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abstract

Despite the UV performance benefits of particulate UV filters, concerns have been raised over their potential percutaneous permeation leading to the requirement of a specific approval for all nano UV filters in Europe. Under the legally binding definition of the Cosmetic regulation No. 1223/2009, nanomaterial means [...] a material [...] on the scale from 1 to 100 nm. The recommendation of the European Commission (2011/696/EU) revised 10th of June 2022 (2022/C229/01) further specifies that a material is nano when at least 50% of the number size distribution of the constituent particles is comprised between 1 and 100 nm. The situation for Titanium Dioxide and Zinc Oxide is confusing since the constituent particles are often part of agglomerates. Some suppliers clearly specify the nano feature of their material, while others declare the non-nano property of their inorganic products. The objective of this study was, therefore, to measure the number-based particle size distribution of three marketed Titanium Dioxide and Zinc Oxide grades using the decision support flow scheme devised by the NanoDefine project. This group was created to support the identification of nanomaterials as required by the European legislation. We employed the tier 1 method asymmetrical flow field-flow fractionation (AF4) coupled with UV and IC-PMS (inductively coupled plasma mass spectroscopy) detectors and tier 2 method transmission electron microscopy (TEM). The study clearly taught that the samples that appeared to be non-nanomaterials with AF4-IC-PMS measurements were finally proven to be nanomaterials using the TEM tier 2 method. Relying only on tier 1 methodologies such as AF4, X-Ray Disc Centrifuge or Dynamic Light Scattering to determine the non-nano property of a material is insufficient, since tier 1 methods are not able to detect constituent particles as part of agglomerates, which is often the case for Titanium Dioxide and Zinc Oxide. The status of being a non-nanomaterial can, in this case, only be certified with a tier 2 technique, particularly electron microscopy.

Introduction

UV filter ingredients used in sun protection products must be approved by the appropriate authority to be used in the respective marketplace; this is true all around the world even if the legislation might differ between the countries. All UV filters absorb UV radiation; filters in particulate form are additionally able to reflect or scatter light. Therefore, it is possible to distinguish between soluble and insoluble or particulate UV filter ingredients. To efficiently absorb UV light, the size of the particles ideally lies in the nano size range [1]. Today, two inorganic materials Titanium Dioxide (TiO₂) and Zinc Oxide (ZnO) and two organic materials Tris-Biphenyl Triazine (TBPT) and Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT) are approved as nano-particulate UV filters for personal care. A third organic one, Bis-(Diethylaminohydroxybenzoyl Benzoyl) Piperazine, obtained a positive scientific opinion and should be placed on the positive list very soon [2-4]. Particulate filters are commonly used in sunscreens; indeed 42% of sunscreens launched in Europe in 2020 contained at least one of the four registered nano UV filters [5]. Despite their huge benefits in term of performance, their use in sunscreens raised concerns over their potential percutaneous permeation. In Europe, this led to the requirement of a specific approval for all nano particulate UV

filters, even for the ones already listed in Annex VI of the Regulation (EC) No. 1223/2009 on cosmetic products, implying a new data submission and a new examination of their safety profile by the Scientific Committee of Consumer Safety (SCCS), which is a commission of independent experts [6]. Under the regulatory definition of the Cosmetic regulation No. 1223/2009 (article 2 (1) (k)), nanomaterial means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm [7]. To define a nanomaterial more precisely, the European Commission published a recommendation (2011/696/EU) revised 10th of June 2022 (2022/C229/01). In this recommended definition, which has in the meantime been adopted under REACH [8], a nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm – 100 nm [9]. There are numerous techniques available to measure the size of particles; their range of applicability, however, depends on their measurement principle and the physico-chemical parameters of the tested material. Not every method is appropriate

to measure the size of every particulate ingredient, yet, the analysis result and drawn conclusion may be incorrect due to the use of an irrelevant measurement method. Therefore, the choice of the method highly depends on the characteristics of the nanomaterial to be tested. From the four approved nano particulate UV filters listed in Annex VI of the Regulation (EC) No. 1223/2009, the two organics TBPT and MBBT exist only as nano and are labelled accordingly. They were shown to be fully safe regarding skin dermal permeation and are allowed up to a use concentration of 10% in ready-to-use preparation excluding spray applications or applications posing a risk of inhalation. Regarding the inorganic UV filters, some suppliers clearly specify the nano feature of their material, while others declare the non-nano property of their inorganics. The situation for TiO₂ and ZnO is confusing regarding their particle size distribution since the constituent particles are often part of agglomerates or aggregates. This poses a difficulty since the Regulation (EC) No. 1223/2009 states the need to consider the internal structure, but not every measurement method is able to identify the constituent particles in a material. To support the implementation of the Cosmetic Regulation regarding nanomaterials, the project "NanoDefine" was funded by the EU's 7th Framework Programme for Research [10]. The NanoDefine consortium has developed a decision tree scheme published by the Joint Research Center (JRC) [11] to guide any operator, firstly in the characterization of the particulate material, then in the choice of the most appropriate measurement method to evaluate any material (powder or dispersion) or finished cosmetic product in order to finally identify if the material is nano or non-nano according to the EC definition of nanomaterials [12].

