Scientific Committee on Consumer Safety

SCCS

OPINION ON
Titanium Dioxide (nano form) as UV-Filter in sprays

The SCCS adopted this Opinion

on 7 March 2017

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems that may pose an actual or potential threat.

These Committees are the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

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1. BACKGROUND

Titanium Dioxide (CAS/EC numbers 13463-67-7/236-675-5, 1317-70-0/205-280-2, 1317-80-2/215-282-2) is authorised both as colorant under entry 143 of Annex IV and as UV-filter under entry 27 of Annex VI to Regulation (EC) No 1223/2009.

In July 2013 the Scientific Committee on Consumer Safety (SCCS) delivered an Opinion on Titanium dioxide (nano) (SCCS/1516/131[1]) to assess the safety of the nano form of Titanium Dioxide. In that Opinion, the SCCS concluded that the use of Titanium Dioxide (nano) as UV-filter in sunscreens, with the characteristics indicated in the Opinion, and at a concentration up to 25 %, can be considered not to pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin.

The SCCS also considered that, on the basis of available information, the use of Titanium Dioxide nanoparticles in spray products cannot be considered safe. In addition, the SCCS indicated, in a further Opinion of 23 September 2014 for clarification of the meaning of the term "sprayable application/products" for the nano forms of Carbon Black CI 77266, Titanium Dioxide and Zinc Oxide[2], that its concern is limited to spray applications that might lead to exposure of the consumer's lungs to Titanium Dioxide nanoparticles by inhalation.

In July 2015, the Commission' services received new data from industry to support the safe use of Titanium Dioxide (nano) when used as UV-Filter in sunscreens and personal care spray products at a concentration up to 5.5%.

2. TERMS OF REFERENCE

1. *In light of the data provided, does the SCCS consider Titanium Dioxide (nano) safe when used as UV-Filter in sunscreens and personal care spray products at a concentration up to 5.5%?*

2. *Does the SCCS have any further scientific concerns regarding the use of Titanium Dioxide (nano) when used as UV-Filter in sunscreens and personal care spray products?*

[1] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_136.pdf
[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_163.pdf

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Titanium dioxide
Titanium dioxide (nano)

3.1.1.2 Chemical names

Titanium dioxide

3.1.1.3 Trade names and abbreviations

PARSOL® TX
PARSOL® TX 50AB
Lot No 401004016
Lot No 401002166

3.1.1.4 CAS / EC number

13463-67-7/236-675-5 (CAS/EC)
1317-70-0/215-280-1 (CAS/EC)
1317-80-2/215-282-2 (CAS/EC)

3.1.1.5 Structural formula

TiO_2

3.1.1.6 Empirical formula

TiO_2

3.1.2 Physical form

Titanium dioxide (nano) used in the enclosed studies is a white powder (Ref-A; Ref-B). It is mainly in the rutile form measured by X-ray diffraction (Ref-C).

3.1.3 Molecular weight

Molecular weight of TiO_2: 79.9 g/mol

3.1.4 Purity, composition and substance codes

According to the Applicant, the titanium dioxide (nano) contained in the batches Lot 401004016 and Lot 401002166 is a yield from regular production.
This material complies with the current US Pharmacopeial Convention specifications set for titanium dioxide as well as with the characteristics as included in the SCCS Opinion SCCS/1516/13 revised on 22 April 2014, and the draft Regulations "15-GROW-COS-

COSCOM-11a Act Titanium Dioxide (nano) and "15-GROW-COS-COSCOM-11b Annex Titanium Dioxide (nano)".

An overview of the characteristics of Lot No 401004016 and Lot No 401002166 are summarised in Table 1.

Table 1: Characteristics of Lot No 401004016 and Lot No 401002166

Characteristics according to *Draft COMMISSION REGULATION (EU) amending Annex VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products*	**Result Lot No 401004016**	**Result Lot No 401002166**
Purity ≥99%	>99% (Ref-C)	99.95% (Ref-C)
Rutile form, or rutile with up to 5% anatase, with crystalline structure and physical appearance as clusters of spherical, needle, or lanceolate shapes	Complies (Ref-C) (Ref-G)	Complies (Ref-C) (Ref-G)
Median particle size based on number size distribution ≥ 30 nm	Complies*	102 nm (Ref-E)
Aspect ratio from 1 to 4.5	Complies (Ref-C)	Complies (Ref-C)
volume specific surface area ≤460 m^2/cm^3	Complies*	Complies*
Coated with silica, hydrated silica, alumina, aluminium hydroxide, aluminium stearate, stearate, stearic acid, trimethoxycaprylylsilane, glycerin, dimethicone, dimethicone/methicone copolymer, simethicone;	Complies (Ref-D)	Complies (Ref-D)
Photocatalytic activity ≤10%	**	8.8% (Ref-F)

*not measured for this specific production lot, however compliance is ensured based on internal measurements performed on other production material.
** not measured for this specific production lot

SCCS comments

The above specifications as reported by the Applicant relate only to the exposure studies conducted. No toxicological studies have been submitted by the Applicant regarding these batches or other similar material.
Further, it should be noted that compliance with the draft commission regulation only relates to dermal application/exposure. Inhalation exposure was not considered in the cited regulation, so that compliance does not mean absence of toxicological concern regarding inhalation exposure.

Only one lot has been tested for photocatalytic activity.

3.1.5 Impurities / accompanying contaminants

Not provided

SCCS comments

Analytical data on impurities were not submitted. Since purity was >99%, hence 1% can be impurity, data on impurities are needed.

3.1.6 Solubility

TiO_2 is insoluble in water and organic solvents. It also has a very low dissociation constant in water and aqueous systems, and thus can in practice be considered as insoluble also under physiological conditions.

(Numerous references in open literature)

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: Not applicable for uncoated TiO_2.

SCCS comments

The partition coefficient only describes materials by and after their dissolution in octanol/water, which is not applicable for uncoated nanoparticles. However, the distribution between polar and non-polar phases should be described for TiO_2 nanomaterials coated with organic substances.

3.1.8 Additional physical and chemical specifications

Melting point:	not provided, not risk relevant
Boiling point:	not provided, not risk relevant
Flash point:	not applicable
Vapour pressure:	not applicable
Density:	not provided
Viscosity:	not provided, not risk relevant (for TiO_2)
pKa:	not applicable for uncoated TiO_2
Refractive index:	not provided
UV_Vis spectrum (….. nm):	not provided

SCCS comments

The data on density and UV/Vis is risk relevant and should be provided.

3.1.9 Homogeneity and Stability

Not provided.

General comments on physicochemical characterisation

The SCCS considers the physicochemical characterisation of the nano-TiO_2 materials under evaluation as insufficient for an assessment of its toxicological effects after inhalation, which is the special focus of this dossier. Particle size distributions of a representative sample of materials to be used in sprays are required. This is even more important because currently the inhalation exposure studies have not been performed with a representative set of formulations. Although the materials evaluated in the exposure studies have been reported by the Applicant to comply with the specifications that have been given in SCCS, 2014, it should be recalled that the cited SCCS Opinion focused on dermal exposure and excluded inhalation. After spraying, the size distribution and agglomeration status of the particles may change, and therefore compliance with the specifications from SCCS, 2014 does not guarantee absence of effects in this case.

3.2 Function and uses

Titanium dioxide is used as a UV-filter in a concentration of up to 25% in cosmetic products. It is regulated in Annex VII, entry 27 of the Cosmetics Directive. In the bulk form it may also be used as a white pigment, while the nano-form is colourless. TiO_2 in the nano-form is primarily used in sunscreens, but might also be used in leave-on products that claim to provide UV-protection. Outside the European market, nano-TiO_2 has been reported to be also used in sunscreens formulated as sprays (e.g. in Brazil, see dossier of the Applicant) and as powder (e.g. US, Lorenz *et al.*, 2010).

The Applicant has submitted a) a market analysis on sunscreen pump sprays that presently contain bulk TiO_2 and therefore may be the ones to contain nano- TiO_2 in future and b) a release study under controlled conditions in a chamber to argue that nano- TiO_2 can safely be applied in sunscreen sprays. The latter study comprises data on nanoparticle release from 4 different (apparently) non-commercial formulations of sunscreens and one commercial sunscreen available in Brazil. The Applicant provided further information in December 2015 upon request of the SCCS.

3.2.1 Occurrence

The Applicant submitted a European market analysis over the last five years (DSM, 2015-Annex 1) which shows that in Europe, most cosmetic sunscreen products placed on the market in the form of sprays, lotions and creams are either oil-in-water (O/W) or water-in-oil (W/O) emulsions.

Further, according to the Applicant the analysis shows that:

a) The sunscreen sprays containing TiO_2 launched within the above-mentioned period are 100% emulsions. About 80% of them are oil-in-water emulsions, and around 20% are water-in-oil emulsions.

b) The composition of the O/W emulsions is either based on hydrocolloid stabilizers like polysaccharide, modified polysaccharide and/or acrylates copolymers or on a combination of hydrocolloid stabilisers and typical O/W emulsifiers like fatty alcohol ethoxylates, fatty acids, fatty acid esters, fatty alcohols, polyglycerin esters, alkylglucosides and/or phosphate acid esters. A limited number of sprayable products are only based on typical O/W emulsifiers without the addition of hydrocolloid stabilizers.

c) The composition of W/O emulsions is generally similar to O/W emulsions as detailed under point b). The main difference is the choice of emulsifier which is much more hydrophobic to be able to disperse the water in the oil phase.

According to the Applicant, sunscreen formulations in pump sprays that could contain nano-TiO_2 will have a low content in ethanol because of the following reasons:

Typical cosmetic macro (simple) emulsions are described using oil (O) and water (W), immiscible fluid pairing stabilised by the use of emulsifiers. In case of an O/W emulsion, oil droplets are dispersed in water. In case of a W/O emulsion, water droplets are dispersed in oil.

Beside O/W and W/O emulsions only ethanol and oil-based spray systems are present on the European sun care market. In the case of the ethanol-based system, the organic UV filters are generally dissolved in different oily emollients/solvents and complemented with ethanol (>30%). In case of the oil-based system, the oil soluble organic UV filters are dissolved in oily emollients/solvents and no ethanol is added or only a limited amount (<15%). Both products finally have a transparent appearance with very low viscosity like an oil or even water. No emulsifier is required in these formulations; ingredients are miscible with and soluble within each other.

According to the Applicant, TiO_2 cannot be stabilised and suspended in low viscous oil based or ethanol based systems. If TiO_2 is added to these systems the product will quickly settle down. To suspend TiO_2 into these kinds of products the viscosity needs to be significantly increased which would result in a non-sprayable product.

According to the Applicant, consequently, TiO_2 cannot be used in sprayable ethanol or oil based systems; they claim that this is also shown by the MINTEL analysis (DSM, 2015). According to the Applicant, no sprayable ethanol or oil-based sunscreen products containing TiO_2 were found in their market analysis ranging from January 2010 to December 2015.
According to the Applicant, the results of the European market analysis over the last five years (Mintel from January 2010 until December 2015 - Annex 1) show that:

a) The composition as indicated on the packaging lists all the ingredients in descending order of weight of the ingredients at the time they are added (Art 19.1.(g)/(EC) 1223/2009); aqua (water) is the first ingredient included in the ingredient list and is expected to be present at a concentration of about 50%.

b) The sunscreen sprays containing TiO_2 launched within the above-mentioned period are 100% emulsion based and consequently water based. Nearly 80% of the sprayable sunscreen products containing TiO_2 marketed in the EU are oil-in-water (O/W) emulsions.
The Applicant states that the market analysis (Annex 1) allows concluding that the sunscreen formulations containing titanium dioxide marketed in pump sprays in the EU are exclusively water-based.

SCCS comments
The SCCS re-evaluated the submitted market analysis and has noted that contrary to the Applicant's statement not all sunscreens on the European market that may contain nano-TiO_2 are water-based.

More specifically, 7 out of the 11 W/O spray formulations are not water-based (either very low or no "aqua" listed in the ingredients list). Instead different emollients (dicaprylyl carbonate, caprylic/capric-triglyceride and others) make up the body of the formulation.

According to a supplier, dicaprylyl carbonate has a very low viscosity of 6-8 mPas at 20°C (BASF, 2016). Another supplier states: 'Its ability to dissolve crystalline UV filters and to disperse pigments makes it particularly suitable for sun care products.' (De Wolf, 2016). Therefore it can be expected that this type of formulation is also relevant for sprayable nano- TiO_2 products. Although water has a lower viscosity than dicaprylyl carbonate, it is not straightforward to calculate the viscosity of a mixture from the viscosities of the components. This also depends on the droplet size in the emulsions (Pal, 1996). As an example, the formulation 'Lubrizol', which is marketed in the US, has a much lower viscosity than the investigated products. It is therefore probable that there are formulations on the EU market with lower viscosities than water-based formulations and, hence, their droplet sizes after spraying may be smaller.

