ANSES Scientific & Technical Support
Request No 2024-AST-0114

The Director General

Maisons-Alfort, (2 September 2024)

Scientific and Technical Support NOTE of the French Agency for Food, Environmental and Occupational Health & Safety

on the analysis of the data provided in the context of the assessment of titanium dioxide (TiO_2) in cosmetic products

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On 14 August 2024, ANSES received a formal request from the Directorate General for Health (DGS) and the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) to provide scientific and technical support for the following: analysis of the data provided in the context of the assessment of titanium dioxide in cosmetic products.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Titanium dioxide (TiO_2) is found in many products that may be used by both professionals and consumers. It is therefore regularly assessed, particularly in its nano forms, under various regulations (REACH, CLP, food additives, cosmetics, etc.).

TiO_2 is a cosmetic ingredient authorised by Regulation (EC) No 1223/2009 as a colorant in its pigmentary form (i.e. at the micrometre scale) and as a UV filter in both its pigmentary and nano forms. In this context, Europe's Scientific Committee on Consumer Safety (SCCS) has published several opinions on the safety of TiO_2 in cosmetic products (SCCS/1516/13, SCCS/1580/16, SCCS/1617/20). Its uses are therefore governed by Annexes IV and VI to Regulation (EC) No 1223/2009.

Following concerns raised by the European Food Safety Authority (EFSA) about the genotoxicity of TiO_2, in 2022 the European Commission's services received a dossier from the industry that contained an updated review of knowledge on the genotoxicity of TiO_2.

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This led the European Commission's services to mandate the SCCS to reassess the safety of TiO_2, focusing on inhalation and oral exposure of consumers and the substance's potential genotoxicity.

In December 2023, the SCCS published a preliminary opinion covering a wide range of TiO_2 grades[1] found in cosmetic products, i.e. 44 pigmentary grades and 40 nano grades. The SCCS concluded that the available evidence was not sufficient to exclude the genotoxicity potential of almost all the TiO_2 grades used in cosmetic products, except for two nano grades (RM09 and RM11). The SCCS also stated that further information was needed on the potential uptake of TiO_2 in the cells of the oral mucosa, in order to consider that cosmetic products containing TiO_2 are safe for use. Comments were submitted by ANSES during the public consultation held between December 2023 and February 2024 (Annex 1). The SCCS's finalised opinion[2] was published in May 2024.

In response to these conclusions, a document[3] from the industry (Cosmetics Europe and the Titanium Dioxide Manufacturers Association (TDMA)) presenting their roadmap was shared with the Member States on 16 May 2024. This roadmap proposes to generate data for certain TiO_2 grades based on grouping approaches.

These nanomaterial grouping approaches include a number of scientific publications (Braakhuis et al., 2021[4]; Di Cristo et al., 2021[5] and 2022[6]) produced as part of the European GRACIOUS framework and shared by the Netherlands with all Member States.

ANSES was therefore asked by the DGS and the DGCCRF to review the roadmap and the grouping approaches used by the industrial sector.

2. ORGANISATION OF THE WORK

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

As it did not involve a risk assessment, this note was prepared with the in-house expertise of ANSES's Risk Assessment Department. Given the time available, it was not possible to include a critical analysis of all the grouping approaches that may be available in the literature. This expert appraisal therefore focused on a critical analysis of the TiO_2 industry roadmap and the

[1] Terminology used by the industry to define the different forms of TiO_2 intended for cosmetic uses (SCCS/1661/23). This term could correspond to "nanoform" according to Annex VI of the REACH Regulation.

[2] SCCS (Scientific Committee on Consumer Safety), Scientific Advice on Titanium dioxide (TiO2), CAS/EC numbers 13463-67-7/236-675-5, 1317-70-0/215-280-1, 1317-80-2/215-282-2, preliminary version of 4 December 2023, final version of 13 May 2024, SCCS/1661/23

[3] Summary for the European Union Member State Competent Authorities (EU MS) including a detailed ROADMAP for the subsequent delivery to data on Titanium Dioxide

[4] Braakhuis HM, Murphy F, Ma-Hock L, Dekkers S, Keller J, Oomen AG, Stone V. 2021. An Integrated Approach to Testing and Assessment to Support Grouping and Read-Across of Nanomaterials After Inhalation Exposure. Appl In Vitro Toxicol. 1;7(3):112-128. doi: 10.1089/aivt.2021.0009.

[5] Di Cristo, Luisana, Agnes G. Oomen, Susan Dekkers, Colin Moore, Walter Rocchia, Fiona Murphy, Helinor J. Johnston, Gemma Janer, Andrea Haase, Vicki Stone et al. 2021. "Grouping Hypotheses and an Integrated Approach to Testing and Assessment of Nanomaterials Following Oral Ingestion" Nanomaterials 11, no. 10: 2623. https://doi.org/10.3390/nano11102623

[6] Di Cristo, Luisana, Gemma Janer, Susan Dekkers, Matthew Boyles, Anna Giusti, Johannes G. Keller, Wendel Wohlleben, et al. 2022. “Integrated Approaches to Testing and Assessment for Grouping Nanomaterials Following Dermal Exposure.” Nanotoxicology 16 (3): 310–32. doi:10.1080/17435390.2022.2085207.

three publications shared by the Netherlands. It also took account of the comments made previously by ANSES during the public consultation on the SCCS's preliminary opinion (Annex 1).

