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Scientific Committee on Consumer Safety

SCCS

**OPINION ON**

**Titanium Dioxide (nano form)**

**COLIPA n° S75**

The SCCS adopted this opinion by written procedure on 22 July 2013

## About the Scientific Committees

Three 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 which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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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ISSN 1831-4767

ISBN 978-92-79-30114-8

Doi: 10.2772/70108

ND-AQ-13-007-EN-N

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## **ACKNOWLEDGMENTS**

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This opinion has been subject to a commenting period of six weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, UV filter, titanium dioxide (nano form), SCCS/1516/13, directive 76/768/ECC, CAS number: 13463-67-7, EC: 236-675-5

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on titanium dioxide (nano form), 22 July 2013, revision of 22 April 2014.

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## 1. BACKGROUND

The first scientific opinion on the safe use of titanium dioxide as a UV-filter at a maximum concentration of 25% in cosmetic products was adopted 24 October 2000 by the SCCNFP (SCCNFP/0005/98).

However, a review of the substance in its nanoform is deemed necessary according to the opinion on Safety of Nanomaterials in Cosmetic Products adopted on 18 December 2007 (SCCP/1147/07), where it is stated that:

"The SCCNFP opinion from 2000 (SCCNFP/0005/98) is on micro-crystalline preparations of TiO<sub>2</sub> and preparations of coarse particles. However, since this opinion, new scientific data on nanosized particles including, TiO<sub>2</sub> has become available. Therefore, the SCCP considers it necessary to review the safety of nanosized TiO<sub>2</sub> in the light of recent information. Also, a safety assessment of nanosized TiO<sub>2</sub>, taking into account abnormal skin conditions and the possible impact of mechanical effects on skin penetration needs to be undertaken".

Supplementary information on nanosized Titanium dioxide was submitted following a meeting with stakeholders on 1 October 2008, where data requirements were agreed.

Titanium Dioxide is currently regulated - irrespectively of its form - as a UV-filter in a concentration up to 25% in cosmetic products in Annex VII, entry 27 of the Cosmetics Directive.

### 1.1 TERMS OF REFERENCE

1. *Does SCCS consider that use of titanium dioxide in its nanoform as a UV-filter in cosmetic products in a concentration up to maximum 25.0 % is safe for the consumers taken into account the scientific data provided?*
  
2. *In order for the COM to differentiate in the regulation between materials in its nanoform and its non-nano form, can the SCCS give quantitative and qualitative guidance on how this differentiation should be given based on the particle size distribution or other parameters?*

## 1.2 OPINION

## 1.3 Chemical and Physical Specifications

### 1.3.1 Chemical identity

Titanium Dioxide

#### 1.3.1.1 Primary name and/or INCI name

Titanium Dioxide

#### 1.3.1.2 Chemical names

Titanium Dioxide

#### 1.3.1.3 Trade names and abbreviations

COLIPA No. S75

#### 1.3.1.4 CAS / EC number

CAS number: 13463-67-7

EC: 236-675-5

Other registry numbers: 100292-32-8; 101239-53-6; 1025343-79-6; 116788-85-3; 12000-59-8; 1205638-49-8; 1236143-41-1; 12701-76-7; 12767-65-6; 12789-63-8; 1309-63-3; 1344-29-2; 1377807-26-5; 1393678-13-1; 1400974-17-5; 158518-86-6; 185323-71-1; 185828-91-5; 188357-76-8; 188357-79-1; 195740-11-5; 221548-98-7; 224963-00-2; 246178-32-5; 252962-41-7; 37230-92-5; 37230-94-7; 37230-95-8; 37230-96-9; 39320-58-6; 39360-64-0; 39379-02-7; 416845-43-7; 494848-07-6; 494848-23-6; 494851-77-3; 494851-98-8; 52624-13-2; 55068-84-3; 55068-85-4; 552316-51-5; 62338-64-1; 767341-00-4; 859528-12-4; 861455-28-9; 861455-30-3; 866531-40-0; 97929-50-5; 98084-96-9.

[Source: ChemIdPlus]

#### 1.3.1.5 Structural formula

TiO<sub>2</sub>

#### 1.3.1.6 Empirical formula

Formula: TiO<sub>2</sub>

### 1.3.2 Physical form

Titanium Dioxide (TiO<sub>2</sub>, COLIPA No. S75, CAS No. 13463-67-7) is described as a solid, white, odourless powder. The TiO<sub>2</sub> materials used in sunscreen products are reported to be composed of two crystalline types: rutile and anatase or a mixture of the two. The different materials included in the dossier have been reported to be needle, spherical, or lanceolate (longer than wide) in shape. The primary particle size of the TiO<sub>2</sub> nanomaterials has been reported to range from around 20 to 100 nm.

Nanoparticles are generally known to have a tendency to stick together to form agglomerates and/or aggregates, and it is claimed by the Applicant that, in sunscreen products, TiO<sub>2</sub> is not present in the form of primary nanoparticles but as aggregates of a

1 size between 30 nm to >150 nm. These aggregates are claimed to be formed during the  
2 manufacturing process.

3  
4 Fifteen (15) TiO<sub>2</sub> nanomaterials have been presented in the submission for evaluation. They  
5 include uncoated as well as surface-coated nanomaterials with various organic and inorganic  
6 coating materials. A range of coating materials has been used which include hydrophilic,  
7 hydrophobic and amphiphilic materials, such as alumina/silica, methicone/silica, aluminium  
8 hydroxide and dimethicone/methicone copolymer, trimethyloctylsilane, alumina/silicone and  
9 alumina/silica/silicone, dimethicone, simethicone, stearic acid, glycerol,  
10 dimethoxydiphenylsilane, triethoxycaprylylsilane (Table 1).

11  
12 The coating materials have been stated by the Applicant to be those that are common  
13 cosmetic ingredients. The purpose of coatings has been stated to include improvement of  
14 the dispersion of TiO<sub>2</sub> nanomaterials within the cosmetic formulation, inhibiting or  
15 controlling photoactivity, and improving compatibility with other ingredients in sunscreen  
16 formulations. The coatings applied to nanoparticle surface are also stated to be not UV  
17 absorbers themselves.

### 18 **SCCS Comment**

19 For this opinion, the trade names of the nanomaterials under assessment have been coded  
20 by the SCCS and are referred to by the relevant codes.

21  
22 It has been stated by the Applicant that '[the stability of coating] is certainly less relevant  
23 from a human-safety aspect, especially since materials used as coating agents for TiO<sub>2</sub> may  
24 be present as constitutive ingredients of the same cosmetic product'. This may be true for  
25 some materials, but it also needs to be considered that a range of materials has been used  
26 for coating the TiO<sub>2</sub> nanomaterials under current assessment. Some of these materials have  
27 been used in a substantially high coating to nanomaterial ratios (e.g. 16% alumina).  
28 Although a few studies showing coating stability have been provided, it is important to know  
29 whether this, for example, could lead to the release of aluminium ions from alumina that  
30 may be present after the coating process and which may dissolve in the final formulation.  
31 Thus, where appropriate, safety of the coating materials should also be considered in their  
32 own right because any significant dissolution of a coating component, such as alumina, may  
33 require a separate safety assessment.

34  
35 Three studies have been provided (submission II – Ref 62 and 63, and Submission III – Ref  
36 68) to indicate that the coatings (e.g. silica/alumina) are stable in formulation, as well as  
37 under different conditions of pH, temperature, shear force, etc. However, from the other  
38 physicochemical data provided, it is less clear how stable the coatings are in final  
39 formulations. The photocatalytic activity data, measured in formulations, indicate that either  
40 some of the materials were not completely coated, or some of the coatings were not stable  
41 in the formulations.

42  
43 Despite the fact that the materials used as coatings to TiO<sub>2</sub> nanomaterials have a wide  
44 diversity, and some of them have been used in substantially high proportions (e.g. 16%  
45 alumina), putative exposure to the coating materials has not been considered in the  
46 assessment. Although a few studies showing coating stability have been provided, it is  
47 important to know the concentration of any dissolved coating materials, e.g. aluminium  
48 ions, in the final formulation. For example, in a recent study, Virkutyte et al (2012) found  
49 that chlorine in swimming pools could potentially strip the coating from titanium dioxide  
50 nanoparticles in sunscreens. The study, however, relates to a specific use scenario – i.e.  
51 where TiO<sub>2</sub> nanomaterials are coated with aluminium hydroxide (Al(OH)<sub>3</sub>), and the product  
52 is used in chlorinated water (e.g. in a swimming pool). It is also likely that the coating-  
53 stripped nanoparticles will be washed off the skin during swimming or bathing after  
54 swimming. Although this specific type of coating/use scenario relates more to risk  
55 management than risk assessment, any significant dissolution of some coating materials  
56 (e.g. alumina) may require a separate safety assessment for the uncoated nanomaterial as  
57 well as the coating material.

## Revision of the opinion on Titanium Dioxide, nano form

1 In view of this, the SCCS has only recommended the types of coatings covered in this  
 2 opinion. Other cosmetic ingredients applied as stable coatings on TiO<sub>2</sub> nanomaterials can  
 3 also be used, provided that they can be demonstrated to the SCCS to be safe and the  
 4 coatings do not affect the particle properties related to behaviour and/or effects, compared  
 5 to the nanomaterials covered in this opinion.

6

7 Table-1: Form and composition TiO<sub>2</sub> nanomaterials \*

8

Material code	TiO <sub>2</sub> crystalline form	Coating material	Doping material	Form	Bulk density (g/cm <sup>3</sup> )	VSSA (m <sup>2</sup> cm <sup>-3</sup> )
S75-A	> 99.5% Rutile	6% silica, 16% alumina	None	Oil dispersion	0.35	460
S75-B	> 99.5% Rutile	6% silica, 16% alumina	None	Aqueous dispersion	0.35	460
S75-C	> 99.5% Rutile	7.5% alumina, 9,5% aluminium stearate	None	Oil dispersion	0.31	220
S75-D	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Oil dispersion	0.58	300
S75-E	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Aqueous dispersion	0.58	300
S75-F	Anatase 85%, Rutile 15%	7.5% trimethoxycaprylsilane	None	Hydrophobic powder	0.2	192
S75-G	Anatase 85%, Rutile 15%	None	None	Hydrophilic powder	0.13	213
S75-H	> 99,5% Rutile	6% alumina, 1% glycerin	None	Hydrophilic powder	0.31	260
S75-I	> 99,5% Rutile	7% alumina 10% stearic acid	None	Hydrophobic powder	0.28	300
S75-J	> 99,5% Rutile	6% alumina 1% dimethicone	None	Hydrophobic powder	0.31	260
S75-K	> 94% Rutile	6-8% aluminium hydroxide, 3.5-4.5% dimethicone/methicone copolymer	None	Hydrophobic powder	0.12-0.28	426
S75-L	> 94% Rutile	6.5-8.5% hydrated silica, 2.5-4.5% aluminium hydroxide, 4.5-6.5% dimethicone/methicone copolymer	None	Hydrophobic powder	0.07-0.2	426
S75-M	> 98% Rutile, <2% anatase	17% silica	None	Hydrophilic powder	0.09	260
S75-N	> 95% Rutile, <5% anatase	Alumina 10% simethicone 2%	1000 ppm Fe	Amphiphilic powder	0.16	400
S75-O	100% Anatase	Simethicone 5%	None	Hydrophobic powder	0.75	400

9

10 \* Regarding purity/impurity all materials are claimed by the applicant to conform with USP  
 11 35 requirements: TiO<sub>2</sub> (99.0-100.5%), Loss on Ignition (≤ 13%), Water-soluble substances  
 12 (≤ 0.25%), Acid-soluble substances (≤ 0.5%), Arsenic (≤ 1 ppm), Residual Solvents (No  
 13 solvents used),  
 14 and FDA requirements: Lead (HCl-soluble) (≤ 10 ppm), Antimony (HCl-soluble) (≤ 2 ppm),  
 15 Mercury (≤ 1 ppm).



1 For purity/impurity, all materials were tested as uncoated and untreated material.

2  
3 **SCCS comment**

4 Analytical data on purity and impurities were not submitted, purity was only referred to USP  
5 and FDA requirements. Analytical data on purity and impurities of each nanomaterial should  
6 be provided.

7 **1.3.3 Molecular weight**

8  
9 Molecular weight of TiO<sub>2</sub>: 79.9 g/mol.  
10

11 **1.3.4 Purity, composition and substance codes**

12 According to the Applicant, the TiO<sub>2</sub> nanomaterials have been produced according to USP  
13 31 specifications, in high purity, with concentration of the active material ≥99.0 %. It is  
14 also stated that the materials do not contain heavy metals (e.g., Hg, Cd, Pb, As or Sb)  
15 beyond the generally accepted limits.  
16

17 **SCCS Comments**

18 The nanomaterials included in the submission have been stated to be manufactured  
19 according to USP-31 specifications, with no heavy metals beyond the 'generally accepted  
20 limits'. The Applicant should provide the contents of heavy metals, such as Hg, Cd, Pb, As  
21 and Sb, which are considered 'acceptable' under USP-31, as they may or may not be  
22 considered acceptable under the EU regulations. In addition, impurities of well-known  
23 metallic contact allergens, such as Cr, Co, Ni, should also be reported.