The objective of the present study was to measure the number-based particle size distribution of several marketed Titanium Dioxide and Zinc Oxide grades using the decision support flow scheme devised by the NanoDefine project. To measure the median value of the particle size and the number-based distribution of the test samples, the two methods were employed: asymmetrical flow field-flow fractionation (AF4) coupled with UV and plasma mass spectrometry (ICP-MS) detectors and transmission electron microscopy (TEM).

Materials and methods

Samples of inorganic UV filters

We measured the median value of the particle size and number weighted distribution of different market products of Titanium Dioxide and Zinc Oxide as listed in **Table 1**.

Sunscreen market product

In parallel, we also evaluated a market product in the form of an emulsion containing Zinc Oxide as solely UV filter. The sunscreen product claimed a SPF of 50+; in the INCI declaration the Zinc Oxide was not listed as a nano UV filter.

Particle size measurement methods

The NanoDefine consortium was founded to support the implementation of the EC definition on nanomaterials. It included European RTD performers, experts from metrology institutes, nanomaterial suppliers, instrument manufacturers, regulators as well as academics. The objective of the group was to develop an approach which allows to identify whether a material is nano or not according to the EC definition using only robust, readily available and standardized methods to provide a reliable analysis of the number-based size distribution. The resulting list exhibits tier 1 (screening purposes) and tier 2 (confirmatory purposes) methods [13].

In our study, we used two complementary measurement methodologies for each material: asymmetrical flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry (ICP-MS) or UV detector as a tier 1 method and Transmission Electron Microscopy (TEM) as a tier 2 method. The two methods are appropriate to evaluate inorganic materials in the relevant nano size range and provide number-sized distribution of the measured materials. Besides the suitability of the methodology itself, the sample preparation is a core aspect to achieve an accurate result and should not modify the size distribution of the original material. Therefore, recommendations on sample preparation exist using standard operation procedures [14].

Asymmetrical flow field-flow fractionation (AF4)

The asymmetrical flow field-flow fractionation (AF4) system (Eclipse separation system AF4, Wyatt Technology, USA) with a trapezoidal channel with a nominal thickness of 350 µm (spacer W350 µm, Wyatt Technology, USA) fitted with a regenerated cellulose membrane with 10 kDa cutoff (PLGC, Reg. Cellulose 10 kD, Millipore, USA) was used. The AF4 was coupled to an UV spectrometer (DAD 1290 Infinity II, Agilent

Sample No.	INCI	Product form	Size indication provided by the supplier
1	Titanium Dioxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina	Dispersion	179 nm with X Ray Disc Centrifuge
2	Titanium Dioxide (nano) (and) Silica	Powder	(nano) indication given by the supplier
3	Zinc Oxide (and) Titanium Dioxide (and) Silica	Powder	Average particle size 455 nm (DLS)*

* Dynamic Light Scattering

Table 1: Investigated Titanium Dioxide and Zinc Oxide market grades.

Technologies) and an ICP-MS (NexION 2000, Perkin Elmer, USA) detector. In this technique, the fractionation occurs in a trapezoidal channel with a semipermeable membrane at the bottom of the channel, called the accumulation wall. After sample injection, the solvent flow transports the sample particle species to the outlet and detectors. The solvent flow has a parabolic velocity profile with the highest velocity at the mid-height of the channel. The fractionation is performed by applying a cross flow perpendicular to the channel flow, which pushes the sample particle species towards the accumulation wall and leaves the channel through the semipermeable membrane. At the same time, the Brownian motion of particles opposes the drag of the cross flow, resulting in the distribution of the sample particles along the vertical axis of the channel. The shape of distribution is then defined by the interplay between the cross flow and the particle diffusion coefficient, D , the latter being inversely proportional to the particle hydrodynamic radius. Therefore, the particle species with smaller radii (larger diffusion coefficients) are further away from the accumulation wall, transported faster and elute first, while the bigger particle species (smaller diffusion coefficients) elute later. The separated particles are then detected using an appropriate detector. The AF4/UV data were analyzed with Astra software, version 7.3 (Wyatt Technology, USA). The ICP-MS data acquisition and analysis were performed using Syngistix software, version 2.4 (Perkin Elmer, USA). The methodology provides a mass-weighted distribution of the particle size, which can be converted to a number-weighted size distribution. The method also provides information on the chemical composition of the particle material via the ICP-MS detector. The AF4 with both detectors was thoroughly evaluated using the NanoDefine Method Manual [11,16]. The calibration of the elution times as function of particle size was done according to the polystyrene size calibration standards (22 ± 2 nm, 51 ± 3 nm, 100 ± 4 nm, 203 ± 5 nm and 345 ± 7 nm, Nanosphere Size Standards, Thermo Scientific, USA).

