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A review of the genotoxicity of titanium dioxide (TiO_2)

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Introduction

- TiO_2 is a ubiquitous white colourant found in a diverse range of products including foods, cosmetics, medicines and paints.
- It has been considered no longer safe for use in foods (E171 which contains both nano and microparticles) by EFSA due to concerns over genotoxicity
- A panel of experts, who are not currently employed by companies that manufacture and sell titanium dioxide (TiO_2) bulk material, was therefore convened to perform an independent review.
- The panel included experts in genetic toxicology, general toxicology, bioavailability, carcinogenicity, and nanoparticle characterisation.
- The panel comprised:
  - David Kirkland (Chair), Marilyn J. Aardema, Rüdiger V. Battersby, Carol Beevers, Karin Burnett, Arne Burzlaff, Andreas Czich, E. Maria Donner, Paul Fowler, Helinor Johnston, Harald F. Krug, Stefan Pfuhler, Leon F. Stankowski Jr.
- The review was sponsored by TDMA, and some panellists received payment for hours worked, but a rigid, approved protocol meant members of TDMA had no influence on the review or its conclusions

Data sources

- The publications on genotoxicity of TiO_2 reviewed by EFSA (the search criteria are described in Appendix A of the 2021 publication) were supplemented with additional publications identified by EBRC using search criteria detailed in the report.
- In addition, unpublished reports conducted by industry or at contract laboratories (sponsored by industry) were included.
- 337 datasets were identified that reported on various genotoxicity investigations with TiO_2
  - Not 337 publications because many contained data on different endpoints
- The various genotoxicity datasets were tabulated separately (in Data Review Tables) according to endpoint and test system, *in vitro* or *in vivo,* with notes as to whether pigmentary or nano-sized TiO_2 was tested (or if it was not clearly stated).

EBRC = EBRC Consulting GmbH

Reliability assessment – the ToxR Tool

- The ToxR Tool (Schneider et al., 2009) assigns a “0” or “1” to a range of parameters to reflect a “no” or “yes” answer (e.g., “1” would be entered if a concurrent negative control was included, but “0” if it was not)
- The scores for the individual parameters are then totalled and the “Tool” calculates a Klimisch score, which the reviewer can either confirm or revise (with justification). The available scores are:
  - 1 (reliable without restrictions),
  - 2 (reliable with restrictions)
  - or 3 (unreliable).
- The standard ToxR Tool template was modified to include nanoparticle characterisation and a “nano score” was also obtained.

Criteria			Evaluator's explanations, comments on criteria, etc.
No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?		
2	Is the purity of the substance given?		
3	Is information on the source/origin of the substance given?		
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?		
		0	
	Criteria Group II: Test system characterisation		
5	Is the test system described?		
6	Is information given on the source/origin of the test system?		
7	Are necessary information on test system properties, and on conditions of cultivation and maintenance given?		
		0	
	Criteria Group III: Study design description		
8	Is the method of administration given (see explanations for details)?		
9	Are doses administered or concentrations in application media given?		
10	Are frequency and duration of exposure as well as time-points of observations explained?		
11	Were negative controls included (give also point, if not necessary, see explanations)?		
12	Were positive controls included (give also point, if not necessary, see explanations)?		
13	Is the number of replicates (or complete repetitions of experiment) given?		
		0	
	Criteria Group IV: Study results documentation		
14	Are the study endpoint(s) and their method(s) of determination clearly described?		
15	Is the description of the study results for all endpoints investigated transparent and complete?		
16	Are the statistical methods for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?		
		0	
	Criteria Group V: Plausibility of study design and data		
17	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?		
18	Are the quantitative study results reliable (see explanations for arguments)?		
		0	

Nano scores (1)