Furthermore, three out of the 43 O/W spray formulations were identified as possibly containing >10% ethanol, because ethanol is listed before a component that may be contained up to 10% (octocrylene) or up to 20% (C12-C15-benzoate). A larger ethanol content in the formulation may also result in smaller droplet sizes because it is readily volatilized, reducing the initial droplet size and enhancing the potential for exposure of the lung alveoli.
Although the Applicant has provided details of a few example formulations, these do not provide adequate account of the types and proportions of the carrier solvents/ emollients that are, or may be, used in sprayable formulations containing nano-forms of TiO_2. Furthermore, the Applicant has not provided information on coatings that may be used for nano-forms of TiO_2 in sprays. The Applicant should therefore lay down precise specifications

for the intended formulations including details of contained solvents/ emollients and coating of nanoTiO_2, which can then be considered by the SCCS.

3.2.2 Experimental studies on particle release

According to the Applicant, the particle size of sprayable products determines whether they can be inhaled and which part of the respiratory tract they can reach. The respiratory tract is divided in three sections: the nasopharyngeal region, the tracheobronchial region and the pulmonary region. The particle fractions reaching these regions are designated as the inhalation, thoracic and respirable fractions which are targeted by particles of the size >30 µm, 10-30 µm and <10 µm, respectively (Steiling *et al.* 2014). Usually particles below 10 µm are considered to be respirable i.e. to reach the alveoli. Initial particle size distribution at spraying will change due to maturation, which is the loss of volatile components and agglomeration. This maturation cannot presently be simulated in computational models. The Applicant has therefore experimentally investigated the maturation of spray particles from titanium dioxide (nano) containing sun-care sprays dispensed from pump-spray and bag-on-valve spray systems. The composition of the sprays is given in section 3.2.1.1. For test item 1 to 8 silica/dimethicone coated titanium (nano) was used as characterised in section 3.1.4. For test item 9 the composition is not known. Further characterisation of particle size etc. in the spray was not performed as these were market-typical sprays and it was the intention to investigate the particle characteristic after spraying. This was performed by determination of the release fraction by mass and analytical titanium-measurements with regard to a) mass in the three inhalation-related fractions, and b) as number of nano and micro-size particles. It was the aim of these studies to determine the potential exposure to the lungs.

3.2.1.1 Test items

According to the Applicant, all the ingredients to formulate the oil-in-water emulsions were chosen primarily for their potential to provide low viscosity emulsions that were both sprayable and stable and secondly for their market relevance. An assessment was done to see if they were used in marketed sprayable sunscreens. The complete information on formulations is given in Annex I.

3.2.1.2 Study setup

According to the Applicant, in a non-GLP study (Schwarz and Koch, 2015a), 9 sprays with different viscosities and different spray heads (volume emitted) covering 5 typical sunscreen formulations were investigated for their release fraction, i.e. the fraction of the mass released from the spray dispenser and found in the inhalable, thoracic and respirable fractions present after maturation of the spray particles. The release fractions are determined by spraying the product over a short time period to achieve a total material release of approximately 9 g into a release chamber with defined control volume, V, and carrying out time resolved measurements of the aerosol concentration (remaining non-volatile part after spraying). The measurement setup enables the determination of the matured particles, i.e. after evaporation of the volatile components. Measurement was performed with two parallel RESPICONs which are commercial aerosol-measuring instruments used for occupational inhalation exposure monitoring of inhalable, thoracic and respirable fraction. Measurements were done via continuous photometric measurement as well as gravimetric measurement on the filter stages of the three fractions. In addition, titanium on the filters was determined by ICP-MS.

According to the Applicant, in a parallel non-GLP study (Schwarz and Koch, 2015b) the same 9 products as used in the above study were analysed for the number fraction of particles generated in the nano-size range and in the micro-size range (<5 µm).
According to the Applicant, the method comprises measuring the release fraction of the number of nano-particles and estimating the number of micro-sized particles with diameters smaller than 5 µm. The release fraction given in units (1/g) is defined as the total number n of particles released into the air per mass of consumed spray formulation. To determine this release fraction, the product is sprayed into a control box (volume 75 L) and nanoparticles are measured with a condensation particle counter. This instrument measures the number concentration of particles with diameters larger than 10 nm. The upper size range captured by the instrument cannot be specified exactly but is in the range between 1 and 2 µm (1000 to 2000 nm). In order to capture only the nanoparticles a pre-separator is introduced into the sampling line to collect particles of 0.12 µm (<120 nm) diameter by the condensation particle counter. For a conservative safety analysis all particles passing the pre-separator are considered as nanoparticles, i.e. are attributed to the class smaller than 0.1 µm (100 nm).

According to the Applicant, in addition to measuring the number concentration of the nanoparticles (<0.12 µm), a number size distribution is measured using an optical particle counter operating in the particle size range between 0.26 µm and 5 µm. For the gap in the size scale from 0.12 to 0.26 µm that is not covered by the two instruments, an extrapolation scheme was used to estimate the particle number in this range based on the cumulative number distribution of the larger particles measured with the aerosol spectrometer.

SCCS comments on the study design

The most relevant information on the formulations tested, frame formulations and other formulations provided by the Applicant are summarised in Table 2.

Table 2: Characteristics of sunscreen formulations containing TiO_2 (italics: Formulations for comparison, not tested)

Formulations	Viscosity (mPa s)	TiO_2 (%)	Organic UV-filters (%)	SPF	Aqua (%)	Ethanol (%)
Recipe 22	2100	3	19	?	52	8
Recipe 35	1080	3	19	?	52	8
E42026503-00-2	3020	4.3	7-21	30	50-75	5-10
E47028018-00-4*	5000	5.5	12-35*	50+	25-50	5-10
Commercial	n.a.	n.a.	n.a.	30	n.a.	0
Frame O/W			*4 – 40*		*40-75*	*3-10*
Frame W/O			*4 – 40***		*0***-75***	*3-10***
Lubrizol (US)	*400-700*	*4.6*	*22*	*70+*	*44*	*0*

n.a. not analysed
* contains octocrylene at 10-25% even though the maximum allowed in the products on the European market is 10%
** in analogy to O/W formulations, as claimed by Applicant
*** based on market analysis

The approximately released mass of 9 g corresponds to the value recommended in the Notes of Guidance, SCCS/1564/15 (SCCS, 2015a) of 18 g per adult daily, which refers to two applications per day.
The SCCS considers that the following points are unclear in the dossier prepared by the Applicant:

- No measurement of TiO_2 content is provided for the commercial product. In order to allow extrapolations to other products, this is needed.

- It is stated that the study used a pre-separator to capture larger particles/droplets, and that the particles/droplets passing through were considered as nanoparticles. As TiO_2 nanoparticles are known to be agglomerative, how was it ensured that the pre-separator did not remove a proportion of nanoparticles along with the larger particles?

- For the spray heads no information on nozzle diameter, pressure generated, etc. is given. The technical details of the nozzles used in the study only refer to the dosage volume per 'throw'. The dosage volume per throw seems to be only a very rough proxy for the nozzle diameter, since it should mainly depend on the size of a reservoir chamber or the length and diameter of the rising pipe. More information on parameters like nozzle diameter or pressure generated would be necessary to conclude on the representativeness of the study for the European market.

- In order to evaluate the representativeness for the European market, the SCCS had requested a market survey on spraying devices used in Europe. Also this overview of spraying devices on the market lacks information on the nozzle diameter and pressure generated of the spraying device. For some devices the length of the rising pipe and the dosage in ml is given. Presumably, the dosage is meant "per throw".

- Although 5 spraying events were performed and averaged to calculate the release fraction, from the point-by-point description on Page 10, Schwarz und Koch, 2015a, it seems that no weighing of the cans was carried out between the 5 spraying events, so that the amount released would not be specific to the single measurements, but would represent an overall average. Therefore, the determined release fractions would not be completely independent and deriving standard deviations for the release fractions would be inadequate. Since a standard deviation for the total masses released is given in Table 2 of the same report, it is not clear whether the point-by-point description is wrong (then individual released masses should be reported somewhere) or which other data form the basis for the standard deviations.

- It is not clear why an upside-down adapter was used for 2 formulations but not for the others.

It should be noted that the measurement devices used in the experimental study could not distinguish between particles and droplets. Therefore, the term "particles" used by the Applicant is misleading. In the SCCS comments the term "particles/droplets" will be used instead.

3.2.1.3 Results from release studies

The RESPICON method was used to separate the respiratory, thoracic and inhalative fractions following the definitions provided in CEN, 1993. The method uses two stage cut-offs at 4 and 10 µm (Schwartz and Koch, 2015a), but these do not provide clear cut-off levels, but sample different fractions of different particle sizes according to Figure 1. The general cut-off of the method for the inhalable fraction is around 68 µm (Koch *et al*., 1999).

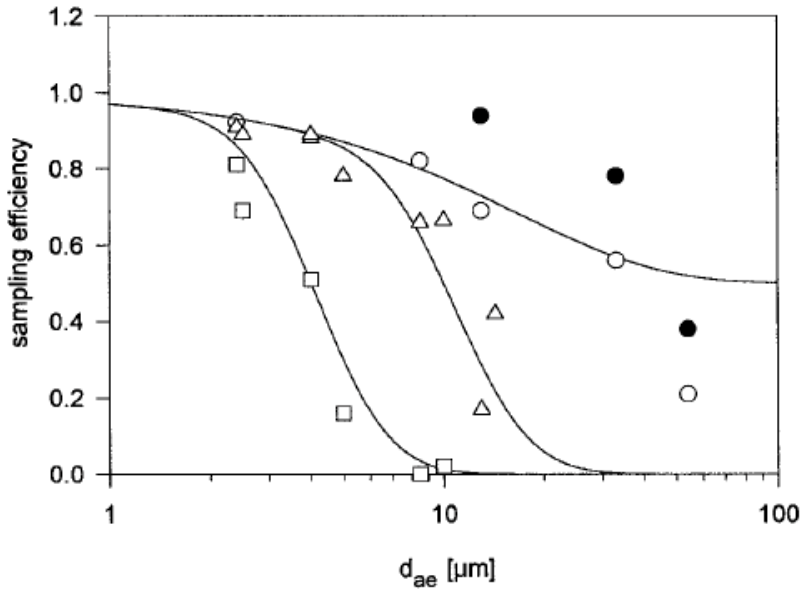

Figure 1: Copied from Koch *et al*., 1999: Experimentally determined sampling and classification characteristics of the RESPICON determined under calm air conditions (squares: respirable, triangles: thoracic, circles: inhalable fraction) compared with the corresponding definition curves after CEN, 1993 (full lines)

According to the Applicant, the respirable fraction for all products was below the optical detection limit related to mass (0.2 mg/m³). Results for the inhalable and thoracic release fractions (R) of non-volatile total mass by photometric determination are given in the following Table 3.

Table 3: Inhalable and thoracic release fractions (R) of non-volatile total mass (photometric determination)

	R [-]*				M[g]*	
	Thor*		Inh*			
Product	Ave.	St. Dev.	Ave.	St. Dev.	Ave.	St. Dev.
2219	1.2E-04	3.9E-05	1.5E-03	3.9E-04	9.09	0.30
2260	4.1E-05	2.7E-05	7.8E-04	2.0E-04	9.14	0.03
2290	9.6E-05	2.4E-05	1.1E-03	3.2E-04	8.85	0.90
3519	8.2E-05	8.0E-06	7.2E-04	1.3E-04	9.18	0.27
3560	1.5E-04	3.1E-05	1.3E-03	2.6E-04	9.03	0.03
3590	8.6E-06	3.0E-06	1.4E-04	2.6E-05	8.75	0.53
E47028018	< LOQ	-	5.6E-04	9.5E-05	9.26	0.09
E42036503	< LOQ	-	7.0E-04	1.5E-04	8.93	0.15
Sunscreen for kids FPS-30	2.6E-05	7.8E-06	1.0E-03	2.9E-04	8.97	0.28

* Abbreviations:
[-] unit-less values (ratio)
Thor = thoracic fraction
Inh = inhalable fraction
M = Mass

According to the Applicant, the aerosol collected on the filters for the three fractions was so small or contained so much semi-volatile mass that the RESPICON filters could not be

evaluated gravimetrically. Analysis of the filters for titanium by inductively coupled plasma mass spectrometry (ICP-MS) resulted in the values given in the Table below.