3. ANALYSIS AND CONCLUSIONS

3.1. Genotoxicity of TiO_2

The mandate given to the SCCS by the European Commission concerned a reassessment of the safety of TiO_2, focusing on genotoxicity and inhalation and oral exposure.

This document therefore focuses primarily on this hazard property for these routes of exposure.

In its response to a Danish comment, the industry[7] stated that the weight of evidence clearly confirms that the genotoxicity of TiO_2 results from an indirect, threshold-dose mechanism. ANSES notes that the data available in the literature mainly support secondary genotoxicity due to the generation of oxidative stress (Charles et al., 2018[8]; Cao et al., 2023[9]; Kirkland et al., 2022[10]). The 12th revision of the SCCS's guidance document on the safety evaluation of cosmetic ingredients (SCCS/1647/22[11]) also indicates that this type of mechanism may be associated with a toxicity threshold. However, according to the data currently available on TiO_2, it is not possible to completely rule out other mechanisms, in particular direct genotoxicity.

In its roadmap, the industry proposes to conduct two types of *in vitro* mutagenicity studies: gene mutation tests (OECD 476) and micronucleus tests (OECD 487), and to combine these studies with an analysis of particle uptake. The industry does not indicate the number or identity of the grades to be tested (see Table 1 in the roadmap).

ANSES reiterates the importance of these data being in line with validated protocols that meet the appropriate test guidelines for assessing the potential genotoxicity of nanomaterials.

For example, during the public consultation on the SCCS's preliminary opinion (Annex 1), ANSES had noted, among other things, that the mutagenicity studies available for grades RM09 and RM11 had been conducted while taking into consideration only a prolonged exposure time (24 hours), whereas a short-term treatment (3-6 hours) is also defined in OECD guidelines 476 and 487. ANSES considered that there was no justified reason for not also conducting the short-term test, in the absence of any OECD recommendation about the irrelevance of this exposure time for nanomaterials. In addition, the validity of a mutagenicity

[7] The term "industry" in this note refers to Cosmetics Europe and the TDMA
[8] Charles S, Jomini S, Fessard V, Bigorgne-Vizade E, Rousselle C, Michel C. Assessment of the in vitro genotoxicity of TiO2 nanoparticles in a regulatory context. Nanotoxicology. 2018 May;12(4):357-374. doi: 10.1080/17435390.2018.1451567. Epub 2018 Mar 19. PMID: 29553842.
[9] Cao, Yue, Jinyao Chen, Qian Bian, Junyu Ning, Ling Yong, Tong Ou, Yan Song, and Sheng Wei. 2023. "Genotoxicity Evaluation of Titanium Dioxide Nanoparticles In Vivo and In Vitro: A Meta-Analysis" Toxics 11, no. 11: 882.
[10] David Kirkland, Marilyn J. Aardema, Rüdiger V. Battersby, Carol Beevers, Karin Burnett, Arne Burzlaff, Andreas Czich, E. Maria Donner, Paul Fowler, Helinor J. Johnston, Harald F. Krug, Stefan Pfuhler, Leon F. Stankowski. A weight of evidence review of the genotoxicity of titanium dioxide (TiO2),Regulatory Toxicology and Pharmacology, Volume 136,2022,105263,ISSN 0273-2300.
[11] https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision_en

test depends on the inclusion of a positive control. In its comments, ANSES therefore reiterated that this control should provide a response that is consistent with the historical control databases. This was not adequately demonstrated in the micronucleus test conducted on RM09.

Furthermore, again in the context of this public consultation, ANSES made a number of comments about the data on the uptake of TiO_2 particles in epithelial cells. ANSES considered that these studies should be carried out at doses consistent with those of the genotoxicity studies and that complete uptake should be demonstrated (using high-resolution microscopy), which was not the case for the two grades tested (RM09 and RM11).

3.2. Interpretation of Entry 27a of Annex VI to the Cosmetics Regulation (EC No 1223/2009)

TiO_2 in nano form is subject to regulatory provisions as a UV filter in the Cosmetics Regulation (Entry 27a in Annex VI). In particular, this Entry 27a states that authorised TiO_2 grades must have a median particle size of ≥ 30 nm based on the number size distribution.

Thanks to advances in analytical techniques for characterising nanomaterials, checks carried out by the French authorities using the currently recommended methods (ECHA[12], JRC[13], SCCS[14], ANSES[15]) show an increasing number of cosmetic products that do not comply with Entry 27a. This point was raised by the French authorities at the March 2024 meeting of the Cosmetics Working Group.

The main arguments put forward by the industry in its response were as follows:

> 1/ The industry data submitted in 2013, which had been used to draw up Entry 27a, were based on particle size distributions obtained using the CPS Disc centrifuge, LUMisizer centrifuge and DLS (dynamic light scattering) techniques. In contrast, the checks carried out by the DGCCRF were based on electron microscopy. The non-compliances can therefore be explained by differences in dispersion protocols and the techniques used to calculate particle size distributions;
>
> 2/ TiO_2 particles exist mainly as aggregates/agglomerates rather than as well-dispersed particles, making it difficult to characterise primary particles.