- Many studies used nano-grade TiO_2, so considered critical to identify whether the physico-chemical (PC) properties of nanoparticles (NPs) had been characterised
- Samples of TiO_2 NPs were obtained from various sources
  - Vary with respect to size, surface area, morphology, agglomeration status, charge, surface chemistry.
- When NPs obtained from reliable sources such as The JRC Nanomaterial Repository, no independent characterisation was required
- However, evidence that PC properties provided by other suppliers may not always be accurate (Luyts et al., 2013)
  - In these cases independent characterisation of their PC properties was considered essential
  - Not sufficient to rely solely upon information provided by the supplier
- Also expected that the PC properties were characterised in media relevant to the study and test conditions
  - PC properties of NPs can change when they are dispersed in biological media
  - Different dispersion methods can also influence PC properties and toxicity

Nano scores (2)

- Quality of studies with TiO_2 NPs determined by addressing whether the following parameters had been characterised as proposed by Card & Magnuson (2010)

- Added a separate tab to the ToxR Tool

Category	**Score**	Comments / Explanation / Justification
Agglomeration and/or aggregation		
Chemical composition		
Crystal structure/crystallinity		
Particle size/particle distribution		
Purity		
Shape		
Surface area		
Surface charge		
Surface chemistry (including composition & reactivity)		
Whether any characterization was conducted in the relevant experimental media		
Total score	0	

Evidence weighting for genotoxicity

The panel's evidence weighting assumptions for the various genotoxicity endpoints reviewed were based on Brusick et al. (2016). The basic weight descriptors are given in the following table:

Weight Descriptor	Definition
Negligible weight	The endpoint is not linked to any adverse effect relevant to genetic or carcinogenic hazard/ risk (e.g., SCE).
Low weight	The end point is indicative of primary DNA damage, not directly linked to mechanisms of tumorigenicity (e.g., DNA breakage or computer-based SAR results), or the endpoints are evaluated in non-mammalian test systems (other than the Ames test).
Moderate weight	The endpoint may be: (a) only potentially relevant to tumor initiation, (b) subject to secondary effects (cytotoxicity), (c) subject to threshold dependent mechanisms of induction (aneugens) or (d) the test system exhibits a high rate of false responses with respect to carcinogenicity predictivity (e.g., mammalian cell *in vitro* clastogenicity and gene mutation tests, particularly in p53-deficient cells).
High weight	The endpoint is one that has been demonstrated to play a critical role in the process of tumorigenicity (gene mutation in bacteria [Ames test*] or *in vivo*, chromosome aberrations or micronuclei *in vivo*).

Default weights for different endpoints

Endpoint*	Negligible Weight	Low Weight	Moderate Weight	High Weight
DNA binding (adduct formation) *in vitro*				
DNA binding (adduct formation) *in vivo*				
SSB/DSB *in vitro (including comet)*				
SSB/DSB *in vivo (including comet)*				
Sister Chromatid Exchanges (SCE) *in vitro*				
Sister Chromatid Exchanges (SCE) *in vivo*				
Oxidative DNA Damage *in vitro*				
Oxidative DNA Damage *in vivo* (detection of 8-OHdG adducts)				
DNA repair effects *in vitro*				
DNA repair effects *in vivo*				
Micronuclei (MN) *in vitro*				
Micronuclei (MN) *in vivo*				
Chromosomal aberrations (CA) *in vitro*				
Chromosomal aberrations (CA) *in vivo*				
Gene mutation in bacteria (Ames Test)**				
Gene mutation in mammalian cells *in vitro*				
Gene mutation *in vivo*				

Notes on weighting

- The principles of this WoE approach are consistent with the endpoint specific guidance document of the European Chemicals Agency (ECHA, 2015), and also with the “Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines” (2015)
- Only those endpoints with a default weighting of “moderate” or “high” (according to the table above) were reviewed in detail. This amounted to 192 datasets.
- The remaining 145 datasets (with default “low” or “negligible” weightings) were not reviewed since they are not considered to contribute meaningfully to the assessment of genotoxic or carcinogenic hazard.
- The Ames test is not recommended for testing insoluble particles such as nano- or micro-particles, because they do not readily pass through the bacterial cell wall and prokaryotes do not perform endocytosis
- Hence the datasets reviewed in detail are given in red in the following slide