Table 4: Analysis of RESPICON filters for titanium by ICP-MS

Product	R [-]*					
	Resp*		Thor*		Inh*	
	Ave.	St. Dev.**	Ave.	St. Dev.	Ave.	St. Dev.
2219	1.7E-07	-	4.9E-06	8.2E-07	6.7E-05	1.8E-05
2260	1.7E-07	-	2.7E-06	1.2E-06	6.9E-05	1.5E-05
2290	2.0E-07	-	3.0E-06	7.5E-07	4.1E-05	1.3E-05
3519	1.6E-07	-	2.9E-06	2.9E-07	2.9E-05	5.1E-06
3560	5.9E-07	-	1.0E-05	2.1E-06	7.0E-05	1.5E-05
3590	2.7E-07	-	5.0E-07	2.0E-07	7.4E-06	1.9E-06
E47028018	2.5E-07	-	5.7E-06	-	1.7E-05	2.9E-06
E42036503	2.6E-07	-	6.3E-06	-	2.4E-05	5.2E-06
Sunscreen for kids FPS-30	3.7E-07	-	2.4E-06	7.6E-07	2.2E-05	5.2E-06

* Abbreviations:
[-] unit-less values (ratio) Resp = respiratory fraction
Thor = thoracic fraction Inh = inhalable fraction
** St. Dev. cannot be calculated for respiratory fraction since photometric signal below detection limit

These data are graphically presented in Figure 2.

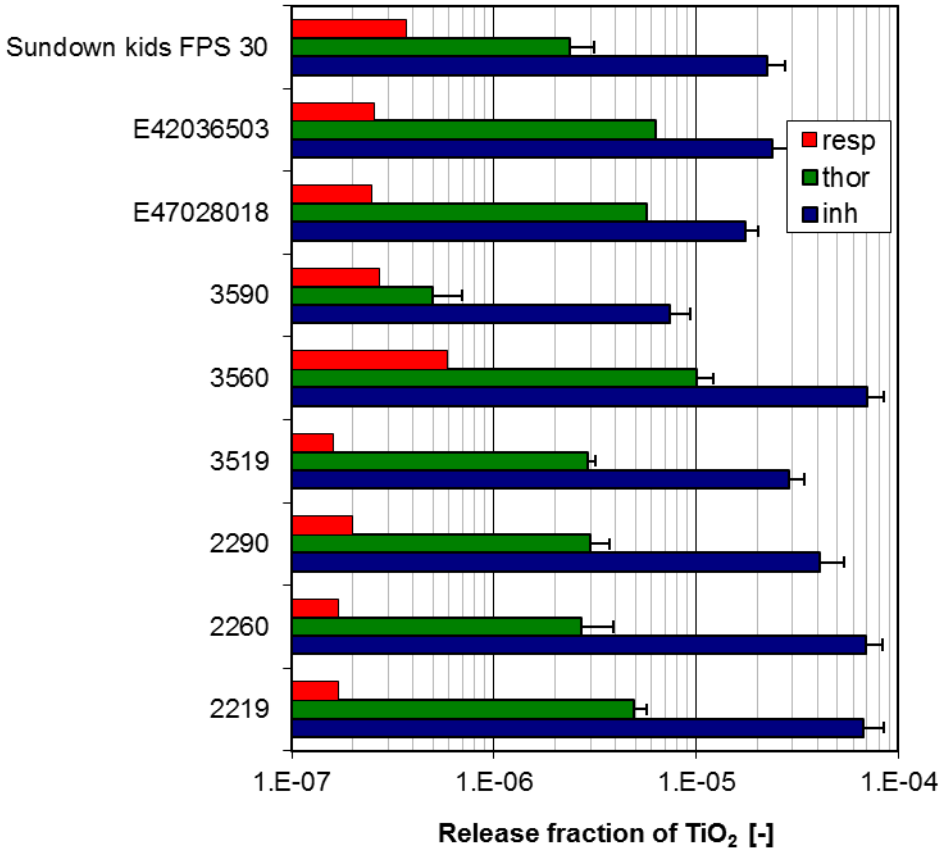

Figure 2: TiO_2 – release fractions of the 9 sunscreen sprays based on direct determination of Ti on RESPICON filters by ICP-MS

According to the Applicant, this study involved measuring the health-related aerosol release fractions for nine sunscreen spray products (5 formulations with different spray heads). Eight dispensers were pump sprays, which were spray bottles with a hand-squeezed trigger that pumps a liquid through a nozzle to generate a spray stream or a mist of the liquid (description of SCCS/1539/14, 23 September 2014), reflecting typical composition of sunscreen sprays available on the market. One product was a spray using bag-on-valve technology, which is commercially available in Brazil (Sunscreen for kids FPS-30). For all 9 sunscreen spray products, the thoracic and inhalable release fractions of total non-volatile mass was smaller than or equal to 0.00015 (0.015%) and 0.0015 (0.15%), respectively. The respirable release fraction was below the limit of quantification of the measurement method (0.00005). Special emphasis was directed to suspended nano-sized titanium dioxide. For this compound the release fractions were smaller than 0.0000006 (0.00006%) for the respirable size range, 0.00001 (0.001%) for the thoracic size range and less than or equal to 0.00007 (0.007%) for the inhalable size range. They are based on chemical analysis of titanium in the material deposited on the RESPICON filters.

Particle-number released per gram of spray formulation released [1/g] and the number concentration of the aerosol in the control box for the nine sunscreen sprays are presented in the following table and Figure 3.

Table 5: Particle-number released per gram of spray formulation released [1/g]

Test Product	Mass released [g]	Concentration [1/L]		Release fraction [1/g]	
		<0.12 µm	< 5 µm	<0.12 µm	< 5 µm
2219	4.75	5.36E+04	2.09E+05	8.48E+05	3.31E+06
2260	4.55	7.80E+03	2.01E+04	1.29E+05	3.32E+05
2290	4.40	9.83E+03	3.89E+04	1.68E+05	6.64E+05
3519	4.57	2.30E+04	4.92E+04	3.78E+05	8.09E+05
3560	4.84	1.16E+04	6.85E+04	1.80E+05	1.06E+06
3590	4.43	3.20E+03	9.55E+03	5.43E+04	1.62E+05
E42026503-00-2	4.65	1.74E+04	7.35E+04	2.72E+05	1.15E+06
E47028018-00-4	4.36	9.38E+04	1.46E+05	1.61E+06	2.52E+06
Sunscreen for kids FPS-30	4.83	1.54E+04	5.34E+04	2.39E+05	8.30E+05

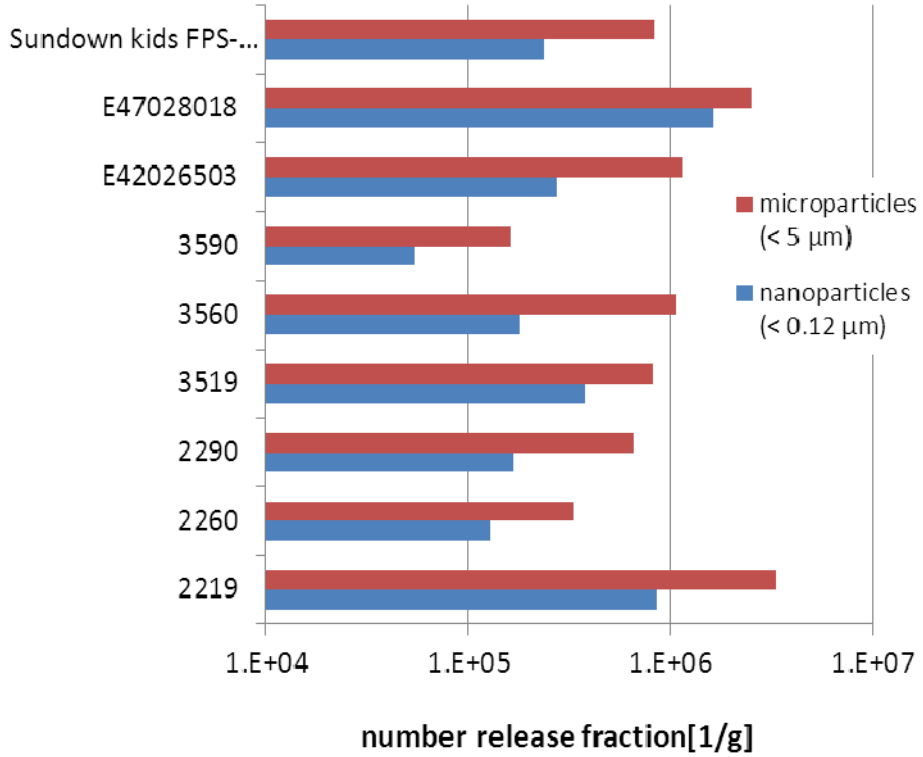

Figure 3: Number release per gram of released spray of the nine sunscreen sprays

According to the Applicant, the nanoparticle release fraction varied between $5.4 \cdot 10^4$ particles/g released spray and $1.6 \cdot 10^6$ particles/g released spray. The micro-particle release ranged from $1.62 \cdot 10^5$ particles/g released spray and $3.31 \cdot 10^6$ particles/g released spray.

SCCS comments

Only limited analytical techniques were used in the experimental studies. Continuous photometric measurements (online-light scattering analysis) were used with a detection limit of 0.2 mg/m³, which in terms of particles may be too high a limit. Hence, the Applicant should estimate the number of particles that corresponds to this detection limit.

Gravimetric measurement on the filter stages of the three fractions was attempted, but according to the Applicant proved to be impossible either because the mass was very small or too "much semi-volatile mass" was contained in aerosol. The Applicant should explain why the semi-volatile mass impairs a gravimetric study (since semi-volatiles are not volatilised immediately).

Total titanium (Ti) was determined in spray using analysis by ICP-MS, which provided identification of the release fraction of Ti for the inhalative, thoracic, respiratory fractions but did not provide information on how the particles were embedded in the particles/droplets after short aging of 15-25 s.

The release fractions above relate to the mass released in either fraction. In a second study the number concentration of the generated and matured particles/droplets was assessed by using a condensation particle counter. From this study, only number concentrations are available, and again no information is provided about the aggregation state.

Therefore, more detailed analysis of the fractions is necessary. Additional analysis of released particles/droplets, e.g. by Cryo-TEM, could provide more detailed information.

The SCCS points out that even after aging, presumably liquid and particles are mixed in the detected "particles". Since (1) smaller-sized nanoparticles could be captured in larger-sized droplets, and (2) also particles with sizes greater than 120nm (up to 1 to 2.5 µm) can deposit in the alveoli, the nanoparticles captured inside the larger droplets can also reach

the alveoli. Therefore, using only the fraction <120 nm for calculating the risk is not conservative.

Regarding representativeness for the European market: In view of the testing of only water-based formulations in the exposure studies presented in chapter 3.2.1, data on exposure to TiO_2 in non-water based sprays (such as Dicaprylyl-based sprays) is missing. Considering that these may have a lower viscosity, the Applicant has not tested the worst case, and is requested to provide further information on the potential exposure.

Since both nozzle type and formulation influence the droplet size distribution of the spray, the Applicant should demonstrate that the market-relevant conditions are being met. The overview of spraying devices on the market requested by SCCS lacks information on the nozzle diameter, generated pressure and other technical details of the spraying device.

Specific points:
- In the table stating the results from ICP-MS analysis, no standard deviation was calculated, "since photometric signal below detection limit". Which photometric signal is involved when performing ICP-MS?
- Figure 2 in Ref-4 shows that different time slots were used for determining the release fraction of the three size fractions. Why were they not done in parallel?

3.2.3 Exposure assessment

The Applicant assessed exposure by mass as described in section 3.2.3.1 and exposure by particle number as described in section 3.2.3.2.

3.2.3.1 Exposure by mass

According to the Applicant, the aim of the experiment was to determine the distribution of spray particles (release fraction) in the three aerosol size fractions, i.e. inhalable, thoracic and respirable fraction. The level and the temporal pattern of the aerosol concentration as measured in the release chamber do not represent any workplace or consumer exposure. The values for the three release fractions serve as input data for indoor air quality models calculating the exposure concentration for defined scenarios of spray application and room conditions, for example room size and ventilation rate.
The data of the TiO_2 analysis are considered most relevant and are used for a simple estimate of inhalation dose of TiO_2 using a worst-case exposure scenario (1-box model): A quantity of nine grams of spray is used twice a day inside a 2 m^3 room (e.g. changing cubicle). It is assumed that all of the particles smaller than 40 µm become airborne. The residence time in the room is 10 minutes and the users' respiratory minute volume is 10 L/min for an adult carrying out light exercise.

These data lead to the inhalation doses listed in the Table below.

Table 6: Inhaled dose (mass-based) per application

	Inhaled dose per application [µg]		
Product	resp.	thor.	inh.
2219	<0.15	4.45	60.90
2260	<0.16	2.47	63.07
2290	<0.18	2.66	36.29
3519	<0.15	2.66	26.62
3560	0.53	9.03	63.21
3590	0.24	0.44	6.48
E47028018	0.23	5.26	16.15
E42036503	0.23	5.67	21.39
Sunscreen for kids FPS-30	0.33	2.12	20.07

SCCS comments
Table 6 seems to indicate the mass-based dose per day, and not per application.