On the first point, ANSES observes that the CPS Disc centrifuge, LUMisizer centrifuge and DLS techniques do not provide information on the primary constituent particles, but rather the agglomerates/aggregates. According to Annex L of the SCCS's 2023 opinion (SCCS/1661/23), 32 out of 40 nano TiO_2 grades have median primary particle sizes below 30 nm (lowest median particle size 9 nm) based on electron microscopy analysis.

On the second point, according to ANSES, even if the sample is agglomerated/aggregated, this does not prevent information being obtained on the distribution of the primary particle

[12] Appendix for nanoforms applicable to the Guidance on Registration and Substance Identification (ECHA, 2022)
[13] Rauscher et al., Identification of nanomaterials through measurements (JRC, 2019)
[14] Guidance on the Safety Assessment of Nanomaterials in Cosmetics, 2nd revision, 6 June 2023 (SCCS/1655/23).
[15] Review of analytical methods available for characterising nano-objects and their aggregates and agglomerates, in order to meet regulatory requirements (ANSES, 2020)
https://www.anses.fr/fr/system/files/AP2018SA0168Ra_0.pdf

sizes. This is because since 2013, the methods and knowledge regarding the physio-chemical characterisation of nanomaterials have evolved.

ANSES therefore notes that the physico-chemical characterisations of nanomaterials presented in the SCCS's 2013 opinion (SCCS/1516/13), and in particular the size measurement methods, are no longer up to date and should no longer be used as a basis for Entry 27a.

Consequently, ANSES recommends:

- taking account of scientific and technical progress in the area of nanomaterial characterisation. ANSES therefore referred to the guidance documents of the SCCS (SCCS/1655/23)[16] and the JRC[17], as well as ANSES's scientific and technical analysis report on the review of analytical methods (link in footnote 15). All this documentation provides guidance on the definition of the term "nanomaterial" and the analytical methods to be considered when applying this definition, as well as on the requirements for data on safety assessment of nanomaterials in cosmetics;
- on the basis of these references, reviewing the physico-chemical characterisation data presented in the 2013 opinion with the current reference methods, in case any grades analysed in 2013 are not covered by the SCCS's 2023 opinion in which the size distributions were established by electron microscopy;
- updating the physico-chemical characteristics of TiO_2 given in the conditions of use in Annex VI, Entry 27a. The reference to "particles" could incorrectly imply that these are not primary particles but aggregated or agglomerated particles. Entry 27a of the Regulation should make reference to the terminology of constituent primary particles as defined by the SCCS's guidance document on the assessment of nanomaterials. These should be measured using the analytical methods listed in the SCCS and JRC guidance documents. Specifying the term "constituent primary particles" in the entry would provide greater clarity.

[16] According to its Guidance on the safety assessment of nanomaterials in cosmetics (SCCS/1655/23), the SCCS recommends that Applicants take the Commission Recommendation of 10 June 2022 (and any resulting revision of the definition) into consideration when assessing the safety of cosmetic ingredients that are comprised of or consist of small particles, even if the 2022 Commission Recommendation has not yet been applied to the definition of a nanomaterial under Cosmetic Regulation (EC) No 1223/2009.
In addition, according to the SCCS, primary particle size must be measured by more than one method, one of which must be high-resolution EM (SEM or TEM).

[17] The JRC has drawn up methodological guides for implementing the European Commission's definition of nanomaterials. According to the JRC, DLS and other methods based on particle sedimentation in a centrifugal field are "screening methods".
- They do not yield external dimensions but provide equivalent hydrodynamic diameters.
- They measure the size of the aggregates/agglomerates and not their constituent particles.
- They do not provide number-based distributions at a reasonable measurement uncertainty.
According to the JRC, "confirmatory methods" such as electron microscopy are able to identify the constituent particles within agglomerates and aggregates and measure their external dimensions.

3.3. Grouping of grades

This analysis focuses on the three publications on the grouping of nanomaterials and the industry roadmap. The principle of grouping nanomaterials has been under discussion for several years, without a validated and/or harmonised methodology for regulatory application having been adopted by the scientific community and/or a European body. Uncertainties and limitations persist, particularly regarding the specificity of the nanoscale, which makes it all the more difficult to establish similarities between nanoforms.

The roadmap considers that it is not necessary to test every one of the 84 TiO_2 grades used in cosmetics, and the industry has proposed to conduct these tests on a more limited selection of grades.

In response to the concerns raised by the SCCS about a grouping approach based on physico-chemical parameters and the generation of toxicological studies, the industry referred to two methodologies:

> 1/ the one described in the ECHA decision (ECHA, 2021[18]) in connection with assessing the inhalation toxicity of TiO_2 under REACH, for which France was the evaluating Member State,
>
> 2/ a publication (Stone et al., 2020) resulting from the European research framework GRACIOUS, funded under the H2020 Programme[19].

The industry therefore proposed to carry out toxicological studies on a selection of 11 grades representative of the 84 grades to be assessed, based on the similarity of their physico-chemical parameters. ANSES notes that the selected grades were neither defined nor justified in the roadmap.

Regarding point 1/, ANSES states that the ECHA decision defining the request for studies to be carried out under REACH focused solely on the inhalation toxicity of TiO_2 and covered some forms of TiO_2 that are not used in cosmetics. In this context, a number of TiO_2 forms were selected on the basis of physico-chemical criteria relevant to inhalation exposure. The aim of the required studies is to investigate the impact of these parameters on toxicity *via* the respiratory route (including pulmonary genotoxicity). It therefore consists of a preliminary step to defining a protocol for repeated *in vivo* inhalation toxicity studies. The applicability of this approach to oral exposure was not assessed.

