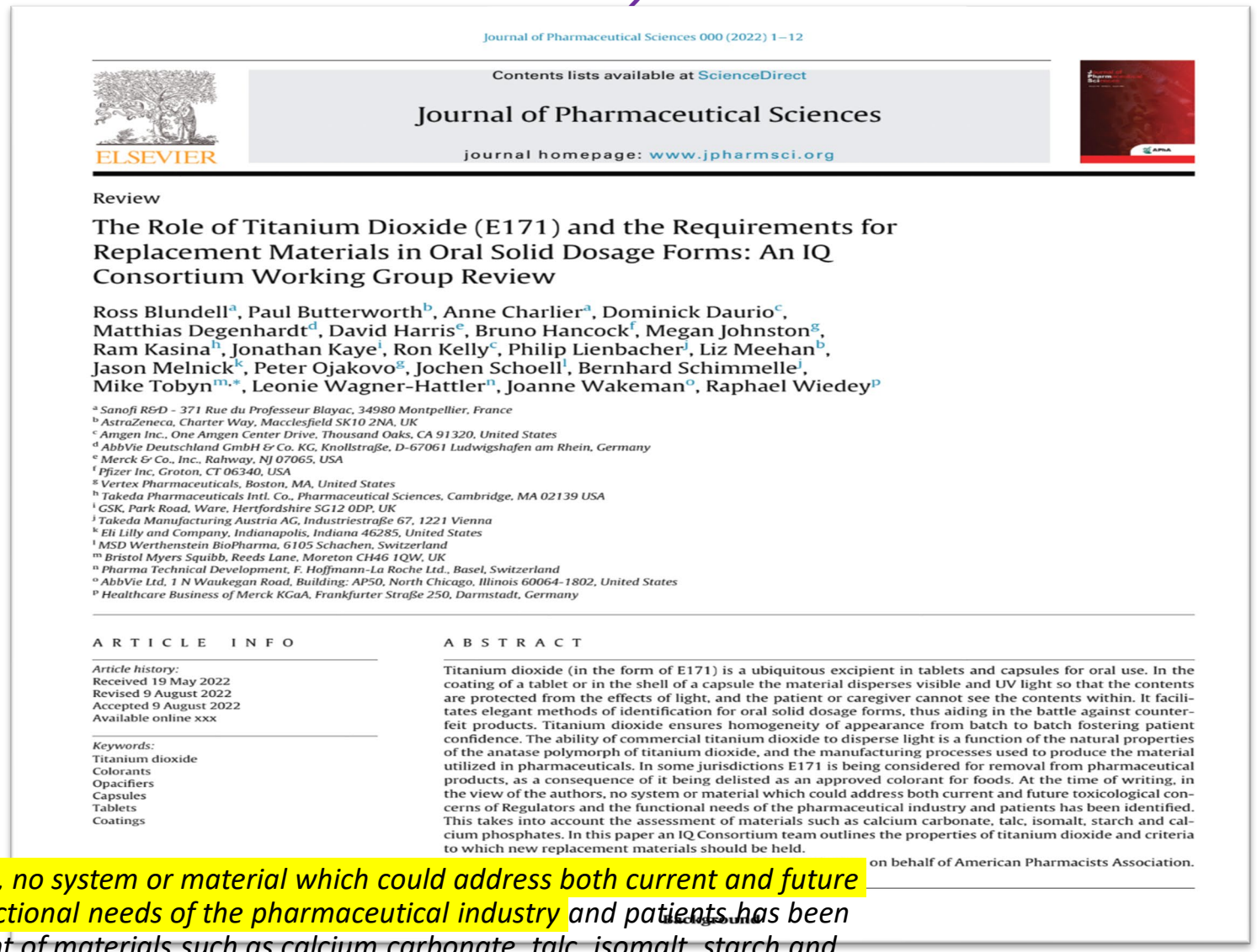
RECENT ANALYSES ON THE REPLACEMENT OF TIO, IN PHARMACEUTICALS

Blundell et al 2022- The Role of Titanium Dioxide (E171) and the Requirements for Replacement Materials in Oral Solid Dosage Forms: An IQ Consortium Working Group Review



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Review

The Role of Titanium Dioxide (E171) and the Requirements for Replacement Materials in Oral Solid Dosage Forms: An IQ Consortium Working Group Review