3.2.3.2 Exposure by particle number

The Applicant states that the same worst-case exposure scenario as in 3.2.3.1 was also applied to the data of number of particles, i.e. daily application of 2x9 g of the sunscreen (according to SCCS, 2012) in a small room of 2 m³ volume (changing booth) and a total residence time of 10 min inside the booth. Table 7 shows the exposure concentration, C_{exp}, and the inhaled number of particles N_{inh} calculated with a respiration rate of 10 L/min.

Table 7: Inhaled dose (particle number-based) per application

Test specimen	Exposure concentration, C_{exp} [1/L]		Inhaled number of particles N_{inh} [-]	
	<0.12 µm	< 5 µm	**<0.12 µm**	< 5 µm
2219	**3.82E+03**	1.49E+04	7.63E+05	2.98E+06
2260	**5.80E+02**	1.50E+03	1.16E+05	2.99E+05
2290	**7.56E+02**	2.99E+03	1.51E+05	5.97E+05
3519	**1.70E+03**	3.64E+03	3.40E+05	7.28E+05
3560	**8.10E+02**	4.78E+03	1.62E+05	9.56E+05
3590	**2.44E+02**	7.29E+02	4.88E+04	1.46E+05
E42026503	**1.22E+03**	5.17E+03	2.45E+05	1.03E+06
E47028018	**7.26E+03**	1.13E+04	1.45E+06	2.27E+06
Sunscreen for kids FPS-30	**1.08E+03**	3.73E+03	2.15E+05	7.47E+05

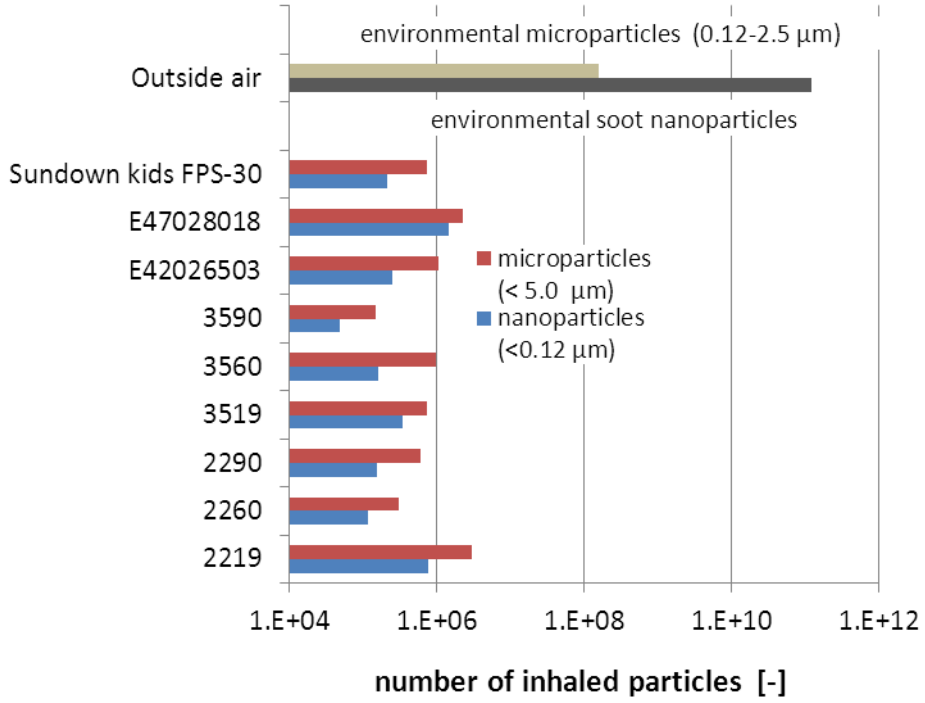

Figure 4: Number of inhaled sunscreen spray particles per application (worst case) in comparison with the daily uptake of environmental soot particles (< 0.10 µm) and PM 2.5 micro particles (0.1-2.5 µm).

SCCS general comments on exposure assessment

The SCCS considers that any study aimed at assessing the exposure from the use of nano-TiO_2 in sunscreen sprays should at least address the following aspects:

I. The tested products and scenarios must be representative of the products on (or intended to be on) the market, and as such cover the range of possible properties that are

relevant for exposure. This needs to encompass the type of formulation and the spraying device used, and, where relevant, a combination of both.

II. The study must show that there is no significant consumers' lung exposure to nanoparticles.

Both points are not met by the presented exposure studies. Representativeness for the European market and exposure determinants need to be assessed more rigorously by the Applicant.

3.3 Toxicological Evaluation

The Applicant has stated that the materials intended for use in sprayable sunscreen formulations comply with the specifications of those already covered in a previous SCCS opinion (SCCS/1516/13). However, the SCCS Opinion in question only addressed the safety of nano-forms of TiO_2 in dermal applications and excluded sprayable products. In fact, that Opinion expressed concerns over the safety of TiO_2 nanomaterials applications that could lead to inhalation exposure of the consumer to TiO_2 nanoparticles. Therefore the conclusions from the previous Opinion can only be considered applicable to this assessment with respect to oral and dermal uptake routes but not for the inhalation route.

As such, the current submission lacks information on inhalation toxicity of TiO_2 nanomaterials that are intended to be used in sprayable sunscreen formulations in support of safety via the inhalation route. In the absence of specific information on inhalation toxicity of the TiO_2 nanomaterials intended to be used in sprayable sunscreen formulations, the SCCS considerations are based on the available information that indicates that inhalation exposure to TiO_2 nanoparticles in general, depending on dose and duration of exposure, may lead to adverse effects in the lungs. Inhalation of TiO_2 has also been considered to be associated with the induction of lung tumours (ECHA, 2016 and the references cited therein).

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

SCCS comments (on acute oral toxicity in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014)

The TiO_2 nanomaterials tested for this endpoint are mainly anatase/rutile mixtures, coated with trimethoxy-n-octyl-silane. The derived LD50 in rats is >2150 mg/kg. One study has determined the approximate lethal dose at >11000 mg/kg.

From the limited data available, the acute oral toxicity of nano- TiO_2 (anatase and rutile mixtures) appears to be very low.

3.3.1.2 Acute dermal toxicity

SCCS comments (on acute dermal toxicity in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014)

From the provided test data, acute dermal LD50 of TiO_2 has been derived at >2000 mg/kg (ultrafine material), and >10,000 mg/kg (natural colour material). However, the provided studies are of no value to the current assessment of nano forms of TiO_2.

3.3.1.3 Acute inhalation toxicity

No data provided by the Applicant.

SCCS comments
Studies acutely exposing the pulmonary system to TiO_2-nanoparticles produced both local and systemic symptoms and aggravate pre-existing symptoms. It is documented that TiO_2-nanoparticles administered through the lung are more inflammatory than fine particles of similar chemistry at equal mass concentrations (Noël *et al.*, 2013). However, it should be noted that mass might not be the optimal dose descriptor for describing respiratory toxicity for nanoparticles in general (Braakhuis *et al.*, 2016). Specifically for TiO_2-nanoparticles it was found that when the dose is described as surface area equalling the amount of administered TiO_2 nanoparticles, the dose response curves of fine and ultrafine (nano) TiO_2 particles indicate equal toxicity that is dependent only on the surface area and not on the mass (Oberdörster *et al.*, 2005).

Relevant data/literature should be provided and discussed.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

SCCS comments (on skin irritation in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014)

From the limited useful data presented in the dossier (supporting SCCS/1516/13), it appears that the TiO_2 nanomaterials are either mild or non-irritant to skin.

3.3.2.2 Mucous membrane irritation / Eye irritation

SCCS comments (on Eye irritation in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014):

From the limited useful data provided (to support SCCS/1516/13), the eye irritation potential of nano- TiO_2 appears to be low.

3.3.2.3 Airways irritation

No data provided by the Applicant.

SCCS comments
Studies suggest that TiO_2 nanoparticles can act as an airway irritant (overview in Shi *et al.*, 2013). Relevant data/literature should be provided and discussed.

3.3.3 Skin sensitisation

SCCS comments (on Skin sensitisation in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014):

From the limited useful data, TiO_2 nanomaterials appear to be weak or non-sensitisers for skin applications. Sensitisation potential of the materials under consideration may however be different from previously evaluated materials because these materials may differ in properties because of different formulation environments.

3.3.4 Absorption

3.3.4.1 Dermal / percutaneous absorption

The studies and literature information evaluated in the previous SCCS Opinion on coated and uncoated nano forms of TiO_2 (SCCS, 2014) indicated that TiO_2 nanoparticles do not penetrate the (simulated) sunburnt skin. However, it was pointed out that such information on flexed or damaged skin is not available, and the evaluated studies were not directed towards hazard identification using either a dose response approach or a worst case scenario (overdosing situation), and that there were certain knowledge gaps in relation to the possible dermal penetration of nano-TiO_2 on repeated or long-term use of cosmetic products, which may not only be used on flexed healthy skin but also on skin that may have lesions or cuts.

3.3.4.2 Absorption by the respiratory tract

No data provided by the Applicant.

In the absence of data, an absorption fraction of 1 has to be assumed.

3.3.5 Repeated dose inhalation toxicity

3.3.5.1 Repeated dose (short-term) inhalation toxicity

Short-term (up to 10 day) repeated inhalation toxicity studies performed in rats and mice (mainly using anatase) pointed to inflammatory responses in the lungs of animals. Changes in biochemical bronchoalveolar lavage (BAL) markers were already observed at concentrations of 2 mg/m^3.

Rossi *et al.* (2010) investigated the inflammatory potential of different types of nano-sized TiO_2 (SiO_2 coated, rutile; nano-TiO_2 anatase, nano- TiO_2 rutile/anatase and nano-TiO_2 anatase/brookite) at 10 mg/m^3 in female BALB/c/SCA mice (n=8/group). Exposure was once for 2 hr (sacrifice 4 and 24 hr after exposure), 2 hr on 4 consecutive days (sacrifice 4 and 24 hr after exposure) and 2 hr on 4 consecutive days for 4 weeks (sacrifice 24 hr after last exposure). Only silica-coated TiO_2 nanoparticles elicited neutrophilic pulmonary inflammation in mice already after 1 week of exposure. Repeated inhalation of silica-coated TiO_2 particles, but not other particles, elicited increased expression of proinflammatory cytokine TNF-a and neutrophil chemoattractant CXCL1.
Further short-term (up to 10 day) repeated inhalation toxicity studies performed in rats and mice (mainly using anatase) pointed to inflammatory responses in the lungs of animals.

Changes in biochemical BAL markers were already observed at concentrations of 2 mg/m^3 (Grassian, 2007, Ma-Hock, 2009, van Ravenzwaay, 2009, Rossi *et al.*, 2010).

3.3.5.2 Repeated dose (subacute – 28 d) inhalation toxicity

Leppänen *et al.* set up acute and repeated TiO_2 exposure models on outbred Crl:OF1 male mice (exposure to 20 nm anatase/brookite generated in situ at 30 mg/m^3 for 4 weeks) finding nano- TiO_2 mainly accumulated in the pulmonary macrophages but did not cause nasal or pulmonary (Leppänen, 2011) inflammation.

Creutzenberg (2013) compared the distribution and toxic effects of three well-characterised TiO_2 nanoforms (UV Titan M212 (rutile, hydrophobic (surface modification with silicone)), UV Titan M262 (rutile, hydrophilic (surface modification with glycerol)), and P25 (80 % anatase/20 % rutile (no surface modification, hydrophilic)). Male Wistar rats (group size: n=12) were exposed at 3, 12 and 48 mg/m^3 for 6 hrs/day, 5 days/week for 28 days. Selected endpoints (e.g. BAL parameters, histopathology of lung) were analysed at days 3, 45 and 94 post-exposure. Only UV Titan M212 and UV Titan M262 induced an increase in polymorphonuclear cells (PMN) (used as inflammation marker in BAL analysis). Histopathologically, only marginal differences in respiratory tract deposition and lesions between the three particle types were observed (e.g. bronchioalveolar hyperplasia, interstitial infiltration and fibrosis, alveolar lipoproteinosis, granulocyte infiltration). Most particles were found clustered within intraalveolar macrophages. In the low- and mid-dose groups, detection within pneumocytes type I became more evident, and in the high-dose group, intraalveolar free particles became more evident. A ranking for the inflammatory potential based on PMN influx was estimated as: UV Titan M262 > UV Titan M212 > P25. For all three materials, an experimental NOAEL of 3 mg/m^3 was derived.