The powder samples (sample 2 and sample 3, **Table 1**) were prepared in sodium hexametaphosphate solution ($c = 2$ g/l) [15] to obtain a sample concentration of $c = 0.5$ g/l according to the standardized dispersion protocols for high priority material groups (Technical Report D2.3 (6.9)). The Titanium Dioxide dispersion (sample 1) and the market sunscreen emulsion were dispersed in a water solution of Triton X-100 (MP Biomedicals, Irvine, CA, USA) ($c = 5$ g/l) to obtain a sample concentration of $c = 10$ g/l. The dispersions were then sonicated (Branson 550 Sonifier, Model 102C converter, Branson Ultrasonics) twice for 15 minutes at 60% of amplitude with a 5 minute pause, and finally purified passing through a $5,0 \mu\text{m}$ filter before injection into the AF4 setup.

Transmission Electron Microscopy (TEM)

Samples for TEM were prepared on ultra-thin carbon-coated TEM grid carriers (ECF200-Cu-50 from Science Services GmbH Munich, Germany). The Titanium Dioxide dispersion (sample

1, table I) was dispersed 1:100 in a water solution of 50 mg/L Triton X-100. The two powder samples (sample 2 and sample 3, **Table 1**) and market sunscreen emulsion were dispersed 1:100 in ethanol. In addition, for the sunscreen emulsion, the inorganic content was separated from the organic oil content by centrifugation using an Eppendorf MiniSpin at 13400 rpm (Eppendorf DE, Hamburg, Germany). The Titanium Dioxide dispersion was analyzed with a Themis Z3.1 TEM (Thermo-Fisher, Waltham, USA); the three other samples with a Tecnai Osiris F200 TEM (Thermo-Fisher, Waltham, USA) in bright-field as well as HAADF-STEM (High-Angle Annular Dark Field with Scanning Transmission Electron Microscope) mode. Both microscopes are equipped with a Super-X detector for Energy-Dispersive X-ray Spectroscopy (EDS) for chemical analysis. The data were analyzed using the Thermo-Fisher Velox 2.1x and Bruker Esprit (Bruker, Billerica, USA, version 2) software packages.

Results

The nano range conventionally referred to a size ranging from 1 to 100nm, corresponding to the scale at which nano-related incidences are most expected to take place. The relevance of taking into account the size of the constituent particles in the definition of the Cosmetic Regulation EC 1223/2009 was explained by the fact that, over time, primary particles may be released from the agglomerates or aggregates due to condition changes as stated in the EC recommendation (2011/696/EU) [6]. The EC recommendation (2011/696/EU) revised June 10th 2022 furthermore states that a material is a nanomaterial when 50% or more of the particles of the material exhibit one or more external dimensions in the size range 1 nm – 100 nm in the number size distribution. The quantitative criterium enables the use of the definition in a regulatory context and became a common basis for regulatory purposes; indeed, the classification of a material as nano has consequences on its approval requirements. Therefore, the identification as to whether a material is nano or not is of prime importance.

Asymmetrical flow field-flow fractionation

The elution fractions were analyzed with two different detectors, the ICP-MS and UV detectors. The ICP-MS detector is element specific and allows to detect the presence of the metal ion in each eluting fraction, i.e. in our study Titanium or Zinc. The UV detector allows to detect UV absorbing species in investigated samples and, furthermore, serves for the establishment of the correlation between elution time and particle size of the calibration latex standards.

Figure 1 displays the result of different sizes of reference polystyrene latex particles (20 nm, 50 nm, 100 nm, 200 nm) to establish the correlation between the elution time from the channel and the corresponding size of the particles.

In the illustrative example presented in **Figure 1**, a latex particle with a size of 50 nm is eluted and detected after 20 minutes.

The results of the AF4 measurements coupled with UV or ICP-MS detectors are obtained in the form of elugrams; **Figure 2** displays the elugram of the Titanium Dioxide dispersion (sample 1) as an example.