Organisation of datasets in review tables

- *In vitro* mutation in bacteria
- *In vitro* mutation in mammalian cells
- *In vitro* chromosomal damage
  - Micronucleus (MN)
  - Chromosomal aberrations (CA)
- Other endpoints *in vitro*
  - Bacteria (e.g., *rec* assay)
  - Mammalian cells (e.g., comet assay, UDS test)
- *In vivo* mutation in somatic or germ cells
  - Transgenic rodent gene mutation (TGR); Pig-a; HPRT
- *In vivo* chromosomal damage in somatic or germ cells
  - MN and CA
- *In vivo* comet assay
- *In vivo* 8-OHdG adducts
- Other endpoints *in vivo*
  - SCE; UDS; DNA adducts, methylation & damage markers; P53 mutation; tests in *Drosophila;* γH2AX

The most relevant endpoints are those given in red.

192 datasets were found covering these endpoints and were reviewed in detail for **reliability** and **relevance**

WoE process (1)

Each dataset was given an initial weighting according to the criteria above. The “weights” were adjusted (if necessary) according to the acceptability of the study design and the quality of the data. For example:

- Source and purity of TiO_2
- Study design relative to OECD guidelines
- Acceptability of criteria for a positive response
- Coding of slides for CA, MN or Comet studies
- Possible interference with nanoparticle uptake (e.g., testing in the presence of cytochalasin B)
- Cytotoxicity measures used
- Level of cytotoxicity where positive responses seen
- Acceptability of top concentration/dose
- Evidence of tissue irritation/inflammation
- Evidence of target tissue exposure
- Concurrent positive & negative controls included
- Acceptability of negative control frequencies
- Formulations properly characterized (are the PC properties of NPs specified in the papers)
- Cellular/tissue uptake of the test material
- Dispersion method for nanoparticles
- Control for exposure to light

WoE process (2)

- As a result of these considerations:
  - An initial “Moderate” weight may have been down-graded to “Low-moderate” or “Low”
  - Or a “High” weight may have been down-graded to “Moderate-high” (or even lower)
- Exposure time in mammalian cell tests was considered particularly important:
  - Latest OECD recommendations for MN studies are that treatment in the absence of cytochalasin B should be for at least 1 cell cycle, or, if shorter, that there is a clear demonstration of cellular uptake
  - Considered equally important for *in vitro* CA and gene mutation studies
  - However, if clear positive results were obtained with TiO_2 following a treatment period of less than 1 cell cycle, it was assumed that intracellular exposure had occurred
  - Therefore, some *in vitro* MN, CA and gene mutation studies that gave positive or equivocal results with short treatments were considered reliable and retained a Moderate weight
  - But studies that gave negative results with short treatments and with no clear demonstration of cellular uptake were considered unreliable and given Low-moderate or Low weights – potential bias??

Consistency checking/Reliability

- All reviews were checked for consistency of Klimisch and nano scores, and final "weights", by other panel members
  - Completed ToxR forms were shared between the different reviewers where there was more than 1 relevant data set for review within a publication
  - Input from Helinor Johnston and Harald Krug on nanoparticle characterization was sought on a regular basis in order to ensure consistent approaches to nano scores
- Any inconsistent calls were discussed either 1-on-1 with the reviewer, or in small groups by videoconference, or by the full panel
- Many older publications gave ToxR Klimisch scores of 3 (unreliable) but this was not used as a primary criterion to exclude a study from further evaluation
- More recent publications and study reports tended to be more reliable with ToxR Klimisch scores of 1 or 2.
- Nonetheless, the quality of available genotoxicity studies with TiO_2 is clearly very variable, and the structured approaches used in this project were considered important.