3.3.5.3 Repeated dose (subchronic – 90 d) inhalation toxicity

Groups (n=4) of male Fischer 344 rats were whole-body exposed to 23.5 mg/m^3 fine (average primary particle diameter 250 µm (TiO_2-F) or 22.3 mg/m^3 ultrafine (average primary particle 21 nm; TiO_2-D) nano- TiO_2 in anatase form for 6 hr/day, 5 days/week for up to 12 weeks. Thereafter, animals were kept in a filtered air environment and killed after 4, 8, 12, 41 and 64 weeks; excised lungs were either subjected to BAL or investigated by light microscopy. Control animals received clean air. The number of PMN in the BAL increased in the TiO_2-D group already after the 1st month of exposure when compared to the control and the TiO_2-S groups. During the exposure-free period, the number of PMN decreased and reached almost control values at week 64. Microscopically, after dust exposure, particles were detected in alveolar macrophages, type I pneumocytes, in the pulmonary interstitium but also in the peribronchial and perivascular connective tissue and in the lymphoid tissue. Cell debris was observed in some alveoli (Ferin *et al*., 1992).

Male Fischer 344 rats were exposed for 6 hr/day, 5 days/week for up to 12 weeks to TiO_2-F (anatase, particle size about 250 nm, concentration 22.3 ± 4.2 mg/m^3), TiO_2-D (anatase, particle size about 20 nm, concentration: 23.5 ± 2.9 mg/m^3) or filtered air. After 4, 8 and 12 weeks of exposure and at week 41 and 64 after cessation of exposure, four rats per group were killed and inflammatory lavage parameters and Ti contents were determined in the lung along with lung histology. The ability of lungs to clear particles was determined at the end of the exposure period in 4 animals/substance by instillation or inhalation of 85Sr-labelled polystyrene particles. Based on total cell numbers and PMNs in lung lavage fluid, both types of TiO_2 caused statistically significant increases (less pronounced for TiO_2-F) returning to control levels 64 weeks after cessation of exposure. Other inflammatory parameters (lavage protein, lavage LDH and lavage ß-glucuronidase) were significantly increased after exposure to TiO_2-D. Particle clearance retention was slightly increased for TiO_2-F and markedly increased for TiO_2-D. Upon histopathology, mild focal interstitial

pneumonia was observed in TiO_2-D exposed animals, a much lower inflammatory reaction was observed in TiO_2-F exposed animals. In addition, in animals exposed to TiO_2-D the beginning of interstitial fibrotic foci was observed in the lungs (Oberdörster *et al.*, 1994a;b).

Male Fischer 344 rats were whole-body exposed for 6 h/d, 5 days/week for 12 weeks to filtered air (negative control), pigment-grade TiO_2 (TiO_2-F, particle size 250 nm) at 22.3 mg/m^3, ultrafine TiO_2 (TiO_2-D, particle size 20 nm) at 23.5 mg/m^3 or cristobalite (positive control fibrogenic particle) at 1.3 mg/m^3. Groups of 3 or 4 animals were sacrificed at 6 and 12 months after the completion of exposure. After completion of the study, lung burdens were 5.22 ± 0.75 mg for TiO_2-D and 6.62 ± 1.22 mg for TiO_2-F. These values decreased to 3.14 ± 0.59 mg and 1.66 ± 0.76 mg 12 months after exposure of TiO_2-D or TiO_2-F, respectively. Interstitial fibrosis in the lung was found in TiO_2 groups at 6 months post-exposure with significant increase of septal collagen levels. Slightly more fibrosis was found in animals treated with nano- TiO_2 compared to those treated with fine TiO_2, suggesting that ultrafine particles can have a greater biological activity than larger ones. One year post-exposure, the amount of interstitial fibrosis in TiO_2 groups was not significantly greater than in the negative control group. However, increased number of alveolar macrophages persisted, usually with retained particles. In comparison, moderate focal interstitial fibrosis and moderately severe focal alveolitis were observed 6 months after exposure to SiO_2 (cristobalite). After 1 year, fibrosis decreased but was still present (Baggs *et al.*, 1997).

Female CDF (F344)/CrlBR rats, B3C3F1/CrlBR mice, and Lak: LVG (SYR) BR hamsters were exposed to aerosol concentrations of 0.5, 2.0, or 10 mg/m^3 ultrafine- TiO_2 particles (P25, average primary particle size 21 nm) for 6 hr/day, 5 days/week, for 13 weeks. Groups of 25 animals for each species and time point were used. Following the exposure period, animals were held for recovery periods of 4, 13, 26, or 52 weeks (49 weeks for the uf- TiO_2–exposed hamsters) and, at each time point, TiO_2 burdens in the lung and lymph nodes were determined and selected lung responses based on BAL parameters, lung cell proliferation and histopathology were examined.

Lung burdens increased in a dose-dependent manner in all three species reaching a maximum at the end of the exposures. Compared to mice and rats, lung burdens expressed as mg TiO_2/mg dry lung were significantly lower in hamsters. Lung burdens in all three species decreased with time after cessation of exposure. The retardation of particle clearance from the lungs in mice and rats of the highest dose group indicated particle overload. Pulmonary inflammation in rats and mice exposed to 10 mg/m^3 was evidenced by increased numbers of macrophages and neutrophils and increased concentrations of soluble markers in BAL. Consistent increases in LDH and protein occurred principally in rats and mice exposed to 10 mg/m^3 and diminished with time post-exposure. Significant changes in cellular response or with markers indicating toxicity were not observed in hamsters. In rats exposed to 10 mg/m^3, progressive epithelial and fibroproliferative changes along with interstitial particle accumulation and alveolar septal fibrosis were observed. Lesions observed became more pronounced during post-exposure. Epithelial, metaplastic, and fibroproliferative changes did not occur in mice or hamsters. Thus, there were significant species differences in the pulmonary responses to inhaled uf- TiO_2 particles. Under conditions of equivalent lung TiO_2 burdens, rats developed more severe responses than mice. Clearance of particles from the lungs was markedly impaired in mice and rats exposed to 10 mg/m^3 TiO_2, whereas clearance in hamsters did not appear to be affected at any of the administered doses (Bermudez *et al.*, 2004).

3.3.5.4 Repeated dose (chronic) inhalation toxicity

Female Wistar rats were exposed to P25 (at 7.5 mg/m3 for the first 4 months, then at 15 mg/m^3 for 4 months and then to 10 mg/m^3) for 2 years (19h/d, 5d/week). Substantial increase in lung weight over time (peaking at 18 months of exposure) and histopathology indicated pronounced proliferative response of lung tissue. Lung burdens of 39.3 mg at the

end of exposure and still 33 mg four months later demonstrated massive overload and only minor recovery. Tracer (85Sr polystyrene) clearance half-time of about 500 days indicated collapse of clearance functions (Creutzenberg *et al.*, 1990).
Exposure of female Wistar rats to P25 for 26 months (95 h/week; about 7-15 mg/m^3) resulted in highly increased lung weight, disturbed function and shallower breathing. Interstitial lung fibrosis was evident after 12 and 18 months of exposure, respectively. Results were attributed to generic pulmonary overload (Muhle *et al.*, 1990).

Female Wistar rats [Crl:(WI)BR] and NMRI mice were whole-body exposed to an aerosol of TiO_2 (P25, primary particle size 15-40 nm, ca. 80% anatase and ca. 20% rutile). Rats were exposed for up to 24 months (intermediate sacrifice 6 and 12 months) and mice for 13.5 months for 18 hr/day, 5 days/week. Exposure concentrations were slightly changed during the study and roughly averaged 10 mg/m^3. After the exposure period, animals were kept under clean air conditions for an additional 6 months for rats and 9.5 months for mice. Mortalities of rats and mice immediately after the exposure phase were 60 % (compared to 40 % in controls) and 33 % (compared to 10 % in controls), respectively. After the complete experimental time, mortality in exposed rats (90 %) was significantly different from controls (85 %). Alveolar lung clearance (only determined in rats) was significantly compromised in exposed animals when compared to controls and impaired lung clearance was not reversible within a 3-month exposure-free period. After 6 months of exposure, slight bronchioalveolar hyperplasia and very slight to slight interstitial fibrosis were found in the lungs of sacrificed rats. After 2 years of exposure, 99/100 rats showed bronchioalveolar hyperplasia and slight to moderate interstitial fibrosis was observed in the lungs of all rats. The presence of non-neoplastic findings in mice was not reported in the publication.
Lung tumours were found in 5/20 exposed rats sacrificed after 18 months of exposure versus 0/18 lung tumours in controls. After an exposure time of 24 months followed by 6 months of clean air, lung tumour rate was 32% (31/100) in rats exposed to TiO_2, whereas only one lung tumour (adenocarcinoma) was found in 217 control rats. Among TiO_2 exposed animals, 8 showed 2 tumours in their lungs. Mostly benign keratinizing cystic squamous cell tumours and some squamous-cell carcinomas were found. Bronchioalveolar adenomas and adenocarcinomas were also observed at a high frequency. In mice, the only types of lung tumours observed were adenomas and adenocarcinomas. The percentage of adenomas/adenocarcinomas was 11.3%/2.5% in TiO_2 group and 25%/15.4% in the control group. The lung tumour rate in the TiO_2 group (13.8 %) was lower than in the control group (30%) but not significantly different (Heinrich *et al.*, 1995).

SCCS comments

After inhalation, nano-TiO_2 causes pulmonary inflammatory responses and enhanced proliferation of pulmonary cells at relatively high doses. Compared to microsized TiO_2, nano-TiO_2 was reported to be of higher potency with respect to pulmonary inflammatory effects. Studies demonstrate that markers of oxidative stress and markers of inflammation are changed in response to inhalation exposure to nano-TiO_2. Studies further indicate that there are modulatory effects on asthmatic responses (Shi *et al.*, 2013). Available studies indicate that surface modification (coating) might have an influence on the toxic potential (ECHA, 2016).
Up to now, systemic effects distant from lung and lung-associated tissue have only insufficiently been investigated (e.g. Huang *et al.*, 2015).

3.3.6 Mutagenicity / Genotoxicity

No data on the specific materials under consideration either on genotoxicity in general or related to inhalation exposure have been submitted or considered by the Applicant.

Information from open literature:

An overview on genotoxicity studies is given in ECHA (2016). In addition, the SCCS considers further studies/aspects important:

There are numerous recent *in vitro* studies on TiO_2 exposure using lung cells such as A549 alveolar epithelial cells, human lung epithelial cells BEAS-2B, 16hbe14o cells, the human bronchial epithelial Calu-3, or Human Pulmonary Microvascular Endothelial Cells, and macrophages-like THP-1 cells showing adverse effects (Cowie *et al.*, 2015, Kansara *et al.*, 2015, Armand *et al.*, 2016; Di Bucchianico *et al.*, 2017; El Yamani *et al.*, 2017; Hanot-Roy *et al.*, 2016). The latest studies showed that both short-term (El Yamani *et al.*, 2017) and long-term exposure of A549 to low concentrations of TiO_2 (Armand *et al.* 2016) lead to induction of DNA damage (especially to DNA oxidation). Induction of single and double strand breaks and micronucleus formation in A549 cells (Kansara *et al.*, 2015; El Yamani *et al.*, 2017), BEAS-2B (Di Bucchianico *et al.*, 2017) and cells representing alveolocapillary barrier (Hanot-Roy *et al.*, 2016) after TiO_2 exposure were also reported. In contrast, Vang *et al.*, (2015) did not find any genotoxicity (detected by the comet and micronucleus assays) but induction of cell transforming activity (measured as anchorage independent growth in agar) in BEAS-2B cells.

In order to understand the possible effects of TiO_2 NPs on the human respiratory system and particularly on cells constituting the air–blood (alveolocapillary) barrier, Hanot-Roy *et al.* (2016) studied the impact of oxidative stress on cytotoxicity and genotoxicity. Cells were, however, exposed in liquid medium supplemented with heat inactivated foetal calf serum. In three cell lines representative of cell types of the air-blood barrier *in vivo* (epithelial A549, Human Pulmonary Microvascular Endothelial Cells endothelial cells and macrophages-like THP-1 cells) exposure to TiO_2 NPs induced genotoxicity via oxidative stress. Oxidative stress responses are signal transducer for further physiological effects including, inflammation, genotoxicity and fibrosis as authors demonstrated by activation of associated cell-signalling pathways (via MAP kinases) (Hanot-Roy *et al.*, 2016).

The uptake of TiO_2 NPs into cells was demonstrated by many *in vitro* and *in vivo* studies. It was demonstrated that TiO_2 NPs are taken up by cells in a concentration-dependent manner (measured by ICP-MS) (Allouni *et al.*, 2015; Hsiao *et al.*, 2016). Translocation across the human bronchial epithelial barrier was dependent on size and charge; uptake was increased with smaller and negatively charged TiO_2 NPs but by binding of NPs to proteins (modifying the NP corona), the ability of NPs to cross the epithelial barrier may change, making positively-charged NPs more prone to translocate (George *et al.*, 2015). An active intracellular transport of TiO_2 NPs was observed either through pinocytosis, with signals of membrane protrusions enclosing extracellular NPs or via endocytosis, with cell membrane invaginations and vesicle formations (Bayat *et al.*, 2015). Expression of proteins involved with endocytosis and exocytosis and the formation of pseudopodia and intracellular vesicles confirmed that internalisation of TiO_2 NPs is mainly mediated by endocytosis (Huerta-García *et al.*, 2015).