The X-axis corresponds to the detection time of the particles after they were separated by asymmetric flow field-flow fractionation and left the channel. The smaller the particles, the faster they leave the channel and are detected. The scatter blue curve (upper curve) corresponds to the signal from Titanium as recorded by the ICP-MS detector, and corresponds in our sample to the Titanium Dioxide particles. Titanium is detected after an elution time of 12 – 16 minutes and again after 20 – 35 minutes. The solid black line corresponds to the signal recorded by the UV detector, and overlaps the ICP-MS signal with a detection of UV absorbing species after an elution time of 13 – 16 minutes and a second population between 20 – 30 minutes corresponding to the Titanium Dioxide particles. According to the calibration with latex standards (**Figure 1**), an elution time of 12 – 16 minutes corresponds to particles with a size of less than 22 nm; the elution time between 20 and 35 minutes of the second population species of Titanium Dioxide corresponds to particles with a size ranging from 50 to 200 nm. Assuming the measurement is representative for the complete sample and the particles are spherical and knowing the density of Titanium Dioxide and Zinc Oxide, the mass-weighted particle size distribution can be converted into a number-weighted particle

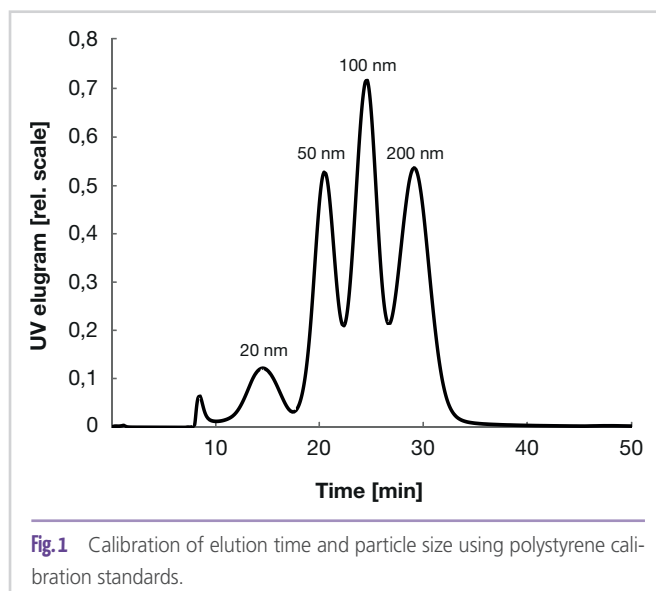


Fig.1 Calibration of elution time and particle size using polystyrene calibration standards.

Sample	Tested inorganic material	Median (D50) (nm) measured by AF4	Size indication provided by the supplier
Dispersion (sample 1)	Titanium Dioxide	13 nm	179 nm with X Ray Disc Centrifuge
Powder (sample 2)	Titanium Dioxide	137 nm	(nano) indication given by supplier
Powder (sample 3)	Titanium Dioxide	120 nm	Average particle size 455 nm (DLS)*
Sunscreen market emulsion	Zinc Oxide	22 nm	Zinc Oxide was not listed as a nano UV filter in the INCI

* Dynamic Light Scattering

Table 2: Median particle size values (AF4 measurements) and size indication provided by the supplier.

size distribution. The results for each sample are displayed in **Figure 3**.

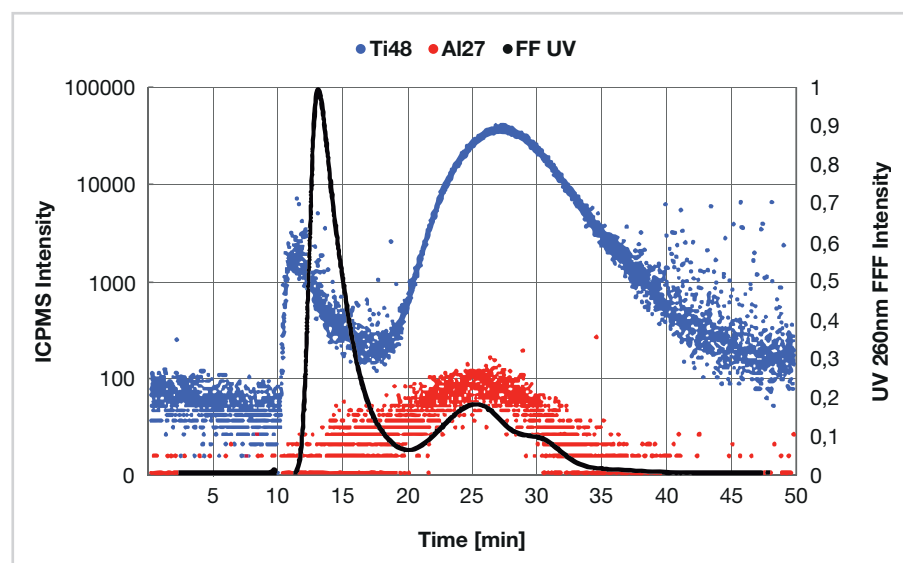


Fig.2 Elugram of Titanium Dioxide dispersion (Titanium Dioxide (and) C 12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina). The solid black line corresponds to the signal detected by the UV detector; the scatter blue (upper) and red (bottom) curves correspond to the signals detected by the ICP-MS detector for the element Titanium and Aluminum, respectively.