24 Purity/impurity has been referred to USP-35 in the additional information provided by the  
25 applicant. USP-31 is an earlier edition of USP-35.  
26

27 **1.3.5 Impurities / accompanying contaminants**

28 See SCCS comment under 1.3.2  
29

30 **1.3.6 Solubility**

31 TiO<sub>2</sub> is insoluble in water and organic solvents. It also has a very low dissociation constant  
32 in water and aqueous systems, and thus can in practice be considered as insoluble also  
33 under the physiological conditions.

34 (Numerous references in open literature)  
35

36 **1.3.7 Partition coefficient (Log Pow)**

37 Log P<sub>ow</sub>: Not applicable for uncoated TiO<sub>2</sub>.

38 (Reference: 137)  
39

40 **SCCS Comment**

41 A method to determine partition coefficient of nano particles coated with organic materials  
42 is not yet available. However, distribution of TiO<sub>2</sub> nanomaterials coated with organic  
43 substances between polar and non polar phases should be described.  
44

45 **1.3.8 Additional physical and chemical specifications**

46 Melting point: Not provided  
47

## Revision of the opinion on Titanium Dioxide, nano form

1	Boiling point:	Not applicable
2	Flash point:	Not applicable
3	Vapour pressure:	Not applicable
4	Density:	The Tap Density of the titanium dioxide powders was
5		measured according to DIN ISO 787/11 (Table 1)
6	Viscosity:	Not provided
7	pKa:	Not applicable for uncoated TiO <sub>2</sub>
8	Refractive index:	Not provided
9	UV_Vis spectrum (200-800 nm):	UV data only (see Table 3)

10  
11 **SCCS Comment**

12 The dissociation kinetics of the materials in acidic media can be potentially modified by  
13 certain coatings. However, considering the physicochemical properties of TiO<sub>2</sub>, it is agreed  
14 that, for TiO<sub>2</sub> nanomaterials, coatings are unlikely by definition to change the dissociation  
15 constant of TiO<sub>2</sub> in water.

16  
17 Table-2: Physicochemical properties of TiO<sub>2</sub> nanomaterials  
18

Material code	Crystal size	Aspect ratio (L/W)	UV Absorption (Extinction coefficient)			Zeta potential (IEP)	Photo-catalytic activity*		Photo-stability	Coating stability
	(XRD)		E308	E360	E400		ΔE	% to Reference		
S75-A	15	3.8	44	20	11	7	3	9	Photo-stable	Stable
S75-B	15	3.8	51	22	12	N/A	3	9	Photo-stable	Stable
S75-C	15	3.7	54	16	7	N/A	7.8	23	Photo-stable	Stable
S75-D	9	4.5	48	7	3	N/A	7.2	21	Photo-stable	Stable
S75-E	9	4.5	50	10	4	N/A	7.2	21	Photo-stable	Stable
S75-F	21	1.2	45	15	8	N/A	11.8	35	Photo-stable	Stable
S75-G	21	1.2	38	16	9	7	25.1	74	Photo-stable	NA
S75-H	21	1.7	30	17	9	7	0.3	1	Photo-stable	Stable
S75-I	15	3.2	38	14	6	N/A	0.8	2	Photo-stable	Stable
S75-J	21	1.5	36	16	9	N/A	0.6	2	Photo-stable	Stable
S75-K	15	3.9	60	12	1	N/A	2.3	7	Photo-stable	Stable
S75-L	15	4.3	55	14	2	N/A	0.8	2	Photo-stable	Stable
S75-M	20	2.6	26	12	5	2	0.6	2	Photo-stable	Stable
S75-N	13	4.1	45	13	5	9	0.7	2	Photo-stable	Stable
S75-O	18	1.2	20	8	5	N/A	15.7	46	Photo-stable	Stable

19  
20 \* Photocatalytic activity 5% TiO<sub>2</sub> formulation irradiated in a Suntest CPS+ solar simulator  
21 for 30 minutes at 300 W/m<sup>2</sup>. Sample measured before and after using a colourimeter.

1 Calculation  $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ ; Reference uncoated TiO<sub>2</sub>  $\Delta E = 34$ .  
2 See Egerton et al. (2007) for more details on the method.

#### 4 **SCCS Comment**

5 The photoreactivity of a chemical is generally determined in terms of degradation of an  
6 organic substance (e.g. iso-propanol, propanone, salicylic acid, or an organic dye such as  
7 methylene blue) on exposure to UV irradiation. Regarding measurement of photocatalytic  
8 activity of nanomaterials, the OECD guidance (2010) provides further information and also  
9 cites the methods described in ISO TC 206/WG37 (Fine ceramics – Test methods for  
10 photocatalytic material).

11 In regard to the TiO<sub>2</sub> nanomaterials under evaluation, the SCCS accepted the applicant's  
12 provided data from a different method used for measuring photocatalytic activity. The  
13 method, which is described by Egerton et al. (2007), is based on photogreying of the TiO<sub>2</sub>  
14 material on exposure to UV irradiation. Although the test is based on a non-standard  
15 method, the SCCS accepted the data in view of the published work by Egerton et al. (2007),  
16 which indicates measurable photogreying of TiO<sub>2</sub> nanomaterials upon UV irradiation. As  
17 such, the method will not be applicable to other nanomaterials because they may not turn  
18 grey on exposure to UV irradiation, and/or may already have a colour.

19 Nanomaterials used in cosmetic products should ideally be non photocatalytic. However, in  
20 view of measurement uncertainties, the SCCS has considered acceptable an arbitrary level  
21 of up to 10% photocatalytic activity of a coated or doped nanomaterial, measured in terms  
22 of % to a reference standard (which is uncoated/undoped form of the same nanomaterial).  
23

### 24 **1.3.9 Droplet size in formulation**

25  
26 According to the information provided by the Applicant, sunscreen spray products containing  
27 nano-sized TiO<sub>2</sub> are available on the EU market. These spray products are formulated with  
28 non-volatile ingredients in pump sprays (without propellant gas) to generate minimal  
29 aerosol cloud. It is stated that these products comply with current standards and  
30 requirements in terms of droplet size, Mass Median Aerodynamic Diameter (MMAD) of at  
31 least 30  $\mu\text{m}$ , with no more than 1% of the droplets having an aerodynamic diameter of 10  
32  $\mu\text{m}$  or less. The Applicant has quoted the Technical Guidance Document on Risk Assessment  
33 of the European Chemical Bureau (2003), which considers aerosols with an MMAD >10-15  
34  $\mu\text{m}$  as not respirable for humans because of deposition mainly in the upper regions of the  
35 lungs (Reference 148). It is also quoted that the U.S. Silicones Environmental, Health and  
36 Safety Council (2001) suggests that a consumer aerosol application for any silicone-based  
37 material, regardless of the method of aerosol generation, should have particle size MMAD at  
38 least 30  $\mu\text{m}$ , with no more than 1% of the particles having an aerodynamic diameter of 10  
39  $\mu\text{m}$  or less (Reference 203). The Applicant has provided droplet size distribution  
40 measurements for a few sprayable products. The technique used for droplet size  
41 measurement was based on Laser Diffraction by Malvern method.  
42

#### 43 **SCCS Comments**

- 44 - The trade name of one sprayable product suggests that it may be for use by children.
- 45 - The droplet size of an aerosolised formulation would affect the entry and uptake of  
46 nanomaterial in the lung. It is therefore noteworthy that whilst droplet size would  
47 depend on nebulizer/ matrix, it may change due to evaporation/sublimation of the fluid  
48 used in the emulsion. Thus, the characteristic dimension of a nanomaterial contained in  
49 the formulation would have little relevance to the droplet size, which is typically much  
50 larger (tens of micron).
- 51 - Although the measurement results indicate that droplet sizes were largely above the  
52 respirable range (>10  $\mu\text{m}$ ), and only 0.24 to 0.37% of the droplets were in the size  
53 range below 20  $\mu\text{m}$ , it should be noted that even a low fraction based on droplet weight  
54 is still relevant because it will contain a large number of nanoparticles. The possibility of  
55 droplets drying and becoming smaller in size following spraying, and the possible lung

1 exposure to dried residual particles after inhalation also needs to be taken into account.  
 2 The measurement of the droplet size distribution therefore needs to be complemented  
 3 by measurements of the size distribution of the dried residual aerosol particles as well, if  
 4 they can dry on the timescale in a practical use scenario.

- 5 - The size distribution of the droplets and dried droplets/ particles should be presented as  
 6 number size distribution.

### 7 **1.3.10 Particle size**

8  
 9 Table 3: Particle size of TiO<sub>2</sub> nanomaterials

Material code	Particle Size Distribution*											
	Lower Cut Off level (nm)				Volume weighted median, X <sub>50,3</sub> (nm)				Number weighted median, X <sub>50,0</sub> (nm)			
	CPS	LUMi-sizer	DLS	Average**	CPS	LUMi-sizer	DLS	Average**	CPS	LUMi-sizer	DLS	Average**
S75-A	20	33	35	29	53	71	111	78	37	48	79	55
S75-B	28	34	47	36	68	76	145	96	47	56	105	69
S75-C	20	25	26	24	52	49	78	60	39	48	59	49
S75-D	17	23	15	18	35	44	56	45	28	34	34	32
S75-E	21	27	41	30	45	51	104	67	37	42	81	53
S75-F	35	49	63	49	75	92	139	102	55	70	115	80
S75-G	25	58	54	46	77	99	129	102	45	79	102	75
S75-H	29	63	41	44	71	120	112	101	50	79	82	70
S75-I	22	58	41	40	73	107	140	107	40	76	103	73
S75-J	33	52	35	40	71	103	125	100	48	69	85	67
S75-K	26	34	30	30	48	52	75	58	41	44	58	48
S75-L	33	37	41	37	56	64	103	74	46	53	80	60
S75-M	42	75	73	63	119	124	173	139	75	99	133	102
S75-N	21	37	26	28	51	61	91	68	41	51	65	52
S75-O	24	71	47	47	354	653	146	384	33	87	85	68

10  
 11 \* The particle size distribution was measured by three different methods - Differential  
 12 Sedimentation Analysis (CPS disc centrifuge); Integral Sedimentation Analysis (LUMiSizer  
 13 centrifuge); and Dynamic Light Scattering (Malvern HPPS). In addition, Electron microscopy  
 14 (SEM and TEM) images of representative nanomaterials have been provided.

15 \*\* average of median values from the three measurement methods.

16  
 17 According to the applicant, all samples were measured in a standardized fashion according  
 18 to specific standard operating procedures as follows:

19  
 20 1. Hydrophilic Powder: 1) Add 30 ml SHMP-solution (0.02 g sodium hexametaphosphate to  
 21 30 ml deionised water) to 0.2 g titanium dioxide powder in the glass beaker and agitate the  
 22 sample gently with an overhead or magnetic stirrer for 15 minutes to ensure homogeneity;  
 23 2) Disperse the probe using an ultrasonic probe (power 50 Watts) for 10 minutes. The  
 24 ultrasonic horn should not touch the side of the glass beaker or the bottom. The  
 25 suspensions should be cooled during the dispersion.

26  
 27 2. Hydrophobic Powder: 1) Add 1 ml isopropyl alcohol to 0.2 g titanium dioxide powder in  
 28 the glass beaker. To wet the powder slew the beaker carefully; 2) Add 1 drop Disperbyk  
 29 190 (BYK Chemie, Germany) after adding isopropyl alcohol; 3) Add 30 ml SHMP-solution  
 30 into the beaker and agitate the sample gently with an overhead or magnetic stirrer for 15

1 minutes to ensure homogeneity; 4) Disperse the probe using an ultrasonic horn (50 Watts)  
2 for 10 minutes. The ultrasonic horn should not touch the side of the glass beaker or the  
3 bottom. The suspensions should be cooled during the dispersion.

4  
5 3. Oil based Dispersion: Dilute the dispersion to 1% solids by cyclohexane (solids content of  
6 dispersion must be supplied by company).