Results of nano assessments

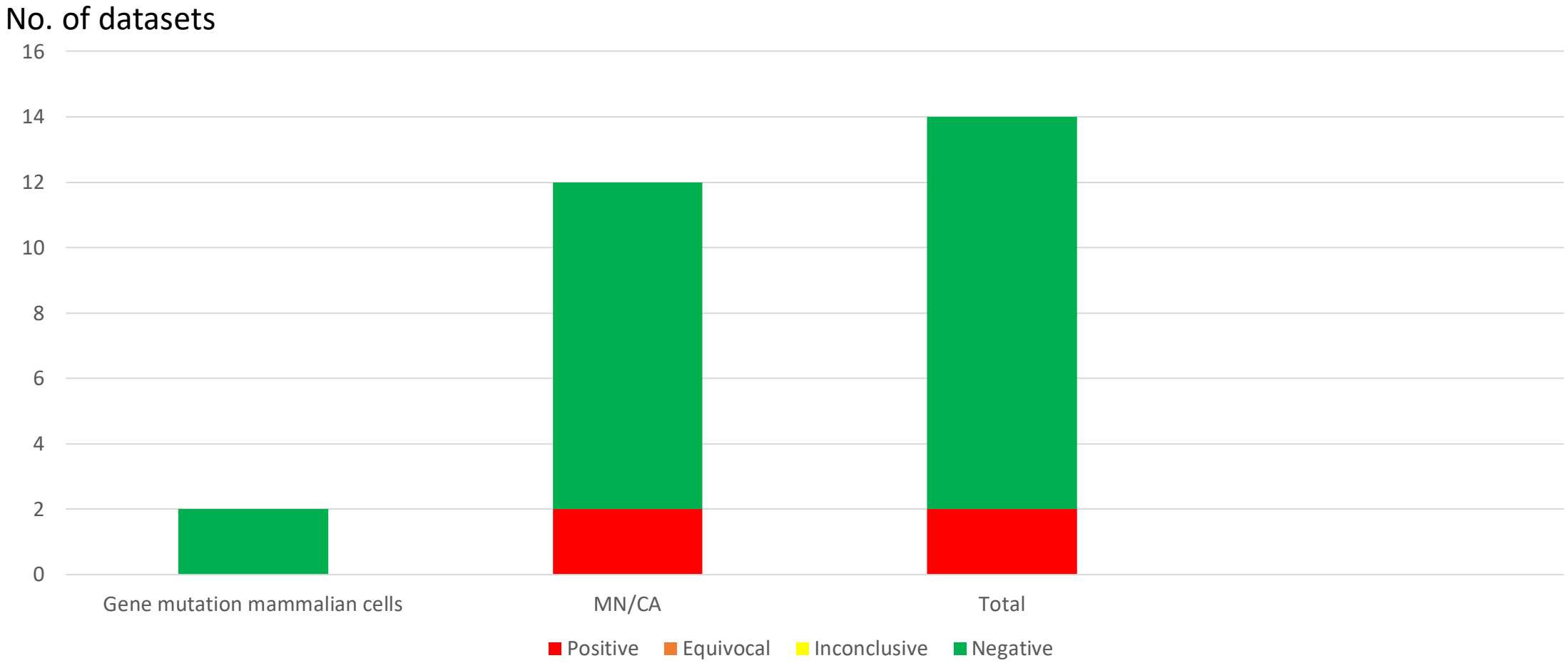
- Detailed characterisation led to high nano scores, but in several cases characterisation was very limited and the nano scores were low, sometimes even zero
- Some studies did report characterisation of NPs in biological media, but many did not.
- Published studies often provided insufficient detail on the methodology employed to characterise the NPs.
  - Details of the concentrations of NPs used and the approaches to disperse them (e.g., media used to suspend NPs, and whether sonication was used and the time of sonication, when used) often not reported
- As a result, nano scores were very variable. However, the nano score was also not used as a primary criterion to exclude a study from further evaluation

Results of genotoxicity assessment

- Conclusions based on overall WoE assessment were used as the primary selection criteria for studies that should be considered most relevant for evaluation of genotoxic potential
- As a result, of the 192 datasets reviewed only 34 achieved final overall weights of "Moderate" or higher