Table 2 summarizes the number-based median particle size obtained by AF4 measurements and the size indication provided by the supplier. The AF4-based result classifies Titanium Dioxide dispersion (sample 1) as nanomaterial with a median particle size of 13 nm, which is in discrepancy with the data provided by the supplier claiming a particle size of 179 nm and no indication of which parameter the value of 179 nm is exactly referring to (median, mean...). Also, the Zinc Oxide contained in the market product is measured as being nano, however, the INCI list for that product does not mention the nano feature of the Zinc Oxide. The Titanium Dioxide particles of sample 2 and 3 show a median size greater than 100 nm and both would

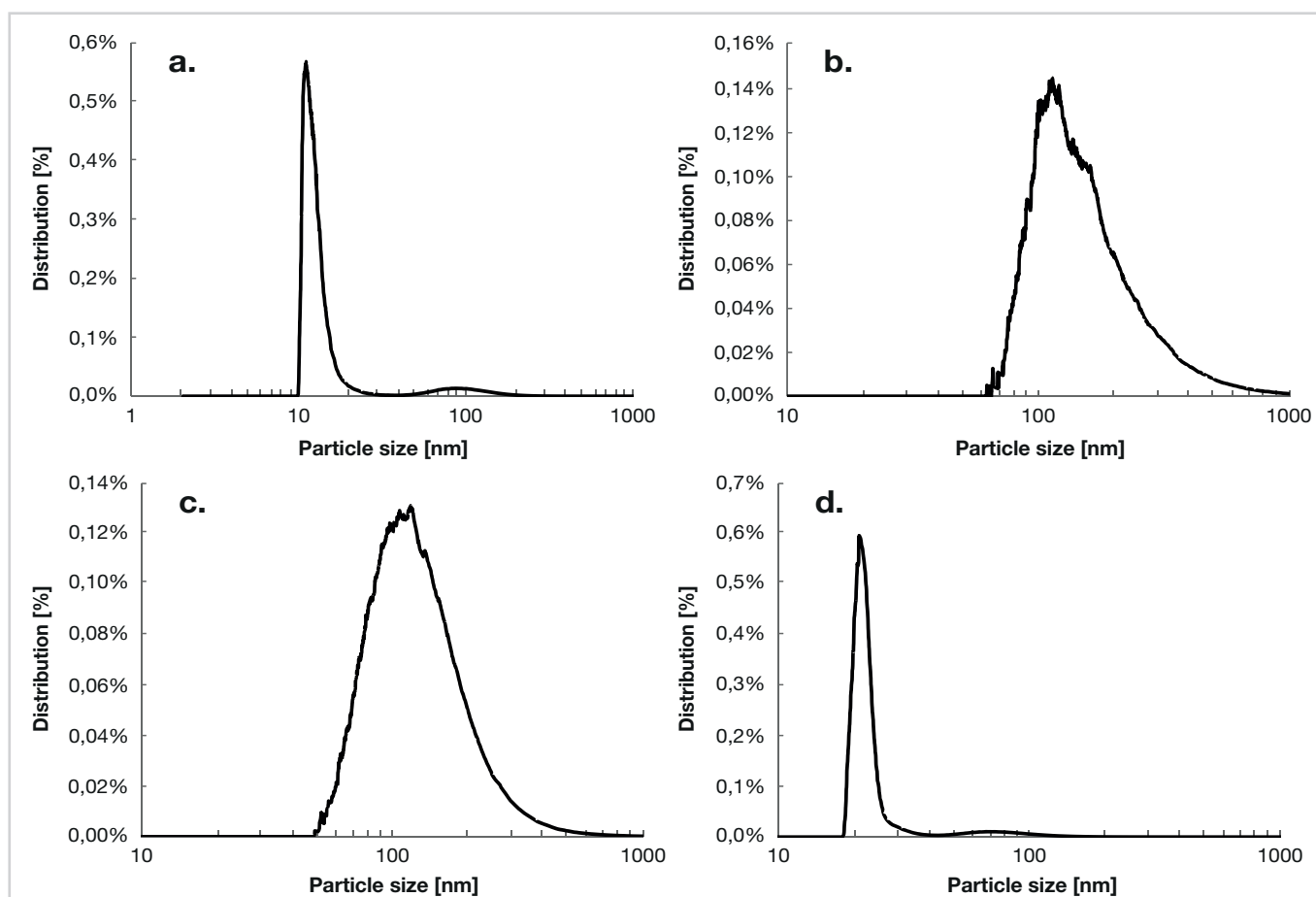


Fig. 3 Number-based particle size distribution using AF4 ICP-MS of: **a.** sample 1 (Titanium Dioxide dispersion); **b.** sample 2 (Titanium Dioxide powder); **c.** Titanium Dioxide in sample 3; **d.** Zinc Oxide in the sunscreen market emulsion.

be classified as non-nanomaterials. The supplier of sample 2, however, mentions the nano status of this Titanium Dioxide grade. The reason of the discrepancy between the AF4 measurements and the nano status claimed by the supplier could be due to the presence of agglomerates or aggregates in sample 2. These discrepancies reveal the complexity of measuring an accurate particle size. In fact, from all methods referenced in part 2 of the NanoDefine Methods Manual [16] and providing a number-based distribution of the size, only electron microscopy techniques can identify and count the constituent particles in aggregates/agglomerates. All other counting methods cannot distinguish between large particles and agglomerates, consisting of smaller primary particles, both implying a risk of misinterpretation and of classifying a nano material as non-nano