TiO_2 NPs have been reported to be localised inside cell nuclei in several studies (both as single particles as well as agglomerates) (Andersson *et al.*, 2011; Lankoff *et al.*, 2012; Ahlinder *et al.*, 2013). Smaller NPs can enter the cell nucleus through a receptor-regulated nuclear pore transport mechanism. Another mechanism occurs during cell division, when nuclear membrane is dissolved. Recent observations show that vesicle/vacuole membranes in which TiO_2 NPs are localised can fuse with or pass via the nuclear membrane. As the presence of TiO_2 NPs in cell nuclei was confirmed in several studies, a primary genotoxic mechanism by direct particle interaction with DNA cannot be totally ruled out.

SCCS comments

In view of the available information, the SCCS considers that where internal exposure of the lungs is possible, there are indications that nano-TiO_2 may have genotoxic activity most likely via a secondary mechanism (e.g. oxidative stress).

3.3.7 Carcinogenicity

No data on the specific materials under consideration either on carcinogenicity in general or related to inhalation exposure have been submitted or considered by the Applicant.

Information from open literature:

The toxicological profile, and in particular the carcinogenic potential, of TiO_2 (bulk and nano) has been reviewed by several scientific and regulatory bodies. The following compilation is mainly taken from ECHA (2016).

In 2006, the IARC (International Agency for Research on Cancer) evaluated carcinogenic risks to humans related to TiO_2 exposure (monograph published in 2010). The IARC assessment was based on epidemiological studies (3 epidemiological cohort studies and one population-based case–control study from North America and western Europe) and on experimental carcinogenicity studies in rats, mice and hamsters by different routes of exposure (oral, inhalation, intratracheal, subcutaneous and intraperitoneal administrations). Briefly, following IARC, human carcinogenicity data do not suggest an association between occupational exposure to TiO_2 and risk for cancer. However, all the studies had methodological limitations and misclassification of exposure could not be ruled out: None of the studies was designed to assess the impact of particle size (fine or ultrafine) or the potential effect of the coating compounds on the risk of lung cancer. Regarding animal carcinogenicity data, the incidence of benign and malignant lung tumours was increased in female rats in one inhalation study while in another inhalation study, the incidence of benign lung tumours was increased in the high-dose groups of male and female rats. Cystic keratinising lesions that were diagnosed as squamous-cell carcinomas but re-evaluated as non-neoplastic pulmonary keratinising cysts were also observed in the high-dose groups of female rats. Furthermore, intratracheally instilled female rats showed an increased incidence of both benign and malignant lung tumours following treatment with two types of TiO_2. In contrast, tumour incidence was not increased in intratracheally instilled hamsters and female mice, and two inhalation studies (one in male and female rats and one in female mice) gave negative results. Moreover, oral, subcutaneous and intraperitoneal administrations did not produce a significant increase in the frequency of any type of tumour in mice or rats. As a conclusion, the IARC has classified TiO_2 as possibly carcinogenic to humans (Group 2B). The classification results from the fact that, although there is a clear indication of carcinogenic potential in animal tests, the epidemiological data situation is inadequate. It should be noted that the IARC classification does not differentiate between ultrafine particles (nano- TiO_2) and fine TiO_2 particles.

In 2008, the German MAK Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area provisionally classified TiO_2 as a Category 3A carcinogenic substance. This means that a carcinogenic mode of action is known, but there is insufficient data to establish a maximum workplace concentration value because a benchmark dose or a NOAEC could not be derived from the existing animal experiments. However, the current MAK classification procedure does not take ultrafine particles (i.e. nanoparticles) into account in its assessment (Becker *et al*., 2011). The proposed mechanism of action for tumour formation is a primarily non-genotoxic mechanism consisting on pulmonary inflammation characterised by the increased infiltration of macrophages, granulocytes and, to a limited extent, lymphocytes. The phagocytes absorb titanium dioxide particles and try to degrade the particles with reactive oxygen and nitrogen species. The intensive production

and release of these species damages the genomic DNA of the immediately adjacent cells, including the DNA of Type II alveolar epithelial cells, precursor cells in lung tumours. The accumulation of genetic changes results in alveolar hyperplasia and metaplasia of type II cells, which are precursor stages of lung tumours.

In 2009, Tsuda published a mini-review of carcinogenic potential of engineered nanomaterials and concluded that nanoparticles, including TiO_2, are clearly potentially toxic/carcinogenic to humans based on the increased lung tumours found in female rats (Tsuda *et al*., 2009). Direct production of ROS by TiO_2 or production of ROS by macrophages to destroy the foreign material in the inflammation is proposed as a possible mechanism of action. The same year, as summaries below, Roller *et al*., 2009 considered that the EU criteria (67/548/EEC) for Carcinogenicity category 2 appear to be fulfilled for bio-durable nanoparticles, including TiO_2, based on a clear positive evidence for the carcinogenicity of nano-GBP (GBP: granular biodurable particles) in one species, together with supporting evidence such as genotoxicity data and structural relationship with granular biodurable particles that are regarded as carcinogens or for which data from epidemiological studies suggest an association.

A summary of a critical review on the carcinogenic potential of nanomaterials, including TiO_2, has been published by Becker *et al*. (2011). It was concluded that inhalation studies in rats point to a possible carcinogenic potential of nano- TiO_2 at high concentration but epidemiological studies are inconclusive. The hypothesised mode of action behind tumour formation favours secondary genotoxicity i.e. oxidative stress and chronic inflammation processes. However, a primary genotoxic mechanism by direct particle interaction with DNA cannot be ruled out. The small size of the nanoparticles and their ability to reach intracellular structures, including the nucleus, point to this possibility. Concerning interspecies comparison, extrapolation of results from inhalation and instillation studies in rats to humans is still subject of controversial discussion. Indeed, it appears that the overload concept holds true for rats and to a lesser extent for mice, but not for hamsters. Hamsters have antioxidant protection mechanisms different from rats and humans and this physiological characteristic should preclude using hamsters for testing particulate substances that may elicit inflammatory oxidative damage. In 2011, the National Institute for Occupational Safety and Health (NIOSH) reviewed animal and human data relevant to assessing carcinogenicity of TiO_2. TiO_2 particles of fine and ultrafine sizes show a consistent dose-response relationship for adverse pulmonary responses in rats, including persistent pulmonary inflammation and lung tumours, when the dose is expressed as particle surface area. NIOSH concluded that TiO_2 is not a direct-acting carcinogen, but acts through a secondary genotoxicity mechanism. The toxicity may not be material-specific but appears to be due to a generic effect of poorly soluble, low-toxicity particles in the lungs at sufficiently high exposure. It was concluded that there are insufficient data at this time to classify fine TiO_2 as a potential occupational carcinogen since the tumorigenic dose (250 mg/m^3) was significantly higher than currently accepted inhalation toxicology practice. Although data on the cancer hazard for fine TiO_2 are insufficient, the tumour-response data are consistent with that observed for ultrafine TiO_2 when converted to a particle surface area metric. NIOSH is concerned about the potential carcinogenicity of ultrafine and engineered nanoscale TiO_2 if workers are exposed at the current mass-based exposure limits for respirable or total mass fractions of TiO_2.

A review of toxicological data on TiO_2 nanoparticles was published by Shi *et al*. in 2013 that reaches a similar conclusion (i.e. carcinogenic effect in animals not confirmed by epidemiological studies). Although the mechanism is not well understood, both genetic and non-genetic factors elicited by TiO_2-NP in cells may predispose to carcinogenicity.

SCCS comments

Various scientific and regulatory bodies have considered TiO_2 as a possible carcinogen to human when inhaled. Recently, a classification proposal of TiO_2 as Carc. Cat 1B – H350i has been submitted to ECHA by France (ECHA, 2016) considering that a causal relationship has

been established between TiO_2 and an increase of both malignant and benign lung tumours in one species (rat), reported in two studies by inhalation and two studies by instillation. Since data provided cannot distinguish if a specific characteristic is linked to such effect, this classification proposal is intended to be applied to all existing possible crystal modifications, morphologies and surface chemistries in all possible combinations of TiO_2.
The proposed classification focuses on the inhalation route because only local tumours were found after respiratory exposure and no carcinogenic concern was identified for the oral and dermal routes. This last assumption is based on the negative results in different carcinogenicity studies that might be explained due to limited absorption reported in other studies and due to the hypothesised mode of action requiring a sufficient accumulation of particles to induce inflammation and proliferative lesions.

Human data do not suggest an association between occupational exposure to TiO_2 and risk for cancer. However, all these studies have methodological limitations and misclassification of exposure could not be ruled out.

Although the full mode of action is still unclear, an inflammatory process and indirect genotoxic effect by ROS production seems to be the major mechanism to explain the effects induced by TiO_2. It is considered that this mode of action is principally due to the biopersistence and poor solubility of the TiO_2 particles. However, a genotoxic effect by direct interaction with DNA cannot be excluded (see section 3.3.6).

3.3.8 Reproductive toxicity

No data provided by the Applicant.

Information from open literature:
Limited *in vivo* and *in vitro* studies suggest that TiO_2 NPs exposure may exert certain reproductive and developmental toxicities (Shi *et al.*, 2013).

3.3.9 Toxicokinetics

No data provided by the Applicant.

Information from open literature:
Depending on size, inhaled nano-TiO_2 is distributed to the nasopharyngeal, tracheobronchial and alveolar regions of the respiratory tract. In part, deposited material is eliminated via mucociliar clearance. Particles having reached the alveolar region are taken up by macrophages and are then eliminated from the body by alveolar clearance. High concentrations have been reported to impair alveolar clearance and to concomitantly increase lung retention half-lives. Compared to microsized TiO_2, nano-TiO_2 was also observed to a greater extent in lung-associated lymph nodes indicating epithelial translocation into the interstitium. There are further reports on the detection of nano-TiO_2 in the cytoplasm of pneumocytes I cells, in the capillary endothelium, the connective tissue or as free particles in the alveolar space (e.g. Ferin *et al.*, 1992; Bermudez *et al.*, 2004; Eydner *et al.*, 2012).
Rapid translocation of a small amount (about 2%) of the lung-deposited material accompanied by subsequent accumulation was reported for a variety of secondary target organs (liver > kidney > blood > spleen > heart > brain) after endotracheal intubation. However, amounts were low compared to those retained in the lung until the end of the observation period. The sum of amounts found in the above-mentioned tissues was lower than that reported for the remainder of the body (Kreyling *et al.*, 2010).

Studies by Wang *et al.* (2008a, 2008b) on murine brain reported that intra-nasally instilled TiO_2 NPs (80 nm rutile, 155 nm anatase; 500 µg/ml; 2, 10, 20, and 30 days) can be taken up by sensory nerves and translocate to the brain.

SCCS comments
A more extensive evaluation of kinetics/deposition of the inhaled nano-TiO_2 in the lung is required.

3.3.10 Photo-induced toxicity

SCCS comments (on photo-induced toxicity in SCCS/1516/13, 22 July 2013, Revision of 22 April 2014):

Only a few studies have been provided that are relevant to the nanomaterials under assessment. These indicate that TiO_2 materials may not be photo-sensitisers.

3.3.11 Human data

No data have been provided by the Applicant.

SCCS comments
Several scientific and regulatory bodies have evaluated the carcinogenic potential of TiO_2 including nano-TiO_2 (IARC, 2006; ECHA, 2016, NIOSH, 2011). These evaluations included human data. Human data did not suggest an association between occupational exposure to TiO_2 and risk for cancer. However, all of the studies have methodological limitations and misclassification of exposure could not be ruled out.

3.3.12 Special investigations and mode of action

Information from literature:
There are many *in vitro* studies that have reported inflammatory effects by ROS generation due to TiO_2 NPs inhalation exposure. ROS-induced signalling and activation of the IL family of cytokines, Bax, caspases 3 and 9, NF-ęB, and p53, as well as phosphorylation of p38 and G2M phase cell cycle arrest, seem to be common findings. With regard to induction of inflammation leading to the production of ROS, inflammatory cytokines seem to play an influencing role. It should be noted that the signalling of IL-1R by TiO_2 NPs is similar to that of asbestos.
By using cell culture models it could be demonstrated that TiO_2 NPs can inhibit cell proliferation, cause DNA damage, and induce apoptosis via a mechanism primarily involving the activation of the intrinsic mitochondrial pathway (Wang *et al.*, 2015). Normal bronchial cells showed a higher susceptibility to cytotoxic effects, however, transformed alveolar cells show higher responsiveness to genotoxic, oxidative and early inflammatory effects induced by tested TiO_2 NPs (Ursini *et al.*, 2014; Grande and Tucci, 2016).