Regarding point 2/, ANSES considers that the grouping approach proposed in the European GRACIOUS framework is useful for prioritising substances in order to select those whose toxicity should be analysed. However, as this type of approach has not been validated, it can only be used as a preliminary step to analysing substance toxicity, without replacing the generation of studies if they are necessary.

[18] https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807ebca5
[19] Home | Gracious (h2020gracious.eu)

ANSES analysed three articles published by the same team (V. Stone[20], H. M. Braakhuis[21], L. Di Cristo[22]) as part of the GRACIOUS framework between 2020 and 2022 (see Annex 2).

These articles offer hypotheses and criteria for grouping nanoforms after exposure by the inhalation, oral or dermal routes, in order to facilitate risk assessment. These groupings are based on detailed hypotheses and criteria using integrated approaches to testing and assessment (IATA). The grouping takes account of the physico-chemical characteristics of the nanoforms ("what they are"), the route of exposure and toxicokinetics ("where they go"), and the hazard ("what they do").

According to these articles, grouping includes important criteria to be analysed, such as:

- the physico-chemical characteristics of the nanoforms, relevant depending on the route of exposure,
- toxicokinetics and behaviour in environmental media,
- relevant hazard parameters depending on the route of exposure.

Conversely, the selection of grades to be tested as proposed by the industry (11 grades out of 84, not specified) is based solely on certain physico-chemical parameters (see table 2 of the roadmap). ANSES considers that this is insufficient for assessing safety. Indeed, the scientific articles show the importance of including parameters other than those selected by industry, such as additional physico-chemical properties, toxicokinetic data and behaviour in environmental media, as well as hazard criteria (Annex 2). In this respect, ANSES points out that the SCCS's guidance document (SCCS/1655/23) provides a structuring framework for the safety assessment of nanomaterials in cosmetic products and identifies a non-exhaustive list of relevant physico-chemical parameters to be characterised. In addition, ANSES notes that there is other work that should be taken into account to define the key criteria, such as ECHA's guidance on read-across strategies for nanoforms (2019)[23] or the OECD's case study (2018)[24] on grouping and read-across for nanomaterials with a focus on the genotoxicity of TiO_2.

To guarantee that the grades tested are representative of the 84 grades used in cosmetic products, ANSES recommends that the industry conduct an exhaustive investigation of the similarities between the different physico-chemical parameters in relation to their influence on reactivity and their fate in biological media. Evidence of similarity is all the more important given the many cosmetic grades with multiple characteristics, varied exposure routes and uses by different types of consumers, including sensitive and vulnerable individuals. Consequently, ANSES considers that the industry should guarantee the representativeness of the nanomaterials tested for all the grades in question, by providing robust scientific justification. This justification should be supported by tests showing that nanomaterials with similar physico-chemical properties have the same reactivity and behaviour in the test media.

Regarding the choice of the proposed grouping method, the industry refers only to the GRACIOUS framework and the ECHA decision. Nevertheless, ANSES feels that the choice should not be limited to these approaches, and points out that other documentation and work

[20] NanoSafety Research Group, Heriot Watt University, Edinburgh, United Kingdom.
[21] Centre for Health Protection and Centre for Safety of Substances and Products, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
[22] Nanoregulatory Platform, Drug Discovery and Development Department, Istituto Italiano di Tecnologia, 16163 Genova, Italy.
[23] Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals
[24] Case study on grouping and read-across for nanomaterials – Genotoxicity of nano TiO_2

in progress should be taken into account to shed light on the feasibility of grouping and the difficulties that remain to be resolved. These include:

- projects under way at ECHA: "NM Risk Assessment: a regulatory way forward for sameness and grouping approaches" and "Methodological developments and data generation for the assessment and building of sets of NFs";
- the OECD's 2018 case study and ECHA's above-mentioned guidance document (2019).

Furthermore, ANSES underlines the need to conduct a detailed analysis of the scientific literature in order to document the various grouping approaches currently available and be able to take a critical look at the industry's proposal.

In conclusion, ANSES considers that the approach defined at this stage by the industry is insufficient for demonstrating the representativeness of the grades that will be tested for all the forms used in cosmetics, especially given the absence of a harmonised approach for grouping nanomaterials, the associated uncertainties and the vast number of grades used in cosmetics.

If the uncertainty is too high, one alternative would be to limit the number of authorised grades to those for which data have been generated and whose safety has been demonstrated.

3.4. Safety assessment

The mandate given to the SCCS by the European Commission's services concerned a reassessment of the safety of TiO_2, focusing on genotoxicity and inhalation and oral exposure.

The SCCS therefore indicated that the previously published opinions (SCCS/1516/13 and SCCS/1580/16) concerning cosmetic products containing TiO_2 and applied by the dermal route remain in force for the TiO_2 grades covered in these opinions. As these opinions pre-date the SCCS's guidance document on the safety assessment of nanomaterials in cosmetics (SCCS/1655/23), ANSES recommends that these assessments be updated in light of developments in knowledge on the physico-chemical characterisation of nanomaterials.

Concerning the other routes of exposure:

ANSES agrees with the SCCS that a safety threshold cannot normally be defined in the case of demonstrated mutagenic/genotoxic potential, as mentioned in table 1 of the roadmap.