material. If the employed method provides a mean or median size of particles below 100 nm, it can be concluded that the tested material is a nanomaterial. This is the case for Titanium Dioxide dispersion (sample 1). The fact that the supplier of sample 1 provides a particle size of 179 nm is most probably due to the X-Ray Disc Centrifuge method used, which is not able to detect the constituent Titanium Dioxide particles in agglomerates/aggregates. The reason for this could be the sample preparation, which was not thorough enough to individualize the constituent particles. Concerning the market product, the producer most probably relied on the information provided by the supplier of the Zinc Oxide, which assumably

also wrongly characterized its ingredient. As there remains uncertainty when using a tier 1 method, a result which indicates that a material is non-nano should then be confirmed using a tier 2 technique such as Electron Microscopy. Therefore, all samples in our study were further evaluated with TEM.

Transmission Electron Microscopy

TEM is a counting methodology and belongs to the tier 2 confirmatory methods. It is adapted to the detection and counting of the particles with sizes ranging from 1 nm to more than 1 μm . For each evaluated particle, the smallest dimension (ferret m_y) was considered. **Figure 4** displays the cumulative number-based size distribution of the TEM evaluation for all samples.

Table 3 summarizes the number metrics (number of particles analyzed, median value of the size distribution, percentage of particles with sizes smaller than 100 nm).

Figure 5 shows a representative TEM image of the dispersion Titanium Dioxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina as an illustrative example.

The TEM evaluation revealed that the Titanium Dioxide and Zinc Oxide particles of all investigated samples are nanoma-