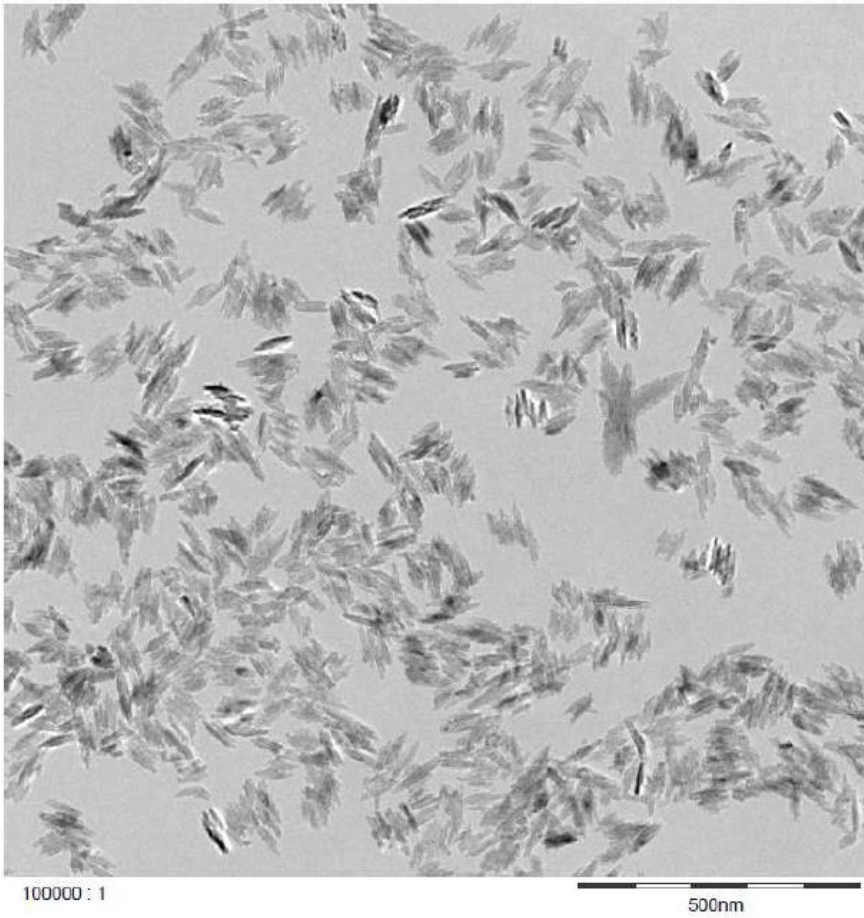
7  
8 4. Water based dispersion: Dilute the dispersion to 1% solids by deionised water (solids  
9 content of dispersion must be supplied by company). Agitate every sample gently with the  
10 stirrer for 1 hour for equilibration before measurement.

#### 11 **SCCS Comment**

12 The different materials included in the dossier have different particle sizes. These range  
13 from ~45 nm to 384 nm on volume weighted median basis (average of 3 measurement  
14 methods), and ~32 nm to ~102 nm on the basis of number weighted median (average of 3  
15 measurement methods). The lower size cut offs (average of 3 measurement methods)  
16 range between 18 nm and 63 nm. Note that different methods are typically characterised by  
17 systematic, or partially systematic, different measurement uncertainties depending on the  
18 size range measured. Therefore the average of different measurement methods on the  
19 same nanomaterial does not necessarily provide a more reliable value than measured by an  
20 individual method, but has been adopted as a practical approach to size determination.  
21  
22  
23

#### 24 **1.3.11 Microscopy**

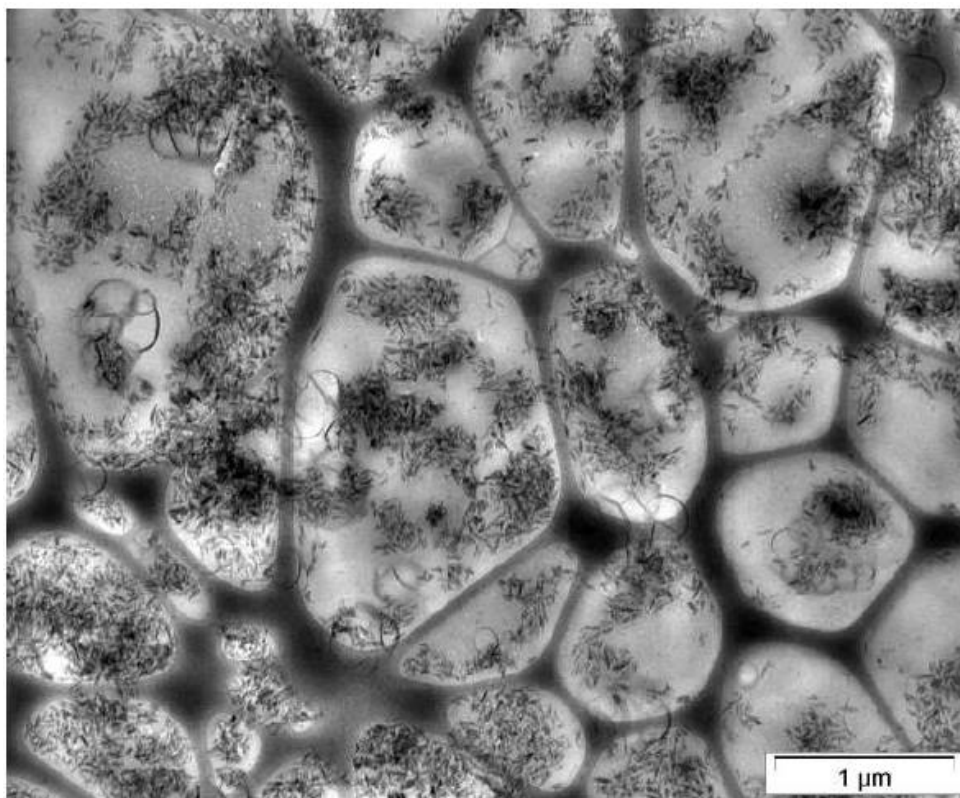
25  
26 An example transmission electron microscopy (TEM) image of TiO<sub>2</sub> nanomaterial is shown  
27 below:  
28



1  
2  
3  
4  
5  
6

An example Cryo-TEM image of TiO<sub>2</sub> nanomaterial in formulation is shown below:





1  
2  
3  
4  
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7  
8

#### **SCCS Comment**

The different nanomaterials included in the dossier have primary particles that have either spherical, needle, or lanceolate (longer than wide) shapes, and appear to be present in aggregated clusters.

#### **1.3.12 Homogeneity and stability**

10  
11 According to the Applicant, the term "dispersion" has been used in relation to the dispersion  
12 of TiO<sub>2</sub> clusters/ aggregates in the cosmetic product, whereas aggregates bound by strong  
13 forces could not be dissociated. They also claim that coating materials on the TiO<sub>2</sub> particle  
14 are stable under various conditions of pH, temperature and shear forces, and that the  
15 materials used as coating agents for TiO<sub>2</sub> may also be present as constitutive ingredients of  
16 the same cosmetic product.

17  
18

#### **SCCS Comments on Physicochemical Characterisation**

19  
20 The physicochemical characterisation data provided in the dossier relates to fifteen (15)  
21 TiO<sub>2</sub> nanomaterials. The data are reasonably extensive, which show that:

- 22 1. Ten out of the 15 materials (S75-A, S75-B, S75-C, S75-D, S75-E, S75-H, S75-I, S75-  
23 J, S75-K, S75-L) are rutile. Two other materials (S75-M, S75-N) are mainly rutile with  
24 a small proportion (2-5%) of anatase.
- 25 2. One material (S75-O) is anatase. Two other materials (S75-F and S75-G) are mainly  
26 anatase (85%) with rutile (15%).
- 27 3. The primary crystal size of the materials range between 9 and 21 nm. The average  
28 particle sizes in dispersions (measured by 3 different methods) range from ~45 nm to  
29 384 nm on volume weighted median basis (average of 3 measurement methods), and  
30 ~32 nm to ~102 nm on the basis of number weighted median (average of 3

- 1 measurement methods). The lower size cut offs (average of 3 measurement methods)  
2 range between 18 nm and 63 nm.
- 3 4. One material (S75-G) is uncoated, all other materials are surface coated with different  
4 coating materials (silica, alumina, organo-silanes).
- 5 5. All coatings are reported to be stable at least in the short-term *in vitro* test systems.  
6 In view of the diversity of the coating materials and some high coating to  
7 nanomaterial ratios, it is important to know the concentration of dissolved coating  
8 materials, e.g. alumina that could release aluminium ions, in the final formulation. A  
9 significant dissolution of a coating material (e.g. alumina) may require a separate  
10 safety assessment for the coating material.
- 11 6. One material (S75-N) is doped with 1000 ppm iron. All other materials are not doped.
- 12 7. The apparent bulk density of the materials ranges between 0.09 to 0.75 g/cm<sup>3</sup>. The  
13 SCCS notes that the lowest density reported for some materials does not fit in the  
14 normal range. As all materials have core particles of TiO<sub>2</sub>, with sizes in the nano-  
15 scale, it is not clear why there is such a large variation in their bulk densities. The  
16 Applicant needs to clarify whether the materials with low bulk densities have a porous  
17 structure, as in such a case they may have different physicochemical properties from  
18 the other TiO<sub>2</sub> materials.
- 19 8. One material (S75-E) is in aqueous dispersion. All other materials are either  
20 hydrophilic or hydrophobic powders, or are in oil dispersions.
- 21 9. The VSSAs of the materials range between 192 to 460 m<sup>2</sup>/cm<sup>3</sup> for the different  
22 materials, indicating that they are indeed nanomaterials (i.e. VSSA ≥60 m<sup>2</sup>/cm<sup>3</sup>).
- 23 10. Aspect ratios of the different materials range between 1.2 and 4.5, indicating that the  
24 high aspect ratio materials have needle or lanceolate shaped particle structures.
- 25 11. All materials are stated to be photostable.
- 26 12. UV absorption data for the materials have been provided.
- 27 13. Zeta potential measurements have been provided for some materials, and not for  
28 others due to difficulties in measuring zeta potential for hydrophobic nanomaterials.
- 29 14. Photocatalytic activity data have been provided for all materials (see Table 2, and  
30 corresponding SCCS comments). The data show that the materials have differing  
31 levels of photocatalytic activity, which ranges from insignificant to weak (S75-A, S75-  
32 B, S75-H, S75-I, S75-J, S75-K, S75-L, S75-M, S75-N), to moderate (S75-C, S75-D,  
33 S75-E), and strong (S75-F, S75-G; S75-O). All 3 nanomaterials with strong  
34 photocatalytic activity are also either anatase form of TiO<sub>2</sub>, or mainly anatase with  
35 some rutile.

36  
37 From the physicochemical characterisation data provided, the materials could be broadly  
38 grouped as shown below for the purpose of this assessment. This grouping is based on the  
39 differences between physicochemical properties and the potential effects of anatase/rutile,  
40 coated/uncoated, and photocatalytic/non-photocatalytic forms of TiO<sub>2</sub> nanomaterials. It is  
41 known that uncoated and non-doped TiO<sub>2</sub> nanoparticles are photocatalytic when exposed to  
42 UV light. The anatase form has been shown to be more photoreactive than rutile or  
43 anatase-rutile mixtures (e.g. Sayes et al., 2006). Another indicator of catalytic activity of  
44 nanomaterials is the increased generation of reactive oxygen species (ROS) in biological  
45 systems and the resulting toxicological effects, such as cytotoxicity. Jiang et al. (2008)  
46 noted that the generation of ROS (per unit surface area) was the highest in amorphous  
47 nano-TiO<sub>2</sub>, followed by anatase, anatase/rutile mixture, and rutile. Anatase form of nano-  
48 TiO<sub>2</sub> has also been reported to be 100 times more cytotoxic under UV than rutile of a  
49 similar size (e.g. Sayes et al., 2006). These aspects have already been highlighted in the  
50 SCCP opinion on Safety of Nanomaterials in Cosmetic Products (SCCP/1147/07) in the  
51 phototoxicity part (page 33):