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**

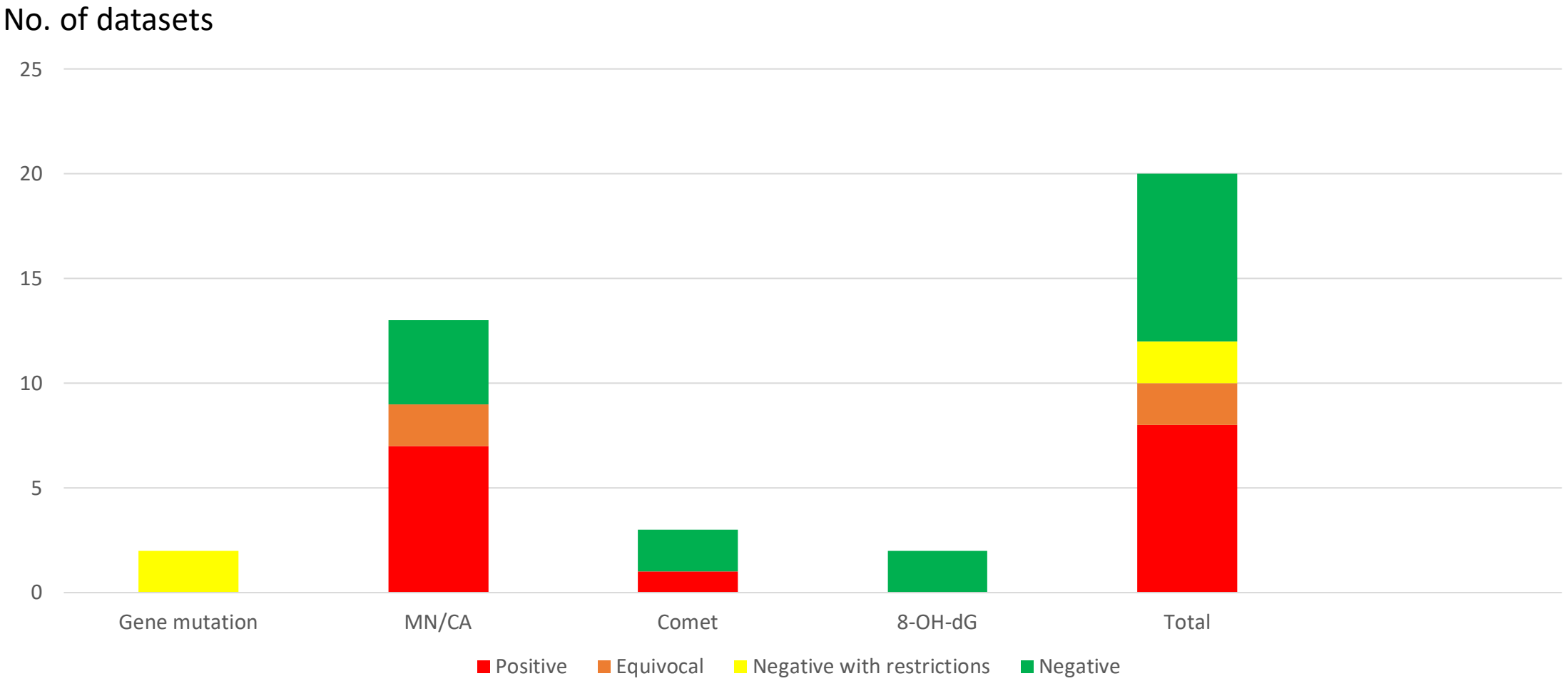
Fig 1: Profile of results for *in vitro* studies



Comments on *in vitro* 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 sometimes seen in negative studies.
  - Therefore, it is highly likely that the increase in MN was secondary to oxidative stress and cytotoxicity.
- The particle concentrations tested in mammalian cells *in vitro* were highly variable, making comparison of effects between studies very challenging
- Despite potential bias by including positive, but excluding negative, mammalian cell tests with short treatments, 10 *in vitro* MN/CA and 2 *in vitro* mammalian cell gene mutation studies that did include sufficiently long exposures to provide robust results were negative.