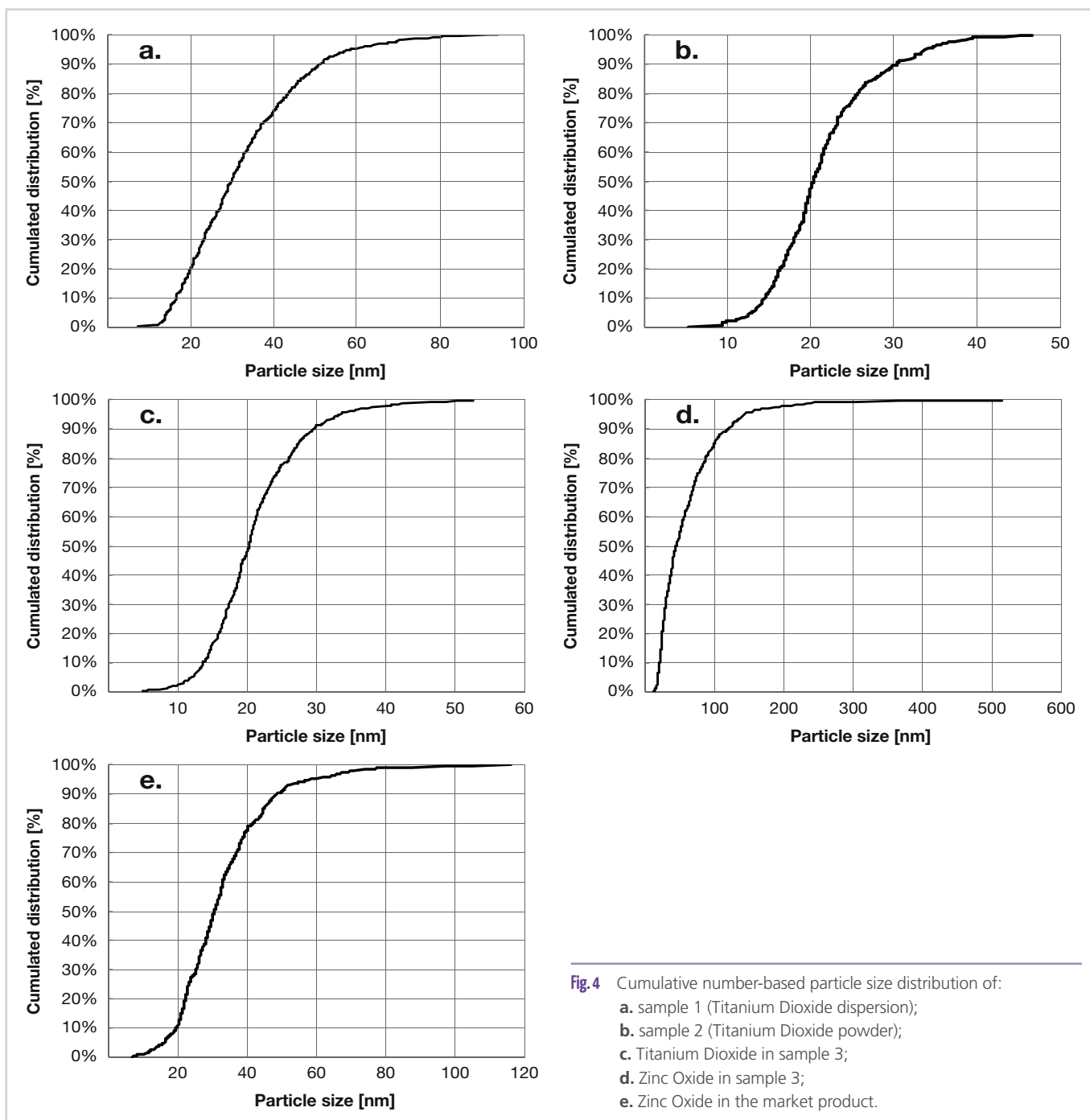


Fig. 4 Cumulative number-based particle size distribution of:
a. sample 1 (Titanium Dioxide dispersion);
b. sample 2 (Titanium Dioxide powder);
c. Titanium Dioxide in sample 3;
d. Zinc Oxide in sample 3;
e. Zinc Oxide in the market product.

terials; the median size value of each being well below 100 nm; for almost all samples, even 100% of the particles lie below the threshold of 100 nm. The difference in the particle size values between tier 1 and tier 2 methodologies for sample 2 and 3 suggests the presence of agglomeration or aggregates and underlines the importance of the correct selection of methodology and sample preparation. Methods which are based on the motion of the particles in a medium tend to measure the size of the aggregates rather than that of the primary particles, since the aggregates are the moving entities [17]. Such methods include Dynamic Light Scattering where the diffusion of

the particles is analyzed; also centrifugation and field-flow fractionation where particles move in a centrifugal force field and flow field, respectively.

Sample	Form	Number of particles analyzed	Median (D50) (nm)	Particles < 100 nm (%)	
1	Dispersion	870	28.0	100.0%	
2	Powder	425	20.4	100.0%	
3	Powder	TiO ₂	477	19.9	100.0%
		ZnO	564	42.8	86.2%
4	Sunscreen market emulsion	509	30.0	99.6%	

Table 3: TEM number metrics of tested samples.

This study reveals that limiting the nano interpretation of a material to a tier 1 methodology such as AF4, X-Ray Disc Centrifuge or Dynamic Light Scattering is clearly insufficient in the case of Titanium Dioxide or Zinc Oxide UV filters, and leads to an erroneous interpretation and classification of the ingredient as a non-nano material.

Conclusion

In the present study we applied the decision support flow scheme devised by the NanoDefine project to identify if investigated materials are nano or non-nano according to the EC definition of nanomaterials. We determined the median value of the particle size and number-based size distribution of several marketed Titanium Dioxide and Zinc Oxide products. The study revealed that when using only a tier 1 methodology, a sample may erroneously appear to be a non-nanomaterial, which may result in misinterpretation. This is explained by the fact that tier 1 methods are not able to detect constituent particles as part of agglomerates. Two samples of Titanium Dioxide tested in the present study appeared to be non-nano with AF4 measurements, but were finally proven to be nanomaterials using the TEM tier 2 method. For these reasons, the non-nano statement provided by a supplier who relies only on a tier 1 method, such as X-Ray Disc Centrifuge or Dynamic Light Scattering, should be doubted until it is verified by using an electron microscopy tier 2 technique.