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ABSTRACT

Titanium dioxide (in the form of E171) is a ubiquitous excipient in tablets and capsules for oral use. In the coating of a tablet or in the shell of a capsule the material disperses visible and UV light so that the contents are protected from the effects of light, and the patient or caregiver cannot see the contents within. It facilitates elegant methods of identification for oral solid dosage forms, thus aiding in the battle against counterfeit products. Titanium dioxide ensures homogeneity of appearance from batch to batch fostering patient confidence. The ability of commercial titanium dioxide to disperse light is a function of the natural properties of the anatase polymorph of titanium dioxide, and the manufacturing processes used to produce the material utilized in pharmaceuticals. In some jurisdictions E171 is being considered for removal from pharmaceutical products, as a consequence of it being delisted as an approved colorant for foods. At the time of writing, in the view of the authors, no system or material which could address both current and future toxicological concerns of Regulators and the functional needs of the pharmaceutical industry and patients has been identified. This takes into account the assessment of materials such as calcium carbonate, talc, isomalt, starch and calcium phosphates. In this paper an IQ Consortium team outlines the properties of titanium dioxide and criteria to which new replacement materials should be held.

on behalf of American Pharmacists Association.

Background

At the time of writing, in the view of the authors, no system or material which could address both current and future toxicological concerns of Regulators and the functional needs of the pharmaceutical industry and patients has been identified. This takes into account the assessment of materials such as calcium carbonate, talc, isomalt, starch and calcium phosphates. In this paper an IQ Consortium team outlines the properties of titanium dioxide and criteria to which new replacement materials should be held

Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the Union code relating to medicinal products for human use, and repealing Directive 2001/83/EC and Directive 2009/35/EC

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(104) The use of colours in human and veterinary medicinal products is currently regulated by Directive 2009/35/EC of the European Parliament and of the Council[23], and restricted to those authorised in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives[24], for which specifications are laid down in Commission Regulation (EU) No 231/2012[25]. Uses of excipients other than colours in medicinal products are subject to the Union rules on medicinal products and are evaluated as part of the overall benefit risk profile of a medicinal product.

(105) Experience has shown the need to maintain to a certain extent the principle of the use in medicinal products of those colours authorised as food additives. However, it is also appropriate to foresee a specific assessment for the use of the colour in medicines when a food additive is removed from Union list of food additives. Therefore, in this specific case, EMA should carry out its own assessment for the use of the colour in medicines, taking into account the EFSA opinion and its underlying scientific evidence, as well as any additional scientific evidence and giving particular consideration to the use in medicines. EMA should also be responsible for following any scientific evidence for the colours retained for specific medicine use only. Directive 2009/35/EC should therefore be repealed.

Opacifiers to be considered other excepients

PHARMACEUTICAL INDUSTRY'S NEXT STEPS ON REQUEST OF EMA

- Under directive 2022/63 and during discussion with the EMA, the EU network have requested that industry collaborate on the investigation of alternatives for titanium dioxide. Industry generally supports this as a **collaborative** exercise.
- The coordination of the safety studies will ensure that 3Rs principles are followed (additional *in vivo* studies are minimised).
- Current main objectives are to generate evidence to support the EMA reassessment in Apr 2024
  - Technical practicality and effectiveness of alternatives in coatings and capsules which are commercially available and sustainable
  - Safety evaluations and gap filling to ensure a comprehensive safety package exist for the alternatives, which can be compared with TiO_2 to ensure safety of potential alternatives

Mechanism-based Genotoxicity Risk Assessment (**MGRA**) Working Group

Mission: Develop a new mechanism-based risk assessment paradigm for genotoxicity

Integrating learnings from Adverse Outcome Pathways (AOPs) into a modern risk assessment "clean sheet" framework

Using data rich case examples, show how the information of KEs for different MIEs can be used to establish a MGRA

Nitrosamine subgroup

Titanium dioxide subgroup

Establish a framework for the MGRA of data poor compounds where the mode of action has not been a priori been aligned with a specific AOP

Agreements so far

Forms of TiO_2 to be included:

- Agreement that all quality TiO_2 data is in scope.
- When considering experimental work, focus should be on E171 and possibly a second TiO_2 candidate in the nano space. (30nm TiO_2 from the EU repository was suggested).
- Crystallinity (rutile vs anatase) should also be kept in mind as a factor.