If this is not the case, the safety assessment should take account of the full toxicological profile, which must be representative of the different grades used in cosmetics. If this is not possible, a safety assessment conducted specifically for each grade in question should be considered.

Furthermore, in its roadmap, the industry only mentions a safety assessment for cosmetics containing TiO_2 and leading to oral exposure (e.g. toothpaste).

ANSES wishes to draw attention to the fact that numerous publications report effects on reproductive and developmental functions with TiO_2 in nano form (e.g. Rolo et al., 2022[25];

[25] Rolo, Dora, Ricardo Assunção, Célia Ventura, Paula Alvito, Lídia Gonçalves, Carla Martins, Ana Bettencourt, et al. 2022. "Adverse Outcome Pathways Associated with the Ingestion of Titanium Dioxide Nanoparticles—A Systematic Review". Nanomaterials 12 (19): 3275. https://doi.org/10.3390/nano12193275

Dianova et al., 2022[26]; Wang et al., 2018[27]). Most of these studies were carried out via the oral route with anatase crystalline TiO_2 and sizes ranging from 6 to 21 nm. No data are available for forms with a surface coating or for rutile forms, which are particularly common in cosmetics. Consequently, in the absence of such data, it is not possible to conclude whether or not the effects observed in the literature can be extrapolated to the grades used in cosmetics. This warrants particular attention from the SCCS.

In addition, the use of TiO_2 as a food additive (E171) has been banned by the European Commission following EFSA's opinion (2021), due to the fact that genotoxic concerns could not be ruled out.

In view of these various points and pending further data, ANSES underlines the importance of reassessing the safety of using cosmetic products containing TiO_2 and leading to oral exposure (e.g. toothpaste).

Furthermore, TiO_2 is currently subject to conditions of use and restrictions for inhalation exposure under the Cosmetics Regulation.

Thus, nano TiO_2 is not to be used in applications that may lead to exposure of the end-user's lungs by inhalation, when the substance is used as a UV filter (Entry 27a in Annex VI). The pigmentary form is subject to restriction in powder form containing 1% or more of particles with aerodynamic diameter ≤ 10 µm (Entry 321 of Annex III and Entry 27 of Annex VI).

As part of the public consultation on the SCCS's preliminary opinion, ANSES had noted that all the pigmentary and nano grades contained less than 1% of particles with aerodynamic diameter ≤ 10 µm, suggesting that the fraction of particles liable to penetrate the lungs after inhalation was low or non-existent. ANSES had questioned the validity of these results in light of the data on particle distribution (particularly for grades with sizes well below 100 nm) and their almost spherical or lanceolate shape.

4. AGENCY'S CONCLUSIONS

The European Commission mandated the Scientific Committee on Consumer Safety (SCCS) to reassess the safety of TiO_2, focusing on inhalation and oral exposure of consumers and the substance's potential genotoxicity. ANSES responded to the public consultation on the SCCS's preliminary opinion of December 2023[28], and supported the SCCS's conclusion on the following points:

- that the available evidence was not sufficient to exclude the genotoxicity potential of TiO_2 used in cosmetic products;
- that further information was needed in order to consider that cosmetic products containing TiO_2 are safe for use.

[26] Dianová, Lucia, Filip Tirpák, Marko Halo, Tomáš Slanina, Martin Massányi, Robert Stawarz, Grzegorz Formicki, Roberto Madeddu, and Peter Massányi. 2022. "Effects of Selected Metal Nanoparticles (Ag, ZnO, TiO_2) on the Structure and Function of Reproductive Organs". Toxics 10 (8): 459. https://doi.org/10.3390/toxics10080459.

[27] Wang, Ruolan, Bin Song, Junrong Wu, Yanli Zhang, Aijie Chen, and Longquan Shao. 2018. "Potential Adverse Effects of Nanoparticles on the Reproductive System". International Journal of Nanomedicine 13: 8487-8506. https://doi.org/10.2147/IJN.S170723.

[28] Response in Annex 1 to this note

The SCCS's opinion has since been finalised and was published in May 2024.

In response to these conclusions, the industry (Cosmetics Europe and the Titanium Dioxide Manufacturers Association (TDMA)) presented a roadmap proposing to generate data on genotoxicity and uptake in the cells of the oral mucosa for certain TiO_2 grades based on grouping approaches.

The Agency focused on an analysis of the TiO_2 industry roadmap and the three publications shared by the Netherlands on a grouping approach cited in the roadmap.

ANSES commented in particular on the following points:

- The genotoxicity of TiO_2;
- The interpretation of Entry 27a of Annex VI to the Cosmetics Regulation;
- The grouping of TiO_2 cosmetic grades.

With regard to genotoxicity, ANSES insists on the need to obtain reliable and relevant studies that meet OECD recommendations and enable conclusions to be drawn on the genotoxicity of all TiO_2 grades used in cosmetics.

ANSES also recommends taking account of scientific and technical progress in the area of nanomaterial characterisation to update the physico-chemical characteristics of TiO_2 given in the conditions of use in Annex VI (Entry 27a) on UV filters.