Furthermore, studies indicate that inhalation of nano- TiO_2 might impair systemic microvascular functions (Nurkiewicz *et al.*, 2006, 2008, 2009; Knuckles, 2012; Husian *et al.*, 2013).

There are also reports on morphological and pathological changes in the brain after intranasal instillation (Wang *et al.*, 2008a, 2008b).

An increasing number of experimental studies have become available highlighting the role of immune-mediated mechanisms in pulmonary inflammation, as well as the adjuvant activity of nano- TiO_2 for known allergic sensitisers or predisposed species (e.g. Gustafsson *et al.*, 2011, 2014).

SCCS general comments on toxicology

The submission lacks an adequate hazard characterisation specific to the materials under consideration. Since the dossier specifically addresses inhalation risk, special emphasis should have been given to evaluate toxicological findings regarding local effects in the respiratory tract and systemic uptake via the inhalation route. Several published studies are available in the scientific literature and a previous SCCS Opinion has also evaluated nano-TiO_2 materials. Where appropriate, this information has been referred to in the sections above. However, although the materials under evaluation have been reported by the Applicant to comply with the specifications that have been given in the SCCS (SCCS/1516/13) these materials have a) not been specifically assessed with respect to the inhalation uptake route and b) may change their properties in response to the formulation environment, which needs to be taken into account in the hazard characterisation.

In conclusion, based on the comments provided in the various subchapters, the SCCS is of the opinion that an adequate toxicological evaluation that makes it possible to derive a point of departure based on inhalation exposure should be provided for the materials that have already been evaluated for dermal and oral exposure in SCCS/1524/13.

3.4 Safety evaluation (including calculation of the MoS)

The Applicant estimated the mass- and particle-based exposure to TiO_2-NP from spray products based on the release fractions determined under a use scenario considered to represent a conservative exposure situation. In this experiment, the respiratory exposure was below the LOD for 4 of 9 sprays and for the other five sprays exposure was shown to be very low (up to about 3.5-fold above LOD). The Applicant concluded that a comparison of the mass-based exposure estimates with occupational exposure limits and of the particle-based exposure estimates with background exposure to environmentally occurring nanoparticles demonstrated large margins of safety and minimal carcinogenic risk. More details on the Applicant's safety evaluation are given in Annex II.

SCCS comments

The SCCS considers the safety evaluation presented by the Applicant as insufficient based on the following reasons:

First, the evaluated formulations cannot be considered representative for the European market, nor as representing a worst case (see SCCS comments in section 3.2).

Second, the Applicant compared the consumer exposure to the occupational exposure limits derived by NIOSH, 2011, including an additional safety factor of 1000. However, this NIOSH report is based only on the literature until 2008 (plus 2 papers from 2009), and there are more recent papers on pulmonary inflammatory properties of TiO_2 which may be used for pulmonary inflammatory risk assessment, some of which have been discussed in the section on toxicology. This literature evaluation should be completed including up-to-date available information. In addition, procedures for consumer risk assessment should be used and not those for workers (see SCCS Notes of Guidance).

Third, it has to be questioned whether the approach for particle-based risk assessment of only considering the fraction <120 nm is a worst-case approach. As shown by a large-scale deposition study (ICRP, 1994) the deposition fraction in the alveoli is largest for particles <100 nm, but fractions also of larger particles up to 1-5 µm are deposited. Since the study design of the present exposure studies did not distinguish between particles and droplets, it may well be that larger droplets transport further nanoparticles into the alveoli. Therefore, the risk assessment also needs to take the larger-sized fractions into account. If this is done, the maximal inhaled number of particles as calculated by the Applicant amounts to 3 x 10^6 particles calculated for a residence time of 10 min in a 2 m^3 cubicle (which, however cannot be regarded as a worst case, see section 3.2).

Fourth, a comparison of exposure to TiO_2-NP from sprays to background exposure to carbon black NP (soot) as presented by the Applicant is only partly meaningful, because the toxicity of nanoparticles is also associated with their chemical nature.

Fifth, as discussed earlier, the toxicological evaluation by SCCS could not take into account that particles may change after spraying (e.g. decrease in size due to drying during air transport) and therefore not assess how many TiO_2 NP reach the lower respiratory tract.

In conclusion: Since the exposure study does not cover the worst case, the recent toxicological literature has not been sufficiently addressed and a toxicological evaluation regarding the inhalation uptake route is missing, no margin of safety can be calculated.

3.5 Discussion

Physicochemical properties

The SCCS considers the physicochemical characterisation of the nano-TiO_2 materials under evaluation as insufficient for an assessment of its toxicological effects after inhalation, which is the special focus of this dossier. Particle size distributions of a representative sample of materials to be used in sprays are required. This is even more important because currently the inhalation exposure studies have not been performed with a representative set of formulations. Although the materials evaluated in the exposure studies have been reported by the Applicant to comply with the specifications that have been given in SCCS, 2014, it should be recalled that the cited SCCS Opinion focused on dermal exposure and excluded inhalation. After spraying the size distribution and agglomeration status of the particles may change, and therefore compliance with the specifications from SCCS/1516/13 does not imply absence of effects in this case.

Exposure assessment

The SCCS has concluded that the submitted exposure study is not representative of the products on the EU market, and the provided information is therefore insufficient to allow assessment of the safety of the use of nano-TiO_2 in sprayable formulations/packaging. Furthermore, as discussed before, the exposure study fails to identify the composition of the inhaled particles, which may consist of smaller nanoparticles that are released in the lungs.

Toxicological Evaluation

Since the focus of this Opinion is on the inhalation route, only toxicological evidence regarding this route is discussed here. For the other routes refer to SCCS, 2014.

The Applicant has not provided any toxicological data for the materials under the current evaluation; therefore the toxicological evaluation was based solely on the open literature. However, it is important that a safety dossier on nanomaterial(s) contains sufficient data and supporting information to enable adequate risk assessment. The dataset should be

complete in relation to physicochemical properties, exposure, toxicological effects, and safety evaluation, as indicated in SCCS, 2012.

Acute toxicity

Studies acutely exposing the pulmonary system to TiO_2 NPs produced both local and systemic symptoms and aggravate pre-existing symptoms. It is documented that TiO_2 NPs administered through the lungs are more inflammatory than fine particles of similar chemistry at equal mass concentrations (Noël *et al.*, 2013). However, it should be noted that mass might not be the optimal dose descriptor for describing respiratory toxicity for nanoparticles in general (Braakhuis *et al.*, 2016). Specifically for TiO_2-nanoparticles it was found that when the dose is described as surface area equalling the amount of administered TiO_2 nanoparticles, the dose response curves of fine and ultrafine (nano) TiO_2 particles indicate equal toxicity that is dependent only on the surface area and not on the mass (Oberdörster *et al.*, 2005).

Irritation and corrosivity

Studies suggest that TiO_2 nanoparticles can act as an airway irritant (overview in Shi *et al.*, 2013).

Absorption by the respiratory tract

In the absence of data, an absorption fraction of 1 has to be assumed.

Repeated dose toxicity

After inhalation, nano-TiO_2 causes pulmonary inflammatory responses and enhanced proliferation of pulmonary cells at relatively high doses. Compared to micro sized TiO_2, nano- TiO_2 was reported to be of higher potency with respect to pulmonary inflammatory effects. Studies demonstrate that markers of both oxidative stress and inflammation are changed in response to inhalation exposure to nano-TiO_2. Studies further indicate that there are modulatory effects on asthmatic responses (Shi *et al.*, 2013).

Up to now, systemic effects distant from lung and lung-associated tissue have only insufficiently been investigated (e.g. Huang *et al.*, 2015).

Mutagenicity

In view of the available information, the SCCS considers that where internal exposure of the lung is possible, there are indications that nano-TiO_2 may have genotoxic activity, most likely via a secondary mechanism (e.g. oxidative stress).

Carcinogenicity

Various scientific and regulatory bodies have considered TiO_2 as a possible carcinogen to humans when inhaled. Recently, a classification proposal of TiO_2 as Carc. Cat 1B – H350i was submitted to ECHA by France considering that a causal relationship had been established between TiO_2 and an increase of both malignant and benign lung tumours in one species (rat), reported in two studies by inhalation and two studies by instillation. Since data provided cannot distinguish if a specific characteristic is linked to such effect, this classification applied to all existing possible crystal modifications, morphologies and surface chemistries in all possible combinations of TiO_2.

Although the full mode of action is still unclear, an inflammatory process and indirect genotoxic effect by ROS production seems to be the major mechanism to explain the effects induced by TiO_2. It is considered that this mode of action is principally due to the biopersistence and poor solubility of the TiO_2 particles. However, a genotoxic effect by direct interaction with DNA cannot be excluded since TiO_2 was found in the cell nucleus in various in vitro and in vivo studies.

Reproductive toxicity

Limited *in vivo* and *in vitro* studies suggest that TiO_2 NPs exposure may exert certain reproductive and developmental toxicities (Shi *et al.*, 2013).

Toxicokinetics

The Applicant should perform a more extensive evaluation of kinetics/deposition of inhaled nano-TiO_2 in the lungs.

Human data

Several scientific and regulatory bodies have evaluated the carcinogenic potential of TiO_2 including nano-TiO_2 (IARC, 2006; ECHA, 2016, NIOSH, 2011). These evaluations included human data. Human data did not suggest an association between occupational exposure to TiO_2 and risk for cancer. However, all studies have methodological limitations and misclassification of exposure could not be ruled out.

General remarks on toxicological evaluation

Several published studies are available in the scientific literature and a previous SCCS Opinion has also evaluated nano-TiO_2 materials. Where appropriate, this information has been referred to in the sections above. However, although the materials under evaluation have been reported by the Applicant to comply with the specifications that have been considered in the SCCS Opinion on TiO_2 (SCCS/1516/13) these materials may change their properties in response to the formulation environment, which needs to be taken into account in the hazard characterisation. The toxicological evaluation performed by the SCCS based on the open literature can therefore only present a starting point: Based on the comments provided in the various subchapters, the SCCS is of the opinion that an adequate toxicological evaluation based on inhalation exposure should be provided by the Applicant.

Safety evaluation

Since the exposure study does not cover the worst case scenario, the recent toxicological literature has not been sufficiently addressed and a toxicological evaluation regarding the inhalation uptake route is missing, no margin of safety can be calculated.

4. CONCLUSION

1. In light of the data provided, does the SCCS consider Titanium Dioxide (nano) safe when used as UV-Filter in sunscreens and personal care spray products at a concentration up to 5.5%?

On the basis of the provided data, the SCCS has concluded that the information is insufficient to allow assessment of the safety of the use of nano- TiO_2 in sprayable application.

The exposure studies have not been conducted using representative sprayable products that may be intended for the EU market. The submission also does not contain a toxicological evaluation for nano-TiO_2 via the inhalation route, which would allow deriving a point of departure for risk assessment using worst-case conditions. It should be emphasised that compliance with the specifications from SCCS/1516/13 will not imply absence of effects after inhalation exposure. The SCCS Opinion in question only addressed the safety of nano-forms of TiO_2 in dermal applications and excluded sprayable products. In fact, that Opinion expressed concerns over the safety of TiO_2 nanomaterial applications that could lead to inhalation exposure of the consumer to TiO_2 nanoparticles.

2. Does the SCCS have any further scientific concerns regarding the use of Titanium Dioxide (nano) when used as UV-Filter in sunscreens and personal care spray products?