Exposure routes:

- Consensus to look at both inhalation and oral (independently). Dermal exposure is out of scope since the majority consensus in the scientific community is that these particles cannot penetrate the skin.

Data published to date:

- Agreement that data do not seem to allow an unequivocal conclusion that direct DNA reactivity can be excluded (MoA)

Work to be done

- Another (streamlined) literature research?
  - *Agreement that a streamlined literature search may not be fruitful given recent work*
  - ***Effort will focus on proposing/designing studies that enable mechanistic conclusions***
- Lead hypothesis is that observed genotoxicity is secondary to inflammation/ox stress. Links to AOP WIKI no. 296 (aopwiki.org) (developed by HESI GTTC)
- Start with *in vitro,* extend to *in vivo* if necessary
- Start with simple tools which provide info on underlying MoA (e.g., biomarkers for oxidative stress, transcriptomic markers, time course important)
- Possibly investigate mutagenicity signatures

Just getting started! Please contact co-leaders Stefan Pfuhler and Paul Fowler if you have questions

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- All members of the Expert Panel for their diligent hard work in a very tight time window
- Andreas Czich for the update from the pharmaceutical industry
- Stefan Pfuhler for the MGRA update

THANK YOU FOR YOUR ATTENTION.

QUESTIONS?

Back-up slides

Comparison of EFSA and Expert Panel approaches

- The EFSA approach can be summarised as follows:
  - The reliability of genotoxicity studies was assessed using criteria published by Klimisch et al. (1997).
  - Then relevance was assessed based on reliability (Klimisch score), some general aspects (e.g., genetic endpoint, route of administration and status of validation), and nano score (NSC).
  - Only studies achieving High or Limited relevance were considered in the overall assessment, but the genotoxicity data in these studies were not independently reviewed and the conclusions of the authors were accepted as published.

	EFSA approach		Expert Panel approach	
Study type	**No. of studies available for evaluation**	**No. of studies achieving High or Limited relevance (No. positive)**	**No. of datasets reviewed**	**No. achieving Moderate or higher weight after WoE assessment (No. positive)**
In vitro				
Ames test	8	0	15	0
Mammalian cell gene mutation	14	7 (3 positive)	16	2 (0 positive)
MN or CA	56	43 containing 67 datasets (26 datasets positive)	62	12 (2 positive)
Comet assay	142	106 containing 142 datasets (102 datasets positive)	0	0
DNA binding	5	5 (unclear whether these considered positive)	0	0
8-OHdG adducts	5	5 (4 positive)	0	0
γH2AX foci	4	4 (2 positive)	0	0
ToxTracker	1	1 (0 positive)	0	0
Sub-totals	**235**	**231 datasets (137 positive)**	**93**	**14 (2 positive)**
In vivo				
Gene mutation	6	6 (1 positive)	9	2 (0 positive)
MN or CA	26	15 (8 positive)	35	13 (7 positive)
Comet	44	18 containing 19 datasets (12 datasets positive)	51	3 (1 positive)
DNA binding	2	2 (unclear whether these considered positive)	0	0
8-OHdG adducts	2	1 (1 positive)	4	2 (0 positive)
γH2AX foci	2	2 (2 positive)	0	0
Sub-totals	**82**	**45 (24 positive)**	**99**	**20 (8 positive)**
Totals	**317**	**276 (161 positive)**	**192**	**34 (10 positive)**

Summary of comparison with EFSA

- EFSA considered many more studies to be “relevant” in the final assessment than the Expert Panel.
  - >50% of those achieving High or Limited relevance were *in vitro* comet assays, of which 71.8% were positive,
  - These were excluded by the Expert Panel on the basis of being only indicator tests.
  - EFSA also included *in vitro* DNA binding, 8-OHdG adducts and γH2AX foci studies which were excluded by the Expert Panel.
- Expert Panel included more *in vivo* studies than EFSA but concluded many fewer studies (in particular *in vivo* comet assays) were positive.
- Expert Panel re-evaluated the data in each dataset included in the final assessment (and sometimes did not confirm the authors findings), whereas EFSA accepted the authors’ conclusions without further review for datasets included in the final assessment.