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



Comments on *in vivo* results (1)

- No evidence of induction of gene mutations *in vivo* from 2 TGR studies, although neither study fully complied with OECD guideline recommendations.
- None of the *in vivo* Pig-a mutation studies met current best practice recommendations or were sufficiently robust to achieve “moderate” or higher weight.
- Of the 13 *in vivo* MN/CA studies, 7 were considered positive, but 2 of these scored Klimisch 3 in the ToxR tool and are therefore considered unreliable
- In these 7 positive studies, dose levels and dosing period were variable even by the same route of administration:
    - 4 oral gavage studies
        - 1 study on nano TiO_2 (rutile, 25 nm) using doses up to 0.8 mg/kg/day for 28 days,
        - Another study on nano TiO_2 (anatase, 5-10 nm) using doses up to 200 mg/kg/day for 60 days,
        - A 3rd study on nano TiO_2 (58 nm) using doses up to 500 mg/kg/day for 90 days,
        - A 4th study on micro TiO_2 using doses up to 1000 mg/kg/day for 7 days.
    - 1 drinking water study on nano TiO_2 P25 using doses calculated up to 500 mg/kg over 5 days
    - 1 IP study on pigmentary TiO_2 using doses up to 1500 mg/kg/day for 3 days
    - 1 IV study on nano TiO_2 NM-105 using a single dose of 5 mg/kg.

Comments on *in vivo* results (2)

- 5 of the 7 positive MN/CA studies used oral gavage or drinking water administration, but 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 of rats
  - Other grades of TiO_2 administered at the same dose, were below the limit of detection in blood, so % absorption was even lower.
- 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 bone marrow exposure assured.
- Of the 7 positive *in vivo* MN/CA studies:
  - 1 was probably an indirect consequence of high bone marrow toxicity since increased CA frequencies only increased at >40% mitotic inhibition
  - 3 showed only weak (approximately 2-fold) increases in MN
  - 1 was positive for MN in rat bone marrow stained with Giemsa, but negative in bone marrow reticulocytes (stained with acridine orange) in the same animals.
  - All other positive responses were associated with inflammation, oxidative stress and/or apoptosis.
- Therefore, there are reasons to question whether any of these positive *in vivo* MN/CA responses are indicative of a direct DNA-damaging effect of TiO_2.

Comments on *in vivo* results (3)

- 3 *in vivo* comet studies in rats achieved “Moderate” weight.
  - Two were negative (one in lung after intratracheal instillation, the other in liver and lung after oral dosing).
  - The third study was positive in lung and liver after endotracheal instillation, but the responses were associated with inflammation and oxidative stress.
- The inconsistent results and use of different routes of administration from the positive MN/CA studies, makes comparing effects across different *in vivo* studies/endpoints challenging.
- Thus, again, there are reasons to question whether the one positive *in vivo* comet response is a biologically relevant indicator of a direct DNA-damaging effect.
- There are 2 *in vivo* 8-OHdG studies that achieved “Moderate” weight that were negative for oxidative DNA damage.

Discussion (1)

- Using a structured WoE approach only 34 out of 337 relevant datasets (10.1%) provided acceptable data for final assessment (other reviews have found even lower % acceptable data)
- Therefore many published studies are too poor to support a robust assessment:
  - The endpoint evaluated is not relevant
  - Study designs and/or the data are not reliable
  - Results are questionable for various reasons.
- 10 of the 34 relevant datasets (29.4%) were positive. All were from DNA or chromosomal damage studies
- Since all of the positive findings were associated with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis, or combinations of these, it is highly likely that the observed genotoxic effects of TiO_2, including those with NPs, 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 - although data from robust *in vivo* gene mutation studies would be useful in reaching firm conclusions.