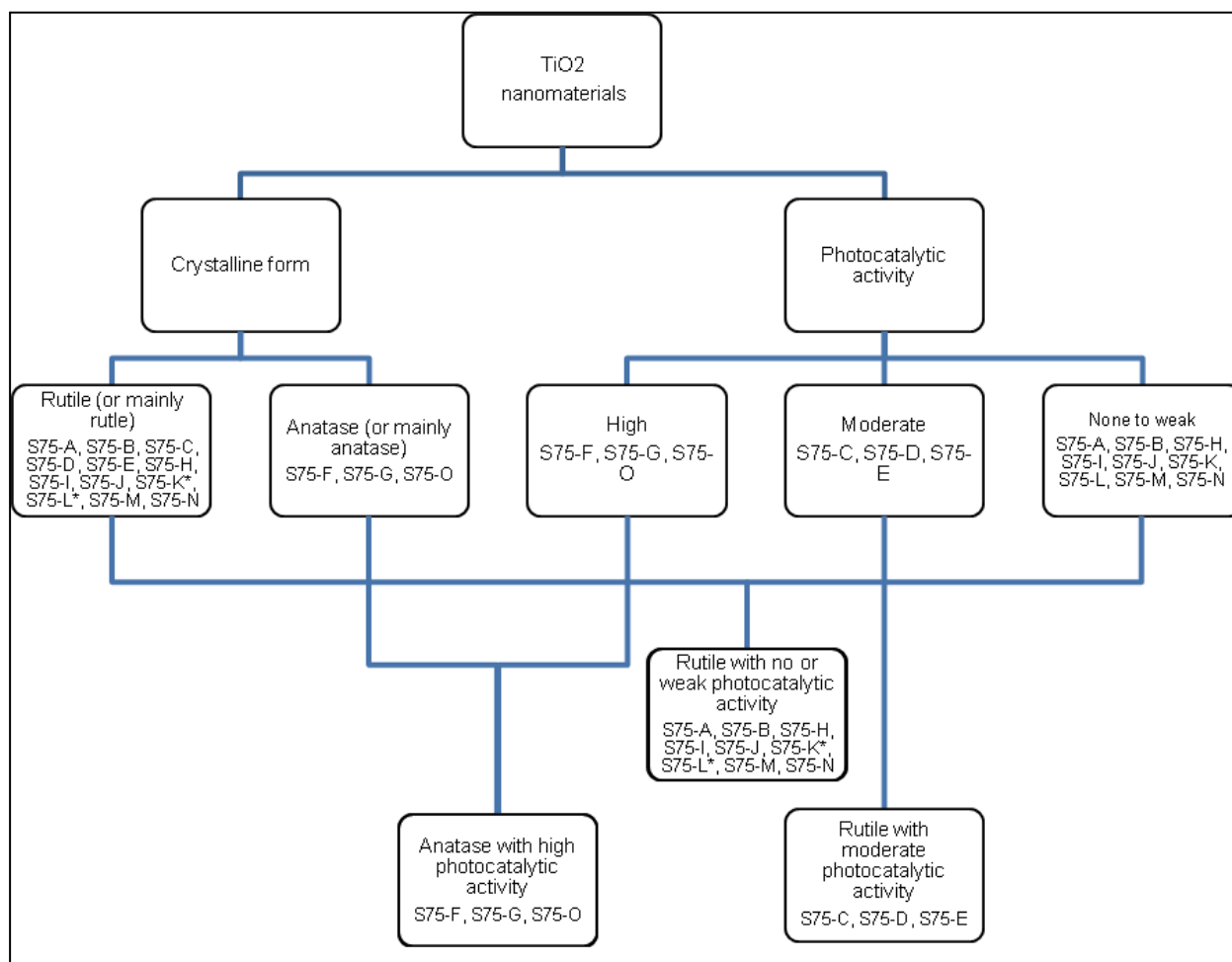
52 *'When coupled with UV irradiation, anatase TiO<sub>2</sub> (hydrophilic, circa 20 nm) was clearly more*  
53 *photogenotoxic than TiO<sub>2</sub> (anatase and rutile, both 255 nm) in mouse lymphoma L5178Y*



## Revision of the opinion on Titanium Dioxide, nano form

1 cells, as measured by the comet assay (Nakagawa et al. 1997). Rutile of larger particle size  
 2 (420 nm) was not photogenotoxic. The nanosized anatase TiO<sub>2</sub> was also photogenotoxic in  
 3 Chinese hamster lung CHL/IU cells, when assessed by chromosome aberration induction,  
 4 but not in *Salmonella typhimurium* or in mouse lymphoma L5178Y tk+/- cells, when studied  
 5 for mutation induction (Nakagawa et al. 1997). Furthermore, this nanosized TiO<sub>2</sub>  
 6 (hydrophilic surface) only induced DNA damage, chromosome aberrations and mutations  
 7 with UV radiation.'

8  
9



10  
11  
12  
13

\* S75-K and S75L have stated purity of >94% with no impurity profile provided.

14 On the basis of above physicochemical considerations, the SCCS has considered the TiO<sub>2</sub>  
 15 nanomaterials in the following 3 groups of for the purpose of this assessment:

- 16 - 9 materials (S75-A, S75-B, S75-H, S75-I, S75-J, S75-K, S75-L, S75-M, S75-N) on the  
 17 basis that they are (mainly) rutile with a relatively low photocatalytic activity. However,  
 18 two of these materials (S75-K and S75-L) have a stated purity of >94%, with no  
 19 impurity profile provided. These two materials (S75-K and S75-L) were considered by  
 20 the SCCS to be not sufficiently pure to include in this opinion.
- 21 - 3 materials on the basis that they are rutile with a moderate photocatalytic activity  
 22 (S75-C; S75-D; S75-E);
- 23 - 3 materials on the basis that they are (mainly) anatase, and also that they have a  
 24 strong photocatalytic activity (S75-F, S75-G, S75-O).

1 In view of the foregoing, it is important to note that this opinion applies to all fifteen (15)  
 2 nanomaterials presented in this submission. The opinion may, however, be also applicable  
 3 to other TiO<sub>2</sub> nanomaterials that have similar characteristics to the 15 nanomaterials in this  
 4 submission in terms of the physicochemical parameters listed in Tables 1-3, and other  
 5 specific provisions laid out in Section 2 below.

#### 7 **1.4 Function and uses**

8  
 9 Titanium dioxide is used as an UV-filter in a concentration up to 25% in cosmetic products.  
 10 It is regulated in Annex VII, entry 27 of the Cosmetics Directive  
 11

#### 12 **1.5 Toxicological Evaluation**

##### 14 **1.5.1 Acute toxicity**

###### 16 **1.5.1.1 Acute oral toxicity**

###### 18 **Acute toxicity, single oral administration, rat**

19 Guideline: OECD Guidelines 401 and EEC Guidelines 92/32/EEC  
 20 Species/strain: 8 week old rats/Hsd-Win: WU  
 21 Group size: 5 male/ 5 female  
 22 Test substance: TiO<sub>2</sub> T805, hydrophobic fluffy white powder, CAS 100209-12-9  
 23 Batch: 27073  
 24 Purity: TiO<sub>2</sub> 96.5%, SiO<sub>2</sub> 3%, carbon approx 4%.  
 25 Vehicle: suspension in peanut oil  
 26 Dose levels: 2150 mg/kg  
 27 Dose volume: 21.5 ml/kg of 100 mg/ml  
 28 Route: Oral  
 29 Administration: single dose  
 30 GLP: yes  
 31 Study period: August 1993

###### 32 **References**

33 Submission I - Evonik (Degussa) 1993 (5) and DHS Evonik (Degussa) 1993 (1)

###### 35 **Results**

36 No signs of toxicity recorded during the observation period, no deaths recorded, necroscopy  
 37 showed no alterations, LD<sub>50</sub> for male and female rats >2150 mg/kg.

###### 39 **SCCS Comment**

40 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
 41 with organic coating of trimethoxy-caprylylsilane, in oily suspension. This study is relevant  
 42 to the nanomaterial group (85% anatase, 15% rutile).  
 43  
 44

###### 45 **Acute toxicity, multiple oral administration, rat**

46 Guideline: OECD Guidelines 401 and EEC Guidelines 92/32/EEC  
 47 Species/strain: 7 week old male rats, 8 week old female rats /Hsd-Cpb: WU  
 48 Group size: 5 male/ 5 female  
 49 Test substance: TiO<sub>2</sub> T817, hydrophobic fluffy white powder, CAS 100209-12-9  
 50 Batch: 04095  
 51 Purity: TiO<sub>2</sub> >97%, Fe<sub>2</sub>O<sub>3</sub> 2±1%, carbon 3.5-4.5%.

1 Vehicle: suspension in olive oil  
 2 Dose levels: Total dose of 2150 mg/kg (dosed twice in equal amount)  
 3 Dose volume: twice dose of 21.5 ml/kg of 50 mg/ml  
 4 Route: Oral  
 5 Administration: single dose  
 6 GLP: yes  
 7 Study period:  
 8 DHS Evonik (Degussa), 1993 (2)  
 9

#### 10 Results

11 No signs of toxicity were recorded during the observation period, no deaths recorded, only  
 12 signs of diarrhoea in 2 male and 1 female rats from day 1 until day 2 after administration.  
 13 Necroscopy showed no alterations, LD50 for male and female rats were >2150 mg/kg.  
 14

#### 15 **SCCS Comment**

16 The study relates to S75-F material included in the dossier, which is a coated, anatase/rutile  
 17 material, with organic coating of trimethoxy-n-octyl-silane, in oily suspension. This study is  
 18 relevant to the nanomaterial group (85% anatase, 15% rutile).  
 19  
 20

#### 21 **Approximate Lethal Dose study, Intra-gastric intubation, Rats**

22 Guideline: OECD Guidelines 401 and EEC Guidelines 92/32/EEC  
 23 Species/strain: 7 week old Male rats/Crl-CD®BR  
 24 Group size: not mentioned  
 25 Test substance: TiO<sub>2</sub> T805, white powder, CAS number 13463-67-7  
 26 Batch: H-20762  
 27 Purity: TiO<sub>2</sub> 100%.  
 28 Vehicle: suspension in deionised water  
 29 Dose levels: 2,300 to 11,000 mg/kg  
 30 Dose volume: not described  
 31 Route: Oral  
 32 Administration: single dose  
 33 GLP: No (not mentioned)  
 34 Study period: August-October 1994  
 35 Reference: Submission I - DuPont, 1994 (1)  
 36

#### 37 Results

38 No signs of toxicity were recorded during the observation period, no deaths recorded,  
 39 pathological examination not performed, weight loss (up to 6%) in some animals after 1  
 40 day of dosing, ALD >11000 mg/kg, considered as very low toxicity.  
 41

#### 42 **SCCS Comment**

43 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
 44 with organic coating of trimethoxy-caprylsilane, in oily suspension. This study is relevant  
 45 to the nanomaterial group (85% anatase, 15% rutile).  
 46  
 47

#### 48 **Exploratory study, acute toxicity, oral, mice (Wang et al., 2007)**

49 Guideline: OECD Guidelines, No. 420  
 50 Species/strain: mice/ CD-1 (ICR)  
 51 Group size: 80 (40 female, 40 male)  
 52 Test substance: TiO<sub>2</sub> nanoparticles (25, 80 and 155 nm) - not mentioned whether  
 53 rutile or anatase  
 54 Batch: not mentioned  
 55 Purity: not mentioned  
 56 Vehicle: 0.5% hydroxypropylmethylcellulose K4M used as a suspending agent.  
 57 Dose levels: 5 gram/kg bw

1 Dose volume: not mentioned  
 2 Route: single oral gavage  
 3 Administration: single high dose 5 g/kg bw gavage.

4 GLP:

5 Study period:

6 Reference 213: (Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., Jia, G., Gao, Y., Li,  
 7 B., Sun, J., Li, Y., Jiao, F., Zhao, Y. and Chai, Z. 2007. Acute toxicity and biodistribution of  
 8 different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 168  
 9 (2): 176-85).

10  
 11 Results  
 12 Retention of a small percentage of titanium (measured by ICP-MS) showed predominantly in  
 13 the liver and spleen. Kidney, liver and heart pathology was observed with all sizes, with  
 14 more pronounced effects for 80 and 155 nm particles. Changes in serum biochemical  
 15 parameters (increased lactate dehydrogenase (LDH) and alpha-hydroxybutyrate  
 16 dehydrogenase (alpha-HBDH) levels) were most pronounced for 80 nm particles.

### 17 18 **SCCS Comment**

19 The study has a number of flaws, and is therefore of little value to this assessment.  
 20 Sufficient characterisation of the nanomaterials used was not carried out, the administered  
 21 dose (5 g/kg/bw) was very high, frequent oesophageal ruptures were reported that led to  
 22 animal deaths, translocation of TiO<sub>2</sub> from GI tract was measured as titanium with no  
 23 evidence that it was in nanoparticulate form. It is not clear whether any of the effects  
 24 observed were due to TiO<sub>2</sub> toxicity, or simply overloading the gut at high dose of the  
 25 particulate material.

### 26 27 28 **SCCS Comment on Acute Oral Toxicity**

29 The TiO<sub>2</sub> nanomaterials tested for this endpoint are mainly anatase/rutile mixtures, coated  
 30 with trimethoxy-n-octyl-silane. The derived LD50 in rat is >2150 mg/kg. One study has  
 31 determined the approximate lethal dose at >11000 mg/kg.