Nanomaterial grouping approaches are still being researched; one example is the study carried out as part of the European GRACIOUS framework, funded under Horizon 2020. Although harmonised approaches are not yet available, ANSES's reading of this work has led it to recommend that other parameters be taken into account in addition to solely the physico-chemical characteristics described in the grouping approach currently proposed by the industry. Otherwise, a high level of uncertainty risks being associated with the results, especially given the vast number of grades proposed for cosmetics. The industry is also expected to demonstrate the representativeness of the grades chosen for testing. If this is not the case, one alternative would be to limit the number of authorised grades to those for which data have been generated that demonstrate their safety.

The critical analysis conducted by the Agency identifies weaknesses in the industry's proposed roadmap to provide additional data. However, it does not prejudge any assessment that may be carried out by the SCCS on the basis of the additional data generated.

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KEY WORDS

Dioxyde de titane, évaluation de la sécurité, génotoxicité, regroupement des nanoformes.

Titanium dioxide, safety assessment, genotoxicity, grouping of nanoforms.

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SCCS. "THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 12TH REVISION", (SCCS/1647/22), 15 May 2023.

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SUGGESTED CITATION

ANSES. (2024). STS Note on the analysis of the data provided in the context of the assessment of titanium dioxide (TiO_2) in cosmetic products (Request No. 2024-AST-0114). Maisons-Alfort: ANSES, 2024, 19 p.

ANNEX 1: ANSES COMMENTS ON SCCS SCIENTIFIC ADVICE ON TITANIUM DIOXIDE

29/01/2024

3.1. Chemical and physical specifications

3.1.4 Purity, composition and substance codes

It is noted that *"it is not possible to know whether aluminium is present as Al_2O_3 or $Al(OH)_3$ or similarly if the analysed elemental silicon is related to silica, silicones or silanes. Only an approximation can be made based on the manufacturing process."* (on page 14 line 34 - 6)

For Anses, it is possible to identify the nature of impurities even at trace levels. Applicants should argue why it is challenging to distinguish impurities and specify if they used a combination of analytical techniques.

Table 3.1.4.A1: Pigmentary grades – categories by composition

It would be useful to add a column specifying the crystallinity of the grades considered.

Table 3.1.9.2: Particle size and distribution

According to TEM results for constituent particles sizes, % of nano is higher than 50 for some pigmentary grades of TiO_2, considering at least one TEM data. Specifically, according to table 3.1.9.1.A2 in Annex L, several grades (RM26, RM27, RM67, RM70a, b, c) were reported with more than 50% of particles by number < 100 nm (determined by TEM). However, these raw materials were reported as pigmentary grades in paragraph 3.1.1.5.

Since TEM gives better resolution than SEM and allow to distinguish the primary particle boundaries, Anses considers as a conservative approach, that these grades (RM26, RM27, RM67, RM70a, b, c) should be considered as nanomaterials, even if other data provided (from SEM and/or TEM) gives a particle size distribution with less than 50% of particle <100 nm.

Table 3.1.9.3: Aerodynamic diameter

Anses questions the very low percentage (close to 0) of particles with an aerodynamic diameter below 10 µm, despite the spheroidal shape of pigmentary samples and certain nanograde samples (RM09, RM11, RM55, RM56, RM57, RM58, RM59, RM60, RM61, RM62, RM64, RM65, RM74a, RM74b, RM74c, RM74d, RM74e, RM78, RM81, and RM82). These results imply that none of these grades fulfil criteria for classification as Carc. 2 by inhalation.

The aerodynamic diameter is defined as the physical diameter of a sphere with a density equal to 1, moving through the air at the same velocity as the particle in question (such as titanium). This parameter determines the behavior of the particle suspended in the air. It is a function of the geometric diameter, shape factor, and the density of the aerosolized particle.

In general, for spheroid particles, the aerodynamic diameter is often close to the physical diameter. Regarding aerodynamic diameter results provided, Anses believes that, if nanoscale titanium samples with a spheroid shape contain less than 1% of particles with an aerodynamic diameter <10 µm, it is primarily due to the particle aerosolization system generating aggregated/agglomerated particles.

As this parameter is a key criteria for classification as Carc. 2 by inhalation, more information is needed regarding the measurement of aerodynamic diameter. Therefore, measurement results' must be accompanied by (i) the characteristics of the dustiness test and (ii) the number-based particle size distribution of aerosols generated during the dustiness test. Moreover, (iii) the aerosolization of the samples during the measurement of the aerodynamic diameter should be representative of the exposure resulting from titanium dioxide used in cosmetics.

Reference:

https://echa.europa.eu/fr/information-on-chemicals/cl-inventory-database/-/discli/details/100661

3.1.11 Dispersibility

It is noted that *"among the 40 nano titanium grades, 3 nano grades have been tested in toxicity studies: RM09, RM11 and RM75".*

While genotoxicity results are provided with RM09 and RM11, it is not clear to what type of studies the SCCS refers to for RM75.

Moreover, the rationale behind the choice of these particular nano grades as representatives of the 40 ones declared with cosmetic uses should be made clearer in the SCCS opinion together with the fact that this rationale is not convincing enough to read-across to the remaining 37 grades.

3.4.1. 1. Mutagenicity / genotoxicity in vitro

Gene mutation assay with RM09

It is noted that a short-term treatment is considered inadequate for nanomaterials as cellular uptake of the test item needs to be demonstrated. However, Anses considers that it would have been relevant to perform a short exposure duration in addition to the 24h treatment (Charles & Jomini et al., 2018).