Discussion (2)

- Within the 34 relevant datasets there was little evidence of reproducible effects for the same endpoint
- This made comparison of effects very challenging due to different non-standardised protocols e.g.:
  - Forms of TiO_2 tested
  - Varied characterisation of the preparations tested
  - Different concentrations or doses
  - Different exposure routes
  - Different cell types showing differences in endocytosis
  - The fact that study designs in many cases differed markedly from, and often fell short of, the recommended approaches in OECD test guidelines.

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.

Conclusions

- **Thus, the conclusions from the 34 robust datasets reviewed here, that achieved "moderate" or higher weight, do 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.**

- The review has now been published in Regulatory Toxicology & Pharmacology https://doi.org/10.1016/j.yrtph.2022.105263

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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-

Acknowledgements

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- EBRC for providing the publications and reports, and to Torsten Grewe (in particular) for setting up SharePoint and helping us when there were problems

Back-up slides

Authors	Assay	Test substance (nano, pigmented, mixture or not clear)	Cell Type/ Concentration	Response (according to authors)	Reliability scores		WoE Considerations	Reviewer's conclusion (based on reliability and WoE considerations)	Weight*	Comments
					Evaluator's Klimisch score (based on ToxR Tool)	Nano score, if relevant (based on Card & Magnuson, 2010)				
Uboldi et al. (2016)	MN	Nanosized anatase (An-10), nanosized rutile (Ru-10), bulk anatase (BAN) and bulk rutile (BRU), synthetized by oxidation of $TiCl_3$ Cellular uptake was determined 72 hours after start of treatment	Immortalized Balb/3T3 mouse fibroblasts (clone A31-1-1) 10 µg/ml	Positive for RU-10, negative for AN10 and the Bulk material	3	6	• Untreated and solvent control MN frequencies within acceptable ranges. • No relevant cytotoxicity was observed for all tested substances • Only one concentration tested • MN induction by RU-10 statistically significant but all values expected to be within historical ranges • 3 replicates and 3 independent experiments • 1000 cells per slide scored, slides not coded • Materials well characterized, • Cytochalasin B added for 24 hours after 24-hour treatment (from Uboldi et al., 2012) • Unclear if treated cultures protected from light	Inconclusive	Low-moderate	

Discussion – relevance to carcinogenicity

- Finally, as shown in the table below (adapted from Brusick et al. (2016); based on Bolt et al. (2004) and Petkov et al. (2015)), the profile of genotoxicity results from the most robust studies with TiO_2 do not fit the pattern expected for a genotoxic carcinogen.

Characteristic	**Carcinogens with a proven genotoxic mode of action**	**TiO_2**
Profile of Test Responses in Genetic Assays	Positive effects across multiple key predictive endpoints (i.e. high weight studies such as gene mutation in bacteria or *in vivo*, chromosomal aberrations or micronuclei *in vivo).*	No valid evidence for gene mutation in mammalian cells or *in vivo*; chromosomal damage in rodents only at doses inducing cytotoxicity, inflammation, oxidative stress.
Structure Activity Relationships	Positive for structural alerts associated with genetic activity.	Not done
DNA binding	Agent or breakdown product are typically electrophilic and exhibit direct DNA binding.	No evidence of DNA binding, and no evidence of 8-OHdG adducts in robust *in vivo* studies
Consistency	Positive test results are highly reproducible both *in vitro* and *in vivo.*	Conflicting and/or non-reproducible responses in the same test or test category both *in vitro* and *in vivo.*
Response Kinetics	Responses are dose dependent over a wide range of exposure levels.	Dose responses in robust, reliable test systems generally not observed.
Susceptibility to Confounding Factors (e.g. Cytotoxicity)	Responses are typically found at non-toxic exposure levels.	Positive responses in robust, reliable test systems typically associated with evidence of apoptosis, necrosis, inflammation and oxidative stress.