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5. MINORITY OPINION

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Annex I

Annex to 3.2.1.1 Test items

In the following the complete information on formulations is given:

Recipe 22 – Viscosity 2100 mpas [RV3/10rpm] - used in:
Test item 1: Sunscreen 2219, spray head 0.19 ml
Test item 2: Sunscreen 2260, spray head 0.60 ml
Test item 3: Sunscreen 2290, spray head 0.90 ml

Ingredient (INCI name)	Concentration (%)
Water (Aqua)	52.05
Octocrylene	8.00
Alcohol	8.00
Glycerin	5.00
Caprylyl Carbonate	5.00
Ethylhexyl Salicylate	5.00
Butyl Methoxydibenzoylmethane	4.00
C12-15 alkyl benzoate	4.00
Titanium dioxide (nano)*	2.54
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	2.00
VP/hexadecene copolymer	1.00
Phenoxyethanol & Ethylhexyl Glycerin (ratio 90:10)	1.00
Microcrystalline Cellulose, Cellulose Gum (ratio 10:90)	0.50
Ethylhexyl Glycerin	0.50
Silica	0.40
Potassium cetyl phosphate	0.30
Cetearyl Alcohol	0.30
Acrylate/C10-30 alkyl acrylate crosspolymer	0.10
Disodium EDTA	0.10
Tocopherol	0.10
Dimethicone	0.07
Xanthan Gum	0.05

*Lot No.401002166

Recipe 35 - Viscosity 1080 mpas [RV3/10rpm] - used in:
Test item 4: Sunscreen 3519, spray head 0.19 ml
Test item 5: Sunscreen 3560, spray head 0.60 ml
Test item 6: Sunscreen 3590, spray head 0.90 ml

Ingredient (INCI name)	Concentration (%)
Water (Aqua)	51.90
Octocrylene	8.00
Alcohol	8.00
Glycerin	5.00
Caprylyl Carbonate	5.00
Ethylhexyl Salicylate	5.00
Butyl Methoxydibenzoylmethane	4.00
C12-15 alkyl benzoate	4.00
Titanium dioxide (nano)*	2.54
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	2.00
VP/hexadecene copolymer	1.00
Phenoxyethanol & Ethylhexyl Glycerin (ratio 90:10)	1.00
Microcrystalline Cellulose, Cellulose Gum (ratio 10:90)	0.50
Ethylhexyl Glycerin	0.50
Potassium cetyl phosphate	0.40
Cetearyl Alcohol	0.40
Silica	0.40
Acrylate/C10-30 alkyl acrylate crosspolymer	--
Disodium EDTA	0.10
Tocopherol	0.10
Xanthan Gum	0.10
Dimethicone	0.07

*Lot No.401002166

Recipe E42026503-00 – Viscosity 3020 mpas, Brookfield 10rpm Spindle 3 used in:

Test item 7: Sunscreen E42026503-00-2, spray head 0.19 ml

Ingredient (INCI name)	FDA CODE
Water (Aqua)	A2
Alcohol Denat.	D
Octocrylene	D
C12-15 alkyl benzoate	D
Glycerin	D
Butyl Methoxydibenzoylmethane	E
Titanium dioxide(nano)*	4.3%
Dicaprylyl Ether	E
Diethylamino Hydroxybenzoyl Hexyl Benzoate	E
VP/hexadecene copolymer	E
Ethylhexyl Salicylate	F
Panthenol	F
Tocopheryl Acetate	F
Silica	F
Microcrystalline Cellulose	F
Caprylyl Glycol	F
Acrylate/C10-30 alkyl acrylate crosspolymer	F
Ethylhexyl Glycerin	F
Disodium EDTA	G
Cellulose Gum	G
Dimethicone	G
Sodium hydroxide	G
Citric acid	G
Galactoarabinan	G
Tocopherol	G

*Lot Nr.401004016

FDA codes: A1 = 75-100%; A2 = 50-75 %; B = 25-50%; C=10-25%; D = 5-10%; E = 1-5%; F = 0.1-1%; G = 0-0.1%; H = Traces

Recipe E47028018-00-4 – Viscosity 5000 mpas, Brookfield 10rpm Spindle 3 used in:
Test item 8: Sunscreen E47028018-00-4, spray head 0.19 ml

Ingredient (INCI name)	FDA CODE
Water (Aqua)	B
Octocrylene	C
Alcohol Denat.	D
C12-15 alkyl benzoate	D
Glycerin	D
Butyl Methoxydibenzoylmethane	E
Ethylhexyl Salicylate	E
Titanium dioxide(nano)*	5.5%
Dicaprylyl Ether	E
VP/hexadecene copolymer	E
Tocopheryl Acetate	F
Silica	F
Panthenol	F
Microcrystalline Cellulose	F
Caprylyl Glycol	F
Ethylhexyl Glycerin	F
Acrylate/C10-30 alkyl acrylate crosspolymer	F
Dimethicone	F
Disodium EDTA	G
Cellulose Gum	G
Sodium hydroxide	G
Aloe Barbadensis Leaf Juice Powder	G
Citric acid	G
Xanthan Gum	G
Tocopherol	G

*Lot Nr.401004016
FDA codes: A1 = 75-100%; A2 = 50-75 %; B = 25-50%; C=10-25%; D = 5-10%; E = 1-5%; F = 0.1-1%; G = 0-0.1%; H = Traces

Recipe of the commercial product (Test item 9) Sunscreen for kids, FPS-30, spray head BOV system (no exact recipe available, only ingredient list printed on the bottle):
INCI: Aqua, Octocrylene, Ethylhexyl Methoxycinnamate, Ethylhexyl Salicylate, C12-15 Alkyl Benzoate, Bis-ethylhexyloxyphenol Methoxyphenyl Triazine, Sorbitan Isostearate, Cetyl Phosphate, Tricontanyl PVP, Titanium Dioxide, Alumina, Simethicone, Phenoxyethanol, Triethanolamine, Isostearic Acid, Dimethicone, parfum, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Disodium EDTA, DMDM Hydantoin, Bisabolol, Chamomilla Recutita Flower Extract (Extract), Glycine Soja Seed Extract (Extract, Seed), Tocopheryl Acetate, Denatonium Benzoate, Iodopropynyl Butylcarbamate

Annex II

Safety evaluation performed by the Applicant

Comparison with a proposed Occupation Exposure Limit

The Applicant compared mass-based exposure to TiO_2 from spray products with the OEL proposed by NIOSH of 300 µg/m3 for chronic exposure to nano-sized titanium dioxide of a respirable size range (NIOSH 2011). NIOSH has set the REL (recommended exposure limit) at 300 µg/m³ based on a risk evaluation targeted to reduce working lifetime risk of lung cancer to below 1/1000. Assuming 8 h exposure and an inhalation rate of 10 L/min the inhaled daily dose is 1440 µg at the OEL. However, for consumers a more conservative estimated cancer risk of $1/10^6$ can be considered as acceptable. Taking this OEL into account and using an inhalation rate of 10 L/min, a daily acceptable exposure for the consumer indicates an exposure to 1.44 µg/day (1/1000 (reduction of risk from $1/10^3$ to $1/10^6$) x 300 µg/m^3 x 0.001 L/m^3 x 10 L/min x 60 min/h x 8 h/day). The estimated respiratory exposure by the use of TiO_2-containing sun care spray products of less than 0.15 to 0.53 µg/application is 2.7 to more than about 10-fold lower. Thus based on mass the use in spray products is considered to have an acceptable risk.
Considering the nanoparticle number aspect, an NRV (nano reference value) for TiO_2 is suggested as 40'000 particles/cm^3 (8-h TWA) for bio-persistent granular nanomaterial in the range of 1-100 nm with a density of <6000 kg/m^3 (Broekhuizen, 2012). Estimating a human exposure at this NRV, assuming an inhalation rate of 10 L/min, corresponds to inhalation of about 192 x 10^9 particle per day (40 x 10^3 particles/cm3 x 1000 cm^3/L x 10 L/min x 60 min/h x 8 h/day). Compared to the estimated exposure from use of sun screen sprays with the highest release fraction of 1.5 x 10^6 nano particles/day is 128'000-fold lower than this NRV. These values are intended for occupational scenarios and the NRV-values should be considered as a warning level, when they are exceeded, exposure control measures should be taken. Therefore, the large margin to the consumer exposure also supports the safe use in sunscreen and personal care spray products.

Lifetime Cancer Risk Approach

Although TiO_2 is not considered to be a direct genotoxic carcinogen (NIOSH 2011), the Lifetime Cancer Risk approach for genotoxic carcinogens as described in the SCCS Notes of Guidance (SCCS 2012) has been applied to the rat carcinogenicity data reported by Heinrich *et al.* (1995). Not only is this a conservative approach, it is, for several reasons, a worst case evaluation as will be explained.
A first consideration is that rats seem to be specifically sensitive to TiO_2 inhalation based on comparison to other species. Specifically, no tumour formation has been observed in mice and hamsters similarly exposed to TiO_2 as were the rats. Response to particulate TiO_2 is dependent on the dose rate as demonstrated by Baisch *et al.* (2014), which does not account for the difference in species' response. Human occupational epidemiologic investigations in TiO_2 manufacturing plants did not suggest any carcinogenic effect associated with workplace exposure to TiO_2. The expected exposure through the use of TiO_2-containing sunscreen spray products is exceedingly lower (0.53 µg/application) than the doses applied in the inhalation carcinogenicity study (9.3 mg/m^3 corresponding to about 0.45 mg/day in the study of Heinrich *et al.* 1995); thus, an extrapolation from animal high dose data to the minute human exposure by the use of TiO_2-containing sunscreen spray products is considered conservative. The carcinogenicity study in rats reported by Heinrich *et al.* (1995) has been performed with non-coated titanium dioxide (P25, Degussa) composed of ca. 80% anatase and 20% rutile, and thus not corresponding to the requirements of SCCS opinion of 2012, i.e. TiO_2 nanomaterial has to be composed of mainly the rutile form.
For our evaluation the exposure of the animals in the carcinogenicity study and that calculated by use of cosmetic spray products from the release fractions (our previous

submission) have been normalised to the specific lung burden as of mg/g lung/day or as cm^2 particle surface/g lung/day as this is more appropriate for particle inhalation exposure of the lung (NIOSH 2011).
Lung tumour incidence (T25) of nano-TiO_2 has been interpolated from the study of Heinrich *et al.* 1995 (cited by Gebel 2012). Tumor incidence observed with nano- TiO_2 was 0.5% in the control and 32% at 9.3 mg/m^3. Interpolation revealed a T25 of about 7.2 mg/m^3. For the relative risk assessment, the following parameters were chosen for rat and human:

	Rat carcinogenicity study	Human cosmetic use	
Specific Surface Area (SSA) of TiO_2	48	50	m2/g
Body weight	0.25	70	kg
Lung weight	2	1300	g
Respiratory minute volume	0.2	10	L/min
Exposure per day (rat), per application (human)	0.45	0.53	µg/application (day)
Applications/day	1	2	/day
Exposure duration/day	4		h/day

On a daily basis the following parameters have been calculated according to SCCS Notes of Guidance (2012) in order to estimate the lifetime cancer risk (LCR)

- T25 - Animal dose-descriptor; chronic dosage rate that will give 25% of the animal's tumours at a specific tissue site after correction for spontaneous incidence
- HT25 Human dose-descriptor, derived from T25 and based on comparative metabolic rates,
- SED - Systemic Exposure Dosage

LCR values have been calculated for humans on two dose metrics:
1. Mass exposure normalized per g lung (first line in the table below)
2. Exposure to particle specific surface area of titanium dioxide normalized per g lung (second line in the table below):

T25	**HT25**	**SED**		**Lifetime cancer risk** (LCR=SED/(HT25/0.25))
0.17	4.24E-02	8.15E-07	mg/g lung/day	4.8E-06
8.33E-03	2.04E-03	4.08E-08	m^2 particle surface/g lung/day	5.0E-06

Using 0.53 µg TiO_2/application to estimate the respiratory fraction, which is the highest value of the amount per application from our studies, will result in a human specific lung burden of 8.15×10^{-7} mg/g lung/day and in a Lifetime Cancer Risk of 4.8×10^{-6}.
Calculation based on the particle specific surface area, considered to be the more relevant dose metric, reveals an LCR of 5.0×10^{-6}. Thus, both dose metrics reveal a similar LCR of less than 10^{-5}, which is considered of little or no concern (SCCS Notes of Guidance, 2012). This is also supported by epidemiological investigations evaluating the mortality statistics at 11 European and 4 US TiO_2 manufacturing plants (total of 20 862 workers), concluding that there was no suggestion of any carcinogenic effect associated with workplace exposure to TiO_2 (Hext *et al.* 2005).

In conclusion, the different approaches and dose metrics considered all reveal an acceptably low risk of carcinogenic lung effects from the use of TiO_2 nano in spray products. In addition, considering the conservative, and worst case daily use scenario, support our conclusion that there is a very low risk associated with the use of TiO_2 in sunscreens and personal care spray products.

Comparison to environmental concentrations of other types of nanoparticles

The inhaled number of sunscreen spray related nanoparticles per day under the worst case scenario can be compared with the daily (24 h) intake of nanoparticles from breathing environmental air in an urban environment. The environmental air quality is approximated by a mass concentration of 2 µg/m³ soot nanoparticles (50 % with diameter of 0.05 µm, and 50 % with diameter of 0.1 µm) and 20 µg/m³ micro-particles (PM 2.5 – particulate matter smaller than 2.5 µm) shared equally between 1 µm and 2 µm particles. These mass concentrations are typical for urban sites at low to moderate pollution conditions [Boogaard *et al.* 2010]. The EU air quality standard for PM2.5 is currently 25 µg/m³ [http://ec.europa.eu/environment/air/quality/standards.htm] annual average value. The number concentration of environmental soot nanoparticles is in the range of 10^6-10^7 [1/L] [Boogaard *et al.* 2010]. It is seen from Figure 3 that the inhalation intake of nanoparticles when using the sunscreen sprays at worst case conditions in a closed changing cubicle is about a factor of 10^4 to 10^5 lower than the daily uptake of soot nanoparticles from the outside air. For the micro-particles the difference in number intake between environmental exposure and exposure due to use of sunscreen spray is two orders of magnitude.