- Reference

Charles & Jomini et al., 2018. Assessment of the in vitro genotoxicity of TiO2 nanoparticles in a regulatory context. Nanotoxicology. Volume 12 – issue 4.
https://www.tandfonline.com/doi/full/10.1080/17435390.2018.1451567

Extract: *Short exposure duration of few hours can allow detecting genotoxicity when the compound is easily absorbed by the cell or when DNA damage is due to oxidative stress. In*

contrast, longer exposure duration (>=24 h) may be necessary to adequately detect NPs genotoxicity in proliferating cells considering that NPs may need more time than other chemicals to penetrate cells and/or nuclei (Karlsson 2010). […] In contrast, tests with long-exposure times may be less sensitive, as they allow the possibility of DNA repair mechanisms to occur.

Moreover, it is stated that "the SCCS is of the opinion that cellular uptake of RM09 was convincingly demonstrated, however, at RM09 concentrations higher than those recommended by the OECD TG 490 (paragraph 29). According to the information on precipitation provided by the Applicant, the highest acceptable concentration tested should be 6.3 µg/mL (Exp I) or 12.5 µg/mL (Exp IA), and these concentrations were not tested for cellular uptake, i.e. the lowest concentration tested by the Applicant for uptake was 25 µg/mL."

In addition, statement provided in Annex R for RM09 Gene Mutation Assay: *"most of the observed V79 cells showed agglomerates of RM09 nanoparticles. Only occasionally separated particles or single small agglomerates can be observed"* cannot be confirmed. Agglomerated particles present inside the cell is due, among other things, to sample preparation through chemical fixation and dehydration. Anses believes that to confirm or refute this statement, images from cryo-TEM need to be provided.

In these conditions (no short-term treatment and no data on cellular uptake at tested doses in the genotoxicity study), it is difficult to conclude firmly that the study is negative.

<u>Gene mutation assay with RM11:</u>

It is noted that the mutation frequency was sporadically statistically significantly increased in the 24-hour experiment without metabolic activation. Even if the results are reported as consistent with historical control and not dose related, they should have been considered as "equivocal" rather than "clearly negative", according to OECD guideline 476 ("evaluation and interpretation of results). In this case, further investigation (as repeat test using same conditions) could have been useful to reach firm conclusion.

The following statement provided in Annex R for RM11 Gene Mutation Assay *"Nevertheless, many cells show no obvious internalization of RM11 nanoparticles and many of the RM11 nanoparticle agglomerates can be observed outside the cells. The majority of the RM11 nanoparticles (inside and outside the cells) are present in agglomerated form. Only occasionally separated particles or single smaller agglomerates can be seen"* based on low resolution TEM micrographs, cannot be accurate. In fact, the low resolution TEM micrographs provided do not clearly distinguish the location of small isolated particles inside or outside the cell. Therefore, Anses believes that high resolution (corresponding to a resolution greater than or equal to the size of the smallest particles in the sample) images need to be provided.

Moreover, the SCCS concludes that cellular uptake was convincingly demonstrated at all concentrations, although applicant stated that obvious internalization of RM11 nanoparticles

was not shown in many cells. Anses asks SCCS to provide more argumentations allowing its conclusion.

Finally, it is noted that *"according to the information on precipitation provided by the Applicant, the highest acceptable concentration tested should be 6.3 µg/mL (4 or 24 h of exposure), and these concentrations were not tested for cellular uptake, i.e. the lowest concentration tested by the Applicant for uptake was 25 µg/mL".* In these conditions, how can it be possible to conclude firmly that the study is negative?

This comment on the lack of demonstration of cellular uptake at concentrations tested in genotoxicity also applies to the micronucleus assay with RM11.

Micronucleus assay with RM09

Anses has the same comments concerning treatment duration and cellular uptake as for the gene mutation assay with RM09.

The SCCS notes that positive control cell cultures treated with griseofulvin showed mean micronucleus frequency which is below the minimal value of the historical control data. This result may raise some uncertainties on the sensibility of the test to detect mutagens of low potency (and thus on the negative conclusion of this test).

3.4.1.2. Mutagenicity / genotoxicity in vivo

Creutzenberg study (2022):

FR notes that this preliminary study was not performed in accordance with criteria/parameters set in the ECHA decision (within Reach Substance Evaluation process), in particular concerning the number of animals, the number of concentrations, the observation times and the Comet assay protocol. New data performed according to requirements of the Reach Substance Evaluation is expected in the next years.

3.4.1.3.2 Published literature search carried out by the SCCS

According to table on page 67: rutile coated (or rutile with up to 2% anatase) nano grades were considered as inclusion criteria. However, it is noted that nano grades used in cosmetics can contain up to 5% anatase. Please clarify.

An additional publication has been identified in the literature:

- Cao et al. Genotoxicity Evaluation of Titanium Dioxide Nanoparticles In Vivo and In Vitro: A Meta-Analysis. Toxics. 2023 Oct 27;11(11):882. doi: 10.3390/toxics11110882. https://pubmed.ncbi.nlm.nih.gov/37999534/

Please consider if this publication is relevant for SCCS opinion.

4. Conclusion

Anses agrees with the SCCS that more experimental data is needed to conclude on safety of TiO_2 used in cosmetic products.