32 In addition, the following two articles have been provided on acute toxicity, but they are of  
 33 no value to this assessment:

34 An article by Ferch, Habersang, 1982 (SI-3) is in fact an old review article (up to 1982)  
 35 which focuses mainly on the possible health effects of amorphous and crystalline silica. It  
 36 also includes literature review on possible effects of Degussa P25 TiO<sub>2</sub> on the formation and  
 37 induction of granulomatous changes in the lungs or the peritoneum. Since these were not  
 38 found, the authors claim that P25 TiO<sub>2</sub> is not toxic. As such the article does not provide  
 39 experimental data, but is solely a review of the literature, with the main emphasis on SiO<sub>2</sub>  
 40 and only a few remarks on P25 TiO<sub>2</sub>.

41 An article by Warheit et al., 2007 (SI-II-215) is a review of different studies on ultrafine  
 42 TiO<sub>2</sub> particles to develop a base set of toxicity tests. As such it does not provide any details  
 43 on the studies or any experimental data that could be used for this assessment.

44 From the limited data available, the acute oral toxicity of nano-TiO<sub>2</sub> (anatase and rutile  
 45 mixtures) appears to be very low.

### 46 **1.5.1.2 Acute dermal toxicity**

#### 47 48 **Exploratory study, Acute toxicity and Skin and Eye irritation tests, Mouse and** 49 **Rabbit**

50  
 51 Guideline: OECD Guidelines 401 and EEC Guidelines 92/32/EEC  
 52 Species/strain: Male albino mice (acute toxicity tests), and male albino rabbits (skin  
 53 irritation tests), male albino rabbits (eye irritation tests)  
 54 Group size: 10 mice for toxicity tests, 4 rabbits for skin irritation tests, 3 rabbits

1		for eye irritation tests
2	Test substance:	TiO <sub>2</sub> (referred to as natural colour)
3	Batch:	
4	Purity:	not stated
5	Vehicle:	suspension in water
6	Dose levels:	up to 10 g/kg for toxicity study, 100mg/square inch for skin patch tests, 100 mg for eye irritation tests
7		
8	Dose volume:	
9	Route:	Oral intubation for toxicity tests, skin patch for irritation test,
10		instillation in lower conjunctival sac of eye,
11	Administration:	7 days for toxicity tests, 48 hours for skin irritation tests, eye washed
12		after 5 minutes of instillation.
13	GLP:	No
14	Study period:	

#### 16 Reference 2

17 (Roy, D. and Saha, J. (1981) Acute toxicity of dyes used in drugs and cosmetics, The  
18 Eastern Pharmacist, May 1981, pages 125-126)

#### 20 Results

21 No mortality recorded in mice, even at 10 g/kg. No sign of skin irritation or eye irritation.  
22 LD<sub>50</sub> >10,000 mg/kg, TiO<sub>2</sub> regarded as non-toxic, non-irritant to both skin and eye.

#### 24 **SCCS Comment**

25 The study is of little value in relation to the current assessment for nano-forms of TiO<sub>2</sub> as  
26 characterisation data (particle size distribution) have not been provided to show that the  
27 tested materials were nanomaterials.

#### 30 **Acute dermal toxicity, limit test, rat**

31	Guideline:	OECD Guidelines 401 and EEC Guidelines 92/32/EEC
32	Species/strain:	8 week old rats/Sprague Dawley
33	Group size:	5 male/ 5 female
34	Test substance:	TiO <sub>2</sub> NP88/296 (ultrafine), fluffy white powder, CAS 35 100209-12-9
36	Batch:	control No. 27073; July 27 <sup>th</sup> , 93.
37	Purity:	TiO <sub>2</sub> 96.5%, SiO <sub>2</sub> 3%, carbon approx 4%.
38	Vehicle:	suspension in peanut oil
39	Dose levels:	2000 mg/kg
40	Dose volume:	
41	Route:	Dermal
42	Administration:	single application under occlusion
43	GLP:	yes
44	Study period:	February 1989
45	Submission I	
46		Croda (Tioxide UK), 1989 (Reference 6)

#### 48 Results

49 No deaths recorded after 24 hour dermal administration, under occlusion, of NP 88/296 at  
50 2000 mg/kg. Clinical signs noted only after day 1 of dosing, and included hypokinesia,  
51 ataxia, chromodacryorrhoea (eyes and nose), animals hot to the touch. All animals were  
52 normal 2 days after dosing. Median Dermal lethal dose (LD<sub>50</sub>) of NO 88/296 in rats is  
53 >2000 mg/kg. No significant abnormalities noted after post-mortem.

#### 55 **SCCS Comment**

56 The study used ultrafine TiO<sub>2</sub>, and lacks data on characterisation (particle size distribution)  
57 of the tested material. According to the Applicant, the material used in this study relates to

1 rutile material coated with alumina/silica (i.e. S75-A, S75-B, S75-C, S75-L). It is however  
2 not clear how the test material relates to those included in the dossier and what proportion  
3 of the micronized material was in the nano-scale.

#### 5 **SCCS Comment on Acute Dermal Toxicity**

6 The TiO<sub>2</sub> material tested in one study is described as 'natural colour'. The other study has  
7 used ultrafine TiO<sub>2</sub>, and it is not clear what proportion of the micronized material (coated  
8 with alumina/silica) was in the nano-scale. Another reference provided in relation to acute  
9 toxicity (Submission I – ref 4, Trochimowicz et al., 1988) is in fact a secondary citation of  
10 the oral lethal dose cited in another article which relates to chronic inhalation toxicity.

11 From the provided test data, acute dermal LD<sub>50</sub> of TiO<sub>2</sub> has been derived at >2000 mg/kg  
12 (ultrafine material), and >10,000 mg/kg (natural colour material). However, the provided  
13 studies are of no value to the current assessment of nano forms of TiO<sub>2</sub>.

#### 15 **1.5.1.3 Acute inhalation toxicity**

16 No study has been provided on acute inhalation toxicity. The SCCS has therefore considered  
17 relevant studies in the open literature:

#### 20 **Respiratory deposition of particles**

21 Inhaled particulate materials may deposit in the lung depending on size (and shape) of the  
22 particles, structure of the lung, and breathing pattern (Sarangapani & Wexler, 2000). The  
23 mammalian respiratory tract is often divided into three regions - the extrathoracic (mouth  
24 or nose and throat), the trachea-bronchial and the alveolar regions with each having a  
25 typical structure and function. In general, particles >10 µm deposit in the extrathoracic  
26 region. Nanoparticles also mainly deposit in the extrathoracic region, but alveolar deposition  
27 has been noted for particles with a size of 300-200 nm down to 3-2 nm (ICRP 1994 –  
28 Oberdorster 2005, Cassee et al. 2002).

29 Particulate materials getting into the lung are generally cleared from the respiratory system.  
30 Large insoluble particles are cleared mechanically, whereas those that dissolve in the lung  
31 are removed via adsorption. Particles in the extrathoracic region are generally removed by  
32 coughing or swallowed into the gastrointestinal tract. Particles deposited into the trachea-  
33 bronchial region are in contact with the mucus layer covering the ciliated cells, and are  
34 generally cleared via the 'mucociliary escalator', which moves the mucus (and the particles)  
35 toward the epiglottis where they are subsequently swallowed and cleared via the GI-tract.  
36 Clearing of particles from the alveolar region is much slower and may take weeks to years.  
37 The most important pathway here involves alveolar macrophages. These phagocytic cells  
38 reside on the alveolar epithelium, and phagocytize the particles. The particle-laden  
39 macrophages can be removed via the mucociliary escalator, or can translocate to the  
40 interstitial tissue – together with free particles. These clearance mechanisms are similar in  
41 humans and most mammals, although clearance rates can significantly differ between  
42 species.

43 Some particles may be retained in the alveoli for long periods (months) before being  
44 cleared. A small fraction of the inhaled particles can reach the systemic circulation by  
45 passing the pulmonary epithelial barrier; another small fraction can probably reach the  
46 brain via olfactory nerve route. It has been shown that ultrafine (including nano) particles  
47 have a longer retention time in the alveoli compared to larger particles (Oberdorster, 1994).  
48 During chronic and/or cumulative exposure nanoparticles in the alveoli potentially  
49 accumulate in the tissue of the entire lungs.

50 Exposure to ultrafine particles has been linked to inflammatory and neurodegenerative  
51 changes in the olfactory mucosa, olfactory bulb, and cortical and subcortical brain structures  
52 (Oberdorster, 2005). So far there are no toxicological studies available which show  
53 extrapulmonary effects when the exposure was performed under relevant occupational or  
54 environmental conditions. Yet there exists a vast epidemiological literature which clearly

1 indicates exposures to urban ambient aerosols containing nano-sized particles at high  
2 number concentrations are associated with cardiovascular morbidity and mortality (Pope et  
3 al., 2009).

4

#### 5 **4-Hour Acute Inhalation Toxicity Study in Rats**

6 Authors: Dekker, U.  
7 Reference: RCC-Report B25007, internal report  
8 Guideline: The following guidelines were considered:  
9 European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity  
10 (Inhalation)", published December 29, 1992 and European Communities  
11 Directive 93/21/EEC, April 27, 1993 amending the aforementioned  
12 Directive.  
13 OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute  
14 Inhalation Toxicity", adopted May 12, 1981.  
15 U.S. Environmental Protection Agency, Health Effects Test Guidelines  
16 OPPTS 870.1300, Acute Inhalation Toxicity, August 1998.  
17 Species/strain: 15 males and 15 females HanRcc:WIST(SPF) rats; 9-10 weeks old  
18 Group size: 15 rats per group, one TiO<sub>2</sub> exposed group, one placebo exposed group  
19 Test substance: TiO<sub>2</sub>;  
20 Batch: /  
21 CAS No. /  
22 Purity: unknown  
23 Dose levels: A mean TiO<sub>2</sub> aerosol concentration of 4.877 mg/L was inhaled by the rats.  
24 TiO<sub>2</sub> particles were resuspended in water and jet nebulized. Median  
25 aerodynamic diameters (MMADs) and geometric standard deviations (GSD)  
26 were 1.4 µm (GSD 2.10)  
27 Route: Acute 4-hour nose-only inhalation. After a 4-hour inhalation BAL was  
28 performed in satellite groups of 5 rats at 14 hours and 2 days after  
29 inhalation. The rats were studied at day 15 after exposure.  
30 GLP: No  
31 Study period:

32

#### 33 **Results**

34 In BALF collected at 14 hours post end of exposure, total cell count (neutrophil numbers)  
35 and total protein were significantly elevated in both sexes of the exposed group compared  
36 to the control group. The changes in BALF were consistent with the histopathology findings  
37 of diffuse alveolar histiocytosis and alveolar lining cell activation seen in all animals of the  
38 exposed group. Significant increases of the absolute and relative lung weights and  
39 histopathology findings of diffuse alveolar histiocytosis and alveolar lining cell activation  
40 were found in the exposed group on day 2. These findings were consistent with TNFα and  
41 IL-6 levels in BALF higher in females of the exposed group than in control group on day 2.

42

#### 43 **SCCS Comments**

44 It is not clear which of the three noted guidelines were followed. The distribution was not  
45 investigated. The deposited TiO<sub>2</sub> particle dose was not determined. The exposed group  
46 showed signs of inflammation based on the methodology applied. The study was poorly  
47 performed and important control parameters are missing. This is by no means a  
48 comprehensive study and is of questionable value to this assessment.

49

#### 50 **Chronic inhalation Exposure of rats to titanium dioxide dust**

51 Authors: Trochimowicz, H.J. et al. (1988)  
52 Reference: Chronic inhalation study ref. No. 4  
53 Guideline: not specified  
54 Species/strain: 3-6 months ChR-CD rats at the begin of the study  
55 Group size: 11 males + 11 females  
56 Test substance: TiO<sub>2</sub> not specified  
57 Batch: not specified.