Acknowledgments:

We would like to thank **Mechtild Petersen-Thiery** and **Prof. Christian Surber** for the discussions on nano particulate filters.

References:

- [1] Sohn M., et al. How to overcome the new challenges in sun care. SOFW-journal (2020) 146.
- [2] Scientific Committee on Consumer Safety, SCCS/1546/15. Revision of 25 June 2015, OPINION ON 2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (nano form). https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_168.pdf Accessed February 2022.
- [3] Scientific Committee on Consumer Safety, SCCS/1429/11. Revision of 13/14 December 2011, OPINION ON 1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_070.pdf Accessed February 2022.
- [4] Scientific Committee on Consumer Safety, SCCS/1634/21. Final opinion on HAA299 (nano), 26 and 27 October 2021. https://ec.europa.eu/health/system/files/2021-11/sccs_o_256.pdf Accessed February 2022.
- [5] Mintel, Global new products database (GNPD) Personal Care portal, Search criteria: sun protection, Europe, 2020. <http://www.gnpd.com/>. Restricted access, Accessed 20 January 2022.
- [6] Scientific Committee on Consumer Safety, Guidance on the safety assessment of nanomaterials in cosmetics, adopted on 30-31 October 2019 https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_233.pdf Accessed February 2022.
- [7] Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. https://ec.europa.eu/health/system/files/2016-11/cosmetic_1223_2009_regulation_en_0.pdf Accessed February 2022.

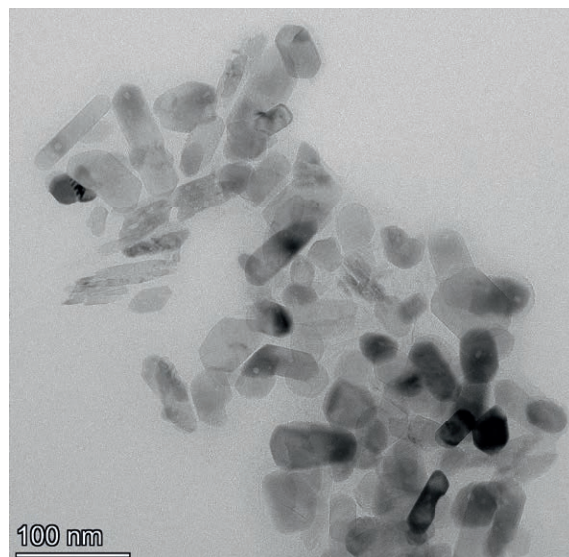


Fig. 5 TEM image of the Titanium Dioxide dispersion (sample 1).

- [8] Commission Regulation (EC) No 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III, VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances (Text with EEA relevance.) <http://data.europa.eu/eli/reg/2018/1881/oj> Accessed February 2022.
- [9] Commission, E., Commission recommendation (2011/696/EU) of 18 October 2011 on the definition of nanomaterial. available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011H0696&from=EN> Accessed February 2022.
- [10] NanoDefine, <http://www.nanodefine.eu>. 2013. Accessed February 2022.
- [11] Rauscher H., et al. Identification of nanomaterials through measurements, EUR 29942 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-76-10372-1, doi:10.2760/053982, JRC 118158. 2019.
- [12] Mech A., et al. The NanoDefine Methods Manual, EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-12336-1, doi:10.2760/58586, JRC 117501. 2020.
- [13] Mech, A., et al. The NanoDefine Methods Manual. Part 1, The NanoDefiner framework and tools. EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2020, <https://data.europa.eu/doi/10.2760/824352>. Accessed February 2022.
- [14] Mech, A., et al. The NanoDefine Methods Manual. Part 3, Standard Operating Procedures (SOPs). EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2020, <https://data.europa.eu/doi/10.2760/778>. Accessed February 2022.
- [15] The NanoDefine consortium 2016, The EU FP7 NanoDefine Project. Standardised dispersion protocols for high priority materials groups. NanoDefine Technical Report D2.3. http://www.nanodefine.eu/publications/reports/NanoDefine_TechnicalReport_D2.3.pdf. Accessed February 2022.
- [16] Mech, A., et al. The NanoDefine Methods Manual. Part 2, Evaluation of methods. EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2020, <https://data.europa.eu/doi/10.2760/06428>. Accessed February 2022.
- [17] Herzog, B. Molecular aggregates in sunscreens – from liposomes to particles. Proceedings 30th IFSCC congress, Munich 2018.

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