Anses recognizes the effort made to provide data on specific nanoforms regarding their genotoxicity. However, based on the above-mentioned limits, we are of the opinion that no firm conclusion can neither be made for RM09 nor RM11. In addition, there is no convincing rationale for considering them as representative of all cosmetic grades.

Regarding question 5 “does the SCCS have any further scientific concerns regarding the use of Titanium dioxide in cosmetic products?”, Anses notes that, in addition to genotoxic concerns, several alerts related to reproductive toxicity (male and female reproduction and development) have been found in the scientific literature with nano grades of TiO_2. Most of these studies are performed by oral route with anatase crystallinity and sizes ranging from 6 to 21 nm. No data are available with coated TiO_2 nor with rutile crystallinity.

Annexes 1, 2, 3:

Some information is lacking for some studies in particular in column “exposure conditions” (e.g. biological medium, FBS serum and statistics “other genotoxicity endpoints – H2AX” (Vignard et al., 2023)), “information on the characteristics of the test substance” and “results” (e.g. see Ferrante et al. 2013). Please check and complete.

ANNEX 2: SUMMARY AND CRITICAL ANALYSIS OF ARTICLES DESCRIBING A NANOFORM GROUPING APPROACH DEFINED IN THE EUROPEAN GRACIOUS FRAMEWORK

In general, ANSES considers that the grouping approach generated in the European GRACIOUS programme is useful for prioritising substances in order to select those whose toxicity needs to be analysed first. This approach should only be a preliminary step to analysing substance toxicity, without replacing the generation of new studies if they are deemed necessary.

ANSES analysed three articles published by the same team (V. Stone[29], H. M. Braakhuis[30], L. Di Cristo[31]) between 2020 and 2022. These articles offer hypotheses and criteria for grouping nanoforms after exposure by the inhalation, oral and dermal routes, in order to facilitate risk assessment.

These groupings are based on detailed hypotheses and criteria using integrated approaches to testing and assessment (IATA). The grouping takes account of the physico-chemical characteristics of the nanoforms ("what they are"), the route of exposure and toxicokinetics ("where they go"), and the hazard ("what they do").

The main analyses and parameters provided by the integrated approach, together with ANSES's critical analysis, are as follows:

I. The integrated approach focuses on the dissolution rate of nanoforms. Depending on their dissolution in biological fluids corresponding to the route of exposure (bronchoalveolar fluid, gastrointestinal fluid, sweat), nanoforms can be grouped into several categories: instantaneous, rapid, gradual or very slow dissolution. Dissolution determines whether the nanoforms remain intact or are transformed into dissolved molecules/ions, and predicts their biopersistence.

ANSES's comment:

ANSES notes that the articles define dissolution categories based on half-lives, for which threshold values are proposed. However, there is no harmonised definition of biopersistence. ANSES also queries the fact that dissolution appears to be the entry point for any grouping, even though it is recognised that this parameter influences the fate and toxicity of nanoforms. Moreover, ANSES notes that the solubility of substances, which must be provided in accordance with the SCCS's guidance document on the safety assessment of nanomaterials, is not taken into account for the dissolution analysis. ANSES adds that there is also no harmonised definition/standard for specifying solubility or dissolution thresholds, which makes it difficult to apply the integrated approach.

II. Other criteria analysed in the decision trees to guide users in grouping include:

[29] NanoSafety Research Group, Heriot Watt University, Edinburgh, United Kingdom.
[30] Centre for Health Protection and Centre for Safety of Substances and Products, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
[31] Nanoregulatory Platform, Drug Discovery and Development Department, Istituto Italiano di Tecnologia, 16163 Genova, Italy.

- Physico-chemical parameters

Certain physico-chemical parameters – such as size, aggregation and agglomeration – are analysed in all cases, regardless of the expected route of exposure. However, other parameters are added specifically according to their relevance to the exposure route in question.

- Hazard parameters and toxicokinetics

The nanoform's hazard properties and its behaviour in the environment in which it is found (for example, surface reactivity and dispersibility in addition to the dissolution rate considered beforehand) must also be taken into account to support the grouping.

The hazard properties to be analysed differ according to their relevance to the exposure route in question.

For inhalation exposure, for example, surface reactivity and the production of reactive oxygen species (ROSs) are key parameters. The potential for inflammation includes the analysis of markers of inflammatory/proinflammatory responses.

The assessment of hazards for oral exposure includes the following parameters: reactivity, inflammation, genotoxicity, cytotoxicity, systemic toxicity, crossing of barriers.

For the dermal route, the assessment of hazards includes skin irritation, skin sensitisation and skin penetration.

ANSES's comment:

ANSES notes that no criteria of physico-chemical similarity were proposed for defining the nanomaterial groupings.

In addition, ANSES observes that the interactions between particles and other components, such as the surface coating, or with other substances present in the environment that could induce synergy or combined effects, were not analysed. These interactions can nevertheless modify the properties and reactivity of nanomaterials and even increase their toxicity.

For the hazard parameters to be analysed in order to determine the groupings, ANSES observes that no similarity criteria were proposed here either.

ANSES notes that genotoxic potential was not proposed as a parameter in the integrated approach for inhalation (unlike in the publications on the dermal and oral routes) for determining the groupings. Similarly, systemic toxicity was not described and only reactive oxygen species were assessed, whereas other reactive species (such as reactive nitrogen species, RNSs) are also involved in oxidative stress.