1 Purity: not specified  
 2 Dose levels: 250 mg/m<sup>3</sup>, 50 mg/m<sup>3</sup>, 10 mg/m<sup>3</sup>, 0 mg/m<sup>3</sup>, 6h/day, 5 days/week, 104  
 3 weeks  
 4 Route: chronic inhalation for 104 weeks;  
 5 Administration: whole body exposure  
 6 GLP: not specified  
 7 Study period: /

## 8 Results

10 After 3 months: alveolar cell hyperplasia at doses of 250 mg/m<sup>3</sup>, 50 mg/m<sup>3</sup>,  
 11 After 6 months: alveolar cell hyperplasia at all dose levels  
 12 After 12 months: additionally minute areas of collagen fiber deposition at 250 mg/m<sup>3</sup> dose  
 13 After 24 months: massive alveolar hyperplasia, focal patches of pneumonia, areas of  
 14 collagenized fibrosis; only at 250 mg/m<sup>3</sup> dose; occurrence of lung tumours  
 15 The authors conclude significant patho-physiological alterations at doses of 250 mg/m<sup>3</sup>, 50  
 16 mg/m<sup>3</sup> but not at 10 mg/m<sup>3</sup>

## 17 SCCS Comment

18 This study is one of the early chronic inhalation studies on titanium dioxide which triggered  
 19 later chronic inhalation studies in the 1980s and 1990s and later investigations into  
 20 biokinetics and more toxicological endpoints.  
 21

## 22 Studies in open literature

23 Several sub-chronic (90 days) TiO<sub>2</sub> inhalation exposure studies have been reported:

- 26 - Rats inhaled a TiO<sub>2</sub> aerosol of 22 mg/m<sup>3</sup> concentration consisting either of  
 27 nanostructured or pigmentary TiO<sub>2</sub> particles for 6h/d 5d/wk for 12 consecutive weeks  
 28 and were followed up for 1 year (Ferin et al., 1992).
- 29 - Rats, mice and hamsters inhaled a nanostructured TiO<sub>2</sub> aerosol at concentrations of 10,  
 30 50 or 250 mg/m<sup>3</sup> for 6h/d 5d/wk for 13 consecutive weeks and were followed up for 1  
 31 year (Bermudez et al., 2002; Everitt et al., 2000).
- 32 - Rats, mice and hamsters inhaled a nanostructured TiO<sub>2</sub> aerosol at concentrations of 0.5  
 33 or 2 or 10 mg/m<sup>3</sup> for 6h/d 5d/wk for 13 consecutive weeks and were followed up for 1  
 34 year (Bermudez et al., 2004)

35 Common findings of these sub-chronic studies were: substantial responses of inflammation  
 36 and overload associated with diminishing particle clearance in a dose dependent manner,  
 37 and histologically clear indications of epithelial hypertrophy and hyperplasia. Most  
 38 pathophysiological responses disappeared after 1 year of recovery and only the very high  
 39 doses led to persistent adverse effects. Rats always responded more sensitively than mice;  
 40 hamsters had the least response. When nanostructured or pigmentary TiO<sub>2</sub> particles were  
 41 compared, stronger effects were observed for the nanostructured particles.

42 Two 5-day inhalation-exposure studies in rats with a follow-up of 28 days as a substitute of  
 43 sub-chronic 90-days studies with a follow-up of 1 year have been conducted:

- 44 - TiO<sub>2</sub> nanoparticles at a concentration of 100 mg/m<sup>3</sup>, and pigmentary TiO<sub>2</sub> particles at a  
 45 concentration of 250 mg/m<sup>3</sup> - with a positive control exposure to quartz particles at 100  
 46 mg/m<sup>3</sup> (van Ravenzwaay et al., 2009) were investigated. Mild inflammation was  
 47 reported in lung histology and BAL with subsequent reversibility. All responses were  
 48 transient but the quartz effects persisted. The authors suggested that the effects seen in  
 49 these short term studies would be similar to those after 90-day exposure studies. It is  
 50 however not clear to the SCCS how the major differences seen in these and the other  
 51 studies can be equated.
- 52 - Nanostructured TiO<sub>2</sub> particles at concentration of 2, 10 and 50 mg/m<sup>3</sup> were  
 53 investigated. Transient inflammatory responses were observed in lung histology and  
 54 BAL. (Ma-Hock et al., 2009).



1 Another intratracheal instillation study used nanostructured anatase TiO<sub>2</sub> particles of 5, 23  
2 and 154 nm (actual hydrodynamic diameters of 19, 28 and 176 nm) at a concentration of 5  
3 mg/kg bw administered to the rats and studied until three months after instillation. The  
4 results showed that the smaller the particles, the larger the inflammatory response and  
5 hypertrophy. However the effects were transient, (Kobayashi et al., 2009). Several other  
6 instillation studies have been published that used nano- and submicron-sized TiO<sub>2</sub> particles  
7 but they have not been considered here because the particles had already formed larger  
8 sized agglomerates.  
9

#### 10 **SCCS Comment on acute inhalation toxicity**

11 No study on acute inhalation toxicity was provided. Studies (including open literature) on  
12 acute and sub-chronic inhalation exposure to TiO<sub>2</sub> nanomaterials have indicated substantial  
13 inflammatory responses, and histologically clear indications of epithelial hypertrophy and  
14 hyperplasia at high exposure dose. In view of this, the SCCS does not recommend the use  
15 of nano TiO<sub>2</sub> in applications that would lead to any significant inhalation exposure (e.g.  
16 powder or sprayable products).  
17

### 18 **1.5.2 Irritation and corrosivity**

19

#### 20 **1.5.2.1 Skin irritation**

21

##### 22 **Skin irritation/corrosion, Patch test, Rabbit**

23 Guideline: OECD Guidelines 404 and EEC Guidelines 92/32/EEC  
24 Species/strain: 11 month old Rabbit/white Russian  
25 Group size: 3 male  
26 Test substance: TiO<sub>2</sub> T805, hydrophobic fluffy white powder, CAS 100209-12-9  
27 Batch: 27073.  
28 Purity: TiO<sub>2</sub> 96.5%, SiO<sub>2</sub> 3%, carbon approx 4%.  
29 Vehicle: Paraffin  
30 Dose levels: 0.5 g in 0.64 ml paraffin to dorsal skin area patch 6.25 cm<sup>2</sup>.  
31 Dose volume:  
32 Route: skin patch  
33 Administration: single application, observation over 3 days  
34 GLP: yes  
35 Study period: August 1993

36

37

38

39

40 Results  
41 Very slight erythema (grade 1 in 2 animals), very slight edema (one animal) after one day  
42 of exposure. Primary Irritation Index is 0.3, TiO<sub>2</sub> was regarded non-irritant on rabbit skin.

43

##### 43 **SCCS Comment**

44 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
45 with organic coating of trimethoxy-caprylylsilane, in oily suspension. This study is relevant  
46 to the nanomaterial group (85% anatase, 15% rutile).  
47

48

##### 48 **Skin irritation/corrosion, Patch test, Rabbit**

49 Guideline: OECD Guidelines 404 and EEC Guidelines 92/69/EEC  
50 Species/strain: 48 month old male, 43 month old female Rabbit/white Russian  
51 Group size: 3 (1 male, 2 female)  
52 Test substance: TiO<sub>2</sub> T817, hydrophobic fluffy white powder, CAS number  
53 100209-12-9  
54 Batch:

54

Submission I

Evonik (Degussa), 1993 (13)

DHS Evonik (Degussa), 1993 (5)

1	Purity:	TiO <sub>2</sub> > 97%, Fe <sub>2</sub> O <sub>3</sub> 2±1%, carbon approx 3.5-4.5%.
2	Vehicle:	
3	Dose levels:	0.5 g in peanut oil to dorsal skin area patch 6.25 cm <sup>2</sup> .
4	Dose volume:	
5	Route:	skin patch
6	Administration:	single application, observation over 3 days
7	GLP:	Yes
8	Study period:	February 1998
9	Reference:	DHS Evonik (Degussa), 1998 (6)

10

11 **Results**

12 No changes observed, neither erythema nor edema observed. Primary Irritation Index was  
 13 0.0, TiO<sub>2</sub> regarded non-irritant on rabbit skin. No systemic effects observed.

14

15 **SCCS Comment**

16 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
 17 with organic coating of trimethoxy-n-octyl-silane, in oily suspension. The study is relevant  
 18 to the nanomaterial group (85% anatase, 15% rutile).

19

20 **Skin irritation/corrosion, Patch test, Rabbit**

21	Guideline:	OECD Guidelines 404 and EEC Guidelines 92/32/EEC
22	Species/strain:	11 month old Rabbit/ New Zealand white
23	Group size:	3 male, 3 female
24	Test substance:	TiO <sub>2</sub> H20762, CAS number 13463-67-7
25	Batch:	
26	Purity:	TiO <sub>2</sub> 100%.
27	Vehicle:	
28	Dose levels:	0.5 g in pre-moistened patch (2 inch square gauze)
29	Dose volume:	
30	Route:	skin patch
31	Administration:	single application, observation over 3 days
32	GLP:	No (not mentioned)
33	Study period:	August-September 1994
34	Reference:	Submission I - DuPont, 1994 (10)

35

36

37 **Results**

38 Three rabbits showed no dermal irritation during the study, no to mild erythema by 1 hour  
 39 after patch removal. By 24, 48 and 72 hours, no to slight erythema observed, no edema  
 40 observed during the study. H-20762 is regarded a mild skin irritant.

41

42 **SCCS Comment**

43 The study is of little value in relation to assessment for nano-form of TiO<sub>2</sub> as there is a lack  
 44 of data on characterisation (particle size distribution) of the tested materials to show that  
 45 they were nanomaterials.

46

47 **Skin irritation/corrosion, Patch test, Rabbit**

48	Guideline:	not mentioned
49	Species/strain:	Rabbit/ albino
50	Group size:	6 male
51	Test substance:	TiO <sub>2</sub> - referred to as Haskell Nos. (H 12684, H 12685, H 12686)
52	Batch:	
53	Purity:	not mentioned
54	Vehicle:	
55	Dose levels:	0.5 g pre-moistened with physiological saline (1½ inch square 56 gauze)
57	Dose volume:	

1 Route: skin patch  
 2 Administration: single application, observation over 2 days  
 3 GLP: No (not mentioned)  
 4 Study period:  
 5 Reference: Submission I DuPont, 1978 (11)  
 6

7 Results  
 8 No skin irritation observed on intact rabbit skin.  
 9

#### 10 **SCCS Comment**

11 The study is of little value in relation to assessment for nano-form of TiO<sub>2</sub> as there is a lack  
 12 of data on characterisation (particle size distribution) of the tested materials to show that  
 13 they were nanomaterials.  
 14

#### 15 **Skin irritation/corrosion, Patch test, guinea pig**

16 Guideline: not mentioned  
 17 Species/strain: guinea pig/ albino  
 18 Group size: 12 male  
 19 Test substance: TiO<sub>2</sub> - referred to as 99.5% active ingredient  
 20 Batch:  
 21 Purity:  
 22 Vehicle:  
 23 Dose levels: 0.5 g powder and 0.1 g 28% paste were slightly rubbed into  
 24 shaved back skin, covered with impervious film and wrapped.  
 25 Dose volume:  
 26 Route: skin patch  
 27 Administration: single application, 24 hours, then rinsed in water, observation  
 28 over 2 days  
 29 GLP: No (not mentioned)  
 30 Study period:  
 31 Reference: Submission I - DuPont, 1969 (12)  
 32

33 Results  
 34 No skin irritation observed on intact guinea pig skin.  
 35

#### 36 **SCCS Comment**

37 The study is of little value in relation to assessment for nano-form of TiO<sub>2</sub> as there is a lack  
 38 of data on characterisation (particle size distribution) of the tested materials to show that  
 39 they were nanomaterials.  
 40

#### 41 **Skin irritation/corrosion, 5 day repeat application study, Rabbit**

42 Guideline: not mentioned  
 43 Species/strain: Rabbit  
 44 Group size: 2 male, 1 female  
 45 Test substance: TiO<sub>2</sub> ultrafine dispersion - referred to as NP 89/97, NP 89/98.  
 46 Batch:  
 47 Purity: not mentioned  
 48 Vehicle:  
 49 Dose levels: around 0.5 g (2.5 cm<sup>2</sup> patch)  
 50 Dose volume: around 0.5 ml  
 51 Route: skin patch  
 52 Administration: 4x repeated (application, removal, skin observation)  
 53 GLP: No  
 54 Study period:  
 55 Reference: Submission I - Croda (Tioxide UK), 1989 (14)

1  
2 Results  
3 One animal died on day 4 (unrelated to the test), 5 day repeat applications produced mean  
4 irritation scores of 1.58 and 1.92 for 89/97, NP 89/98 respectively. NP 89/98 considered  
5 slightly more irritant than NP 89/97.  
6

### 7 **SCCS Comment**

8 The study used ultrafine TiO<sub>2</sub>, however, data on characterisation (particle size distribution)  
9 of the tested material has not been reported. It is therefore not clear whether the material  
10 had a nano-sized fraction, and if so, in what proportion.  
11

### 12 **Skin irritation/corrosion, 5 day repeat application study, Rabbit**

13 Guideline: not mentioned  
14 Species/strain: Rabbit/ New Zealand white  
15 Group size: 3 (2 male, 1 female)  
16 Test substance: TiO<sub>2</sub> ultrafine dispersion - referred to as NP 88/296.  
17 Batch:  
18 Purity: not mentioned  
19 Vehicle:  
20 Dose levels: 2 dispersions tested (40% A.I. and 10% A.I. which was diluted  
21 with carrier oil NP88/310)  
22 Dose volume: around 0.5 ml  
23 Route: skin patch  
  
24 Administration: 4x repeated (application, removal, skin observation)  
25 GLP: No (not mentioned)  
26 Study period:  
27 Reference: Submission I - Croda (Tioxide UK), 1989 (15)  
28

29 Results  
30 5 day repeat applications produced mean irritation scores of 0.13 for both dispersions  
31 tested (i.e. no dose response). Neither the undiluted or diluted test material NP 88/296  
32 produced significant reactions. One rabbit did not react, and the other 2 rabbits showed only  
33 slight to non persistent erythema.  
34

### 35 **SCCS Comment**

36 The study used ultrafine TiO<sub>2</sub>. However, there is a lack of data on characterisation (particle  
37 size distribution) of the tested material. According to Applicant, the material used in this  
38 study relates to rutile material coated with alumina/silica (i.e. S75-A, S75-B, S75-C, S75-L).  
39 However it is not clear how the test material relates to the nanomaterials included in the  
40 dossier and what proportion of the micronized material was in the nano-scale.  
41

### 42 **SCCS Comment on Skin irritation**

43 The study by Warheit et al., 2007 (SI-II-215) is of no use to this assessment because it is a  
44 detailed literature review on the possible effects of different TiO<sub>2</sub> ultrafine particles. As such  
45 it does not provide details on the studies, or any experimental data, that could be used for  
46 this assessment.

47 Two studies provided in the submission are relevant to the TiO<sub>2</sub> nanomaterials. They relate  
48 to anatase/rutile mixture, coated with trimethoxy-n-octyl-silane. In one of the studies, the  
49 test animals showed signs of very slight erythema and oedema. The primary irritation index  
50 was estimated to be zero and 0.3, and the materials regarded as non-irritant on rabbit skin.

51 Two other studies used ultrafine grade materials and showed the mean irritation scores of  
52 0.3 and 1.58-1.92 during 5 day repeat applications on rabbit skin, but the proportion of  
53 nano-scale fraction in the materials used has not been reported.

1 The remaining 3 studies showing the tested materials as either mild irritant or non irritant  
2 to rabbit and guinea pig skin are of little value to this assessment because there is a lack of  
3 data on characterisation (particle size distribution) of the tested materials, and it is not clear  
4 whether they were in fact nanomaterials.

5 From the limited useful data presented in the dossier, it appears that the TiO<sub>2</sub>  
6 nanomaterials are either mild or non-irritant to skin.

7

#### 8 1.5.2.2 Mucous membrane irritation

9

##### 10 **Eye irritation, single application, rabbit**

11 Guideline: OECD Guidelines 405 (1) and EEC Guidelines 92/32/EEC  
12 Species/strain: 10-11 month old Rabbits/ white Russian (albino)  
13 Group size: 3 (males)  
14 Test substance: TiO<sub>2</sub> T805, hydrophobic fluffy white powder, CAS 100209-12-9  
15 Batch: 27073  
16 Purity: TiO<sub>2</sub> 96.5%, SiO<sub>2</sub> 3%, carbon approx 4%.  
17 Vehicle:  
18 Dose levels: 22.8 to 24.3 mg  
19 Dose volume: 0.1 ml  
20 Route: eye instillation  
21 Administration: single application, 3 days observation period  
22 GLP: Yes  
23 Study period: August 1993  
24 Reference: Submission I - Evonik (Degussa), 1993 (9); DHS Evonik  
25 (Degussa), 1993 (3)

26

##### 27 Results

28 No alterations detected in cornea, iris and conjunctiva, primary irritation index is zero, TiO<sub>2</sub>  
29 (805) regarded as non-irritant on rabbit eye. No systemic toxic effects detected.

30

##### 31 **SCCS Comment**

32 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
33 with organic coating of trimethoxy-caprylylsilane, in oily suspension. This study is relevant  
34 to the nanomaterial group (85% anatase, 15% rutile).

35

##### 36 **Eye irritation, single application, rabbit**

37 Guideline: OECD Guidelines 405 (1) and EEC Guidelines 92/69/EEC  
38 Species/strain: 35 month old Rabbits/ white Russian (albino)  
39 Group size: 3 (females)  
40 Test substance: TiO<sub>2</sub> T817, hydrophobic fluffy white powder, CAS number  
41 100209-12-9  
42 Batch: 04095  
43 Purity: TiO<sub>2</sub> >97%, Fe<sub>2</sub>O<sub>3</sub> 2±1%, carbon 3.5-4.5%.  
44 Vehicle:  
45 Dose levels: 11.5 to 16.8 mg  
46 Dose volume: 0.1 ml  
47 Route: eye instillation  
48 Administration: single application, 3 days observation period  
49 GLP: Yes  
50 Study period: February 1998  
51 Reference: DHS Evonik (Degussa), 1993 (4)

52

##### 53 Results

1 Some blood vessels definitely hyperaemic in two animals after one hours of application.  
 2 Primary irritation index is 0.3, TiO<sub>2</sub> regarded as non-irritant on rabbit eye. No systemic  
 3 toxic effects detected.

#### 5 **SCCS Comment**

6 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
 7 coated with organic coating of trimethoxy-n-octyl-silane, in oily suspension. This study is  
 8 relevant to the nanomaterial group (85% anatase, 15% rutile).

#### 11 **Eye irritation, single application, rabbit**

12 Guideline: OECD Guidelines 405 (1) and EEC Guidelines 92/69/EEC  
 13 Species/strain: Rabbits/ New Zealand white  
 14 Group size: 2 (females)  
 15 Test substance: TiO<sub>2</sub> H-20762, CAS number 13463-67-7  
 16 Batch:  
 17 Purity: TiO<sub>2</sub> 100%.  
 18 Vehicle:  
 19 Dose levels: approx. 10 mg  
 20 Dose volume:  
 21 Route: eye instillation  
 22 Administration: single application, eye washed after 20 seconds of application. 3  
 23 days observation period  
 24 GLP: yes  
 25 Study period: September 1994  
 26 Reference: Submission I - DuPont, 1994 (7)

#### 28 Results

29 Moderate redness and slight chemosis observed in both treated and untreated washed eyes  
 30 (normal after 1 and 3 days respectively). No clinical signs of toxicity observed, TiO<sub>2</sub>  
 31 (H20762) regarded as moderate eye irritant but could be classified as non-irritant under the  
 32 EEC Directive 93/21, Annex VI.

#### 34 **SCCS Comment**

35 The study is of little value in relation to assessment for nano-form of TiO<sub>2</sub> as there is a lack  
 36 of data on characterisation (particle size distribution) of the tested materials to show that  
 37 they were nanomaterials.

#### 40 **Eye irritation, single application, rabbit**

41 Guideline: OECD Guidelines 405 (1) and EEC Guidelines 92/69/EEC  
 42 Species/strain: Rabbits/ New Zealand white  
 43 Group size: 3 (2 male, 1 female)  
 44 Test substance: TiO<sub>2</sub> NP 88/296 (ultrafine)  
 45 Batch: not mentioned  
 46 Purity: not mentioned  
 47 Vehicle:  
 48 Dose levels: not mentioned  
 49 Dose volume: 0.1 ml  
 50 Route: eye instillation  
 51 Administration: single application, eye washed after 20 seconds of application. 3  
 52 days observation period  
 53 GLP: Yes  
 54 Study period:  
 55 Reference: Submission I - Croda (Tioxide UK), 1989 (8)

#### 57 Results

1 No corneal or iridial reactions, slight conjunctival redness (score 1) which disappeared after  
2 72 hours of treatment. TiO<sub>2</sub> (NP88/296) is regarded slightly irritant to rabbit eyes.

#### 4 **SCCS Comment**

5 The study relates to ultrafine TiO<sub>2</sub>. However, information on the characterisation (particle  
6 size distribution) of the tested material has not been reported. According to the Applicant,  
7 the material used in this study relates to rutile material coated with alumina/silica (i.e. S75-  
8 A, S75-B, S75-C, S75-L). It is however not clear how the test material relates to the  
9 nanomaterials included in the dossier and what proportion of the micronized material was in  
10 the nano-scale.

#### 12 **SCCS Comments on Eye Irritation**

13 The following two articles provided with the submission on acute toxicity are of no value to  
14 this assessment:

- 15 1. An article by Frosch and Kligman (Reference 16 - S75 irritation skin) refers mainly to  
16 the development of a scarification chamber test for irritancy of materials. It does  
17 refer irritancy of titanium dioxide as low, but it is not clear whether the tested TiO<sub>2</sub>  
18 was a nanomaterial.
- 19 2. An article by Warheit et al., 2007 (SI-II-215) is a review of different studies on  
20 ultrafine TiO<sub>2</sub> particles to develop a base set of toxicity tests. As such it does not  
21 provide any details on the studies or any experimental data that could be used for  
22 this assessment.

23 Two other studies provided used TiO<sub>2</sub> anatase/rutile mixtures, coated with trimethoxy-n-  
24 octyl-silane. From these studies, primary irritation index was between zero and 0.3. Another  
25 study has regarded the tested material (TiO<sub>2</sub>-NP88/296) as slightly irritant to rabbit eye. In  
26 this study, the material used has been described as ultrafine rutile material coated with  
27 alumina/silica (relating to S75-A, S75-B, S75-C, S75-L) but information on characterisation  
28 (particle size distribution) has not been reported to indicate what proportion was in the  
29 nano-scale. Similarly, another study has regarded the tested material (TiO<sub>2</sub>-H20762)  
30 moderately irritant to rabbit eye, but it is not clear whether the tested material was a  
31 nanomaterial.

32 From the limited useful data provided, eye irritation potential of nano-TiO<sub>2</sub> appears to be  
33 low.

### 35 **1.5.3 Skin sensitisation**

#### 37 **Skin sensitisation, Guinea Pig, maximisation test**

38	Guideline:	OECD Guidelines 406 and EEC Guidelines 84/449/EEC
39	Species/strain:	8 week old 12 male, 10 female guinea pigs/Pirbright white
40	Group size:	3 (1 male, 2 female)
41	Test substance:	TiO <sub>2</sub> T805, hydrophobic fluffy white powder, CAS 100209-12-9
42	Batch:	030492
43	Purity:	TiO <sub>2</sub> 96.5%, SiO <sub>2</sub> 3%, carbon approx 4%.
44	Vehicle:	paraffin oil, Freund's Complete Adjuvant for immunisation
45	Dose levels:	0.5 g in paraffin oil to dorsal skin area 5 cm <sup>2</sup> patch.
46	Dose volume:	0.1 ml of 0.5% dispersion, 0.2 ml of 5% dispersion for
47		challenge
48	Route:	Induction application intradermal and epidermal, challenge
49		application epidermal
50	Administration:	single application, 48 hours, challenge on day 22 for 24 hours,
51		observation over 48 hours
52	GLP:	yes
53	Study period:	June 1992
54	Reference:	Submission I Evonik (Degussa), 1992 (19); DHS Evonik
55		(Degussa), 1992 (7)



1  
2  
3 **Results**  
4 Following epidermal challenge neither treated nor control animals showed any changes at  
5 the skin. TiO<sub>2</sub> regarded as non-sensitiser in maximisation test on guinea pig skin. No  
6 systemic effects observed.  
7

### 8 9 **SCCS Comment**

10 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
11 with organic coating of trimethoxy-caprylylsilane, in oily suspension. This study is relevant  
12 to the nanomaterial group (85% anatase, 15% rutile).  
13

### 14 **Skin sensitisation, Guinea Pig, Buehler test**

15 Guideline: OECD Guidelines 406 and EC Guidelines 96/54/EC  
16 Species/strain: 8 week old guinea pigs/PsdPCC: DH  
17 Group size: 20 male, 20 female (2 vehicle control groups of 10, and 1 test  
18 group of 20)  
19 Test substance: TiO<sub>2</sub> T817, hydrophobic fluffy white powder, CAS 100209-12-9  
20 Batch: 04095  
21 Purity: TiO<sub>2</sub> > 97%, Fe<sub>2</sub>O<sub>3</sub> 2±1%, carbon approx 3.5-4.5%.  
22 Vehicle: paraffin oil  
23 Dose levels: 0.5 g applied, 3 applications on day 1,8,15.  
24 Dose volume:  
25 Route: Induction phase duration 15 days, epidermal, challenge  
26 application epidermal (occlusive patch)  
27 Administration: epidermal, 48 hours, challenge on day 29 for 6 hours,  
28 observation over 48 hours.  
29 GLP: yes  
30 Study period: November-December 1997  
31 Reference: DHS Evonik (Degussa), 1992 (8)  
32

33 **Results**  
34 Following first challenge, 3 out of 10 animals reacted with an erythema and 1 in 10 animals  
35 showed edema. Following epidermal challenge neither treated nor control animals showed  
36 any changes at the skin. TiO<sub>2</sub> regarded as non-sensitiser in Buehler test on guinea pig skin.  
37 No systemic effects observed.  
38

### 39 **SCCS Comment**

40 The study relates to S75-F material included in the dossier, which is anatase/rutile material,  
41 coated with organic coating of trimethoxy-n-octyl-silane, in oily suspension. This study is  
42 relevant to the nanomaterial group (85% anatase, 15% rutile). Due to the absence of skin  
43 penetration of TiO<sub>2</sub> as demonstrated by many studies included in this dossier, the  
44 usefulness of the Buehler test for assessing sensitisation potency of nanomaterials is  
45 doubtful as it is based on exposure to intact skin.  
46

### 47 **Skin sensitisation, Guinea Pig, Magnusson-Kligman maximisation test**

48 Guideline:  
49 Species/strain: guinea pigs/Dunkin Hatley strain  
50 Group size: 20 test group, 16 control group  
51 Test substance: TiO<sub>2</sub> NP89/145  
52 Batch:  
53 Purity: TiO<sub>2</sub> 96.5%, SiO<sub>2</sub> 3%, carbon approx 4%.  
54 Vehicle: Freund's Complete Adjuvant for immunisation  
55 Dose levels: 2 cm x 4 cm patch, 2cm x 2 cm patch for challenge  
56 Dose volume:



1	Route:	Induction with NP 89/145 at 10% v/v in NP 88/310 (injection)
2		and 100% (topical), challenge application at 100% and 50% v/v
3		in NP88/310.
4	Administration:	Patch, 48 hours (induction patch), 24 hour (challenge patch),
5		observation period 24 and 48 hours
6	GLP:	Yes
7	Study period:	April-May 1989
8	Reference:	Submission I - Croda (Tioxide, UK), 1989 (20)

## 10 Results

11 At challenge, none of the test or control group animals treated with NP 89/145 at 100% or  
 12 50% v/v (in NP 88/310) showed a positive response. No evidence that NP 89/145 is a  
 13 sensitiser in guinea pigs. Classified as a weak sensitiser according to the Magnusson-  
 14 Kligman classification. No clinical signs were noted, body weight gains were acceptable.

### 16 SCCS Comment

17 The study used ultrafine TiO<sub>2</sub>, however, there is a lack of information on the  
 18 characterisation (particle size distribution) of the tested material. According to Applicant,  
 19 the material used in this study relates to rutile material coated with alumina/silica (i.e. S75-  
 20 A, S75-B, S75-C, S75-L). It is however not clear how the test material relates to the  
 21 nanomaterials included in the dossier because the proportion of the nano fraction in the  
 22 micronized material has not been provided.

### 24 SCCS Comment on Skin Sensitisation

25 The article by Warheit et al., 2007 (SI-II-215) is a review of different studies on ultrafine  
 26 TiO<sub>2</sub> particles to develop a base set of toxicity tests. As such it does not provide any details  
 27 on the studies or any experimental data that could be used for this assessment.

28 From two of the other studies, TiO<sub>2</sub> nanomaterials (anatase/ rutile mixture, coated with  
 29 trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane) have been regarded non-sensitiser.  
 30 Another material (rutile, coated with alumina/silica) is classified as a weak sensitiser  
 31 according to the Magnusson-Kligman classification (that considers 0 to 8% response a weak  
 32 sensitizer category). The material used in this study is described as ultrafine rutile material  
 33 coated with alumina/silica (relating to S75-A, S75-B, S75-C, S75-L) but information on  
 34 characterisation (particle size distribution) of the tested materials has not been reported to  
 35 indicate what proportion was in the nano-scale.

36 Due to the absence of skin penetration of TiO<sub>2</sub> as demonstrated by many studies included  
 37 in this dossier, the usefulness of the Buehler test for assessing sensitisation potency of  
 38 nanomaterials is doubtful as it is based on exposure to intact skin.

39 From the limited useful data, TiO<sub>2</sub> nanomaterials appear to be weak or non- skin sensitiser.

40

## 41 1.5.4 Dermal / percutaneous absorption

42

### 43 *In vitro* studies:

44

45 Guideline/method:

46 Species:

human abdominal epidermis

47 Test substances:

Titanium dioxide T805, comprising 5% micronized titanium  
 48 dioxide; not radiolabelled.

49 Particle size:

not given

50 Group sizes:

2 female donors in experiment 1, 1 male and 1 female donor in  
 51 experiment 2

52 Dose applied:

3.6g/cm<sup>2</sup> of cream with a content of 5% micronized titanium  
 53 dioxide (actual dose 3.55 mg/cm<sup>2</sup>)

1	Skin area:	0.32 cm <sup>2</sup>
2	Skin temperature:	30-32°C
3	Test chamber:	flow through diffusion cells
4	Receptor fluid:	0.9% saline
5	Exposure period:	6 hours
6	GLP:	yes
7	Published:	no
8	Study period:	1995
9	Reference:	Reference 24 submission 1

10  
11 Method  
12 The amount applied to each cell was 3.55 mg/cm<sup>2</sup>. Skin integrity was checked. The  
13 penetration through the skin membranes was determined over a period of 6 hours under  
14 non-occluded conditions. The receptor fluid was delivered at a flow rate of about 1.5 mL/h  
15 during the testing period. The perfusate from each cell was collected separately at ambient  
16 temperature for 0-8h post application. Eight hours post application the perfusate sampling  
17 was terminated. All skin membrane rinse fractions were combined according to the  
18 individual cells and added to the 0-8h perfusate.

#### 19 20 Results

21 The perfusate samples were analysed by IPCMS, the TiO<sub>2</sub> content ranged from 2.6 to 4.8  
22 ng/ml. These concentrations were reported to be in the same range as the 'blind' solutions  
23 (2.-2.9 ng/ml). Transmission electronic microscopy of titanium dioxide in the skin samples  
24 showed presence only in the outer skin layers and not in the deeper layers of the epidermis.  
25 Thus TiO<sub>2</sub> nanoparticles did not penetrate through human skin under the experimental  
26 conditions described above.

#### 27 28 **SCCS Comments**

29 The study shows lack of detectable skin penetration of the test nanomaterial which relates  
30 to S75-F included in the dossier (anatase/rutile material, with organic coating of  
31 trimethoxy-caprylsilane, in oily suspension). This study is relevant to the nanomaterial  
32 group (85% anatase, 15% rutile).

33 The particle size of the tested nano-material was not determined in this study. It is assumed  
34 that the particle size is similar to the data shown in Table 1.3. However most likely the  
35 particles were present as agglomerates as the test item was used in a cream formulation.

36	Study Design:	
37	Guideline/method:	-
38	Species:	human abdominal epidermis
39	Test substances:	micronized TiO <sub>2</sub> : Eusolex TA (5% O/W lotion),
40	micronized TiO <sub>2</sub> :	Eusolex TC (5% W/O cream)
41	vehicle	(O/W lotion and W/O cream)
42	Particle size:	particle sizes not provided, Eusolex TA: BET= 84.2 m <sup>2</sup> /g Eusolex TC:
43		BET= 58.8 m <sup>2</sup> /g
44	Group sizes:	4 cells per donor; 4 donors
45	Dose applied:	between 3.19 and 4.28 mg/cm <sup>2</sup>
46	Skin area:	0.32 cm <sup>2</sup>
47	Skin temperature:	30-32°C
48	Test chamber:	flow through diffusion cells
49	Receptor fluid:	0.9% saline
50	Exposure period:	6 hours
51	GLP:	yes
52	Published:	no
53	Study period:	1995
54	Reference:	Reference 25 submission 1

55  
56  
57 Method

1 The amount applied to each cell was 3.19-3.31 mg/cm<sup>2</sup> (Eusolex TC and TA, respectively;  
2 applied amount of vehicle only was slightly higher). Skin integrity was checked. The  
3 penetration through the skin membranes was determined over a period of 6 hours under  
4 non-occluded conditions. The receptor fluid was delivered at a flow rate of about 1.5 mL/h  
5 during the testing period. The perfusate from each cell was collected separately at ambient  
6 temperature for 0-8h post application.

7 Eight hours post application the perfusate sampling was terminated. All skin membrane  
8 rinse fractions were combined according to the individual cells and added to the 0-8h  
9 perfusate.

## 10 Results

11 The perfusate samples were analysed by ICP-OES, the TiO<sub>2</sub> content were below  
12 0.05ug/sample. No, or only slight traces of TiO<sub>2</sub> particles were detectable on the skin  
13 samples treated with Eusolex® TA under the light microscope. The refracting colourless  
14 TiO<sub>2</sub> particles were localized on the outer surface of the stratum corneum. One skin sample  
15 revealed two particles sited intracellularly at one location at the stratum granulosum.  
16 Whether these were refracting particles of TiO<sub>2</sub> could not be resolved unequivocally under  
17 the optical microscope. Multiple foci of TiO<sub>2</sub> particles were observed on most of the skin  
18 samples that had been treated with Eusolex® TC. The refracting particles were localized on  
19 the outer surface of the stratum corneum. It was concluded that titanium dioxide  
20 nanoparticles did not penetrate through human skin under the experimental conditions  
21 described above.  
22

## 23 SCCS Comments

24 The study shows lack of detectable dermal penetration of TiO<sub>2</sub> nanoparticles. The test  
25 material possibly (as it is not clear from the different code) relates to S75-M, S75-N, and/or  
26 S75-O. The particle size of the tested nano-material was not determined in this study.  
27  
28  
29

## 30 Test for penetration of micronized TiO<sub>2</sub> through the egg membrane or the chorio- 31 allantoic membrane (CAM).

32 Guideline:

33 Species/strain: White Leghorn chicken eggs, freshly fertilized

34 Group size: 3 eggs per group (control group: 2 eggs)

35 Test substance: micronized Eusolex TC (TC);

36 Batch: TO 118279

37 Purity: not reported

38 Particle size: not reported

39 GLP:

40 Reference: Reference 26 submission I

## 41 Method

42 The testing material was prepared on the day of exposure. The concentration was 5 g/100  
43 ml carrier. The carrier used was water for injection to which 0.01 % of the cationic tenside  
44 UCARE 10 had been added. To enable the test material to be applied to the egg membrane,  
45 the eggshell was opened with the aid of a dentist's drill and the material was introduced  
46 with the aid of a needle. The volume introduced was 0.06 ml per egg. To enable the  
47 material to be applied to the CAM (chorio-allantoic membrane), the eggshell was taken off,  
48 the egg membrane removed and the material introduced onto the exposed CAM. The  
49 volume introduced was 0.3 ml. After the prescribed period of exposure, the treated surface  
50 was fixed for 24h with approximately 10% formaldehyde solution. The fixed CAM or egg  
51 membrane with CAM was removed, embedded in paraffin, sliced, and then stained with  
52 nuclear fast red and H. E. The sections were evaluated under an optical microscope.  
53  
54

## 55 Results

1 No signs of penetration by TiO<sub>2</sub> through the egg membrane or the chorio-allantoic  
2 membrane were seen under an optical microscope. The introduction of TiO<sub>2</sub> was fully  
3 tolerated in this sensitive model.

#### 4 5 **SCCS Comments**

6 The test report is very concise. No positive control was used in this test. This test is  
7 therefore of very limited use for this assessment.

#### 8 9 10 **Study Design:**

11 **Guideline/method:**

12 **Species:** human abdominal skin

13 **Test substance:** J&J Baby Sunblock SPF 30 (2723L) containing microfine titanium  
14 oxide (Hombifine 535) (conc unknown)

15 **Particle size:** not reported.

16 **Group sizes:** not reported (1 donor?)

17 **Dose applied:** 400 um formulation

18 **Skin area:** not reported

19 **Skin temperature:** not reported

20 **Test chamber:** flow through diffusion cells

21 **Receptor fluid:** 0.9% saline

22 **Exposure period:** 24h hours

23 **GLP:** no

24 **Published:** no

25 **Study period:** 1990

26 **Reference:** Reference 28 submission 1

#### 27 28 **Method**

29 A layer of about 400 um of formulation was applied on each human cadaver skin sample  
30 and left to dry for 15 minutes. The treated skin samples with the epidermis side facing up  
31 were then mounted on each of the modified diffusion cells. The receptor compartment was  
32 filled with 0.9% NaCl adjusted to pH 7.4 and 5 respectively. The permeation was conducted  
33 for 24 hrs and the receptor solutions were collected at the end of the experiment. The  
34 amount of cream left on the skin surface was then recovered using wipes and rinsed with  
35 methanol (methanol washings).

#### 36 37 **Results**

38 In these diffusion cell based tests, samples of stripped human cadaver skin and mouse skin  
39 were used. The stripped skin does not have a stratum corneum and can thus be regarded to  
40 simulate injured skin. The study showed that only a negligible amount of titanium  
41 permeated through either whole skin or the simulated "damaged skin". About 15% of  
42 titanium oxide was found in the skin tissue and most of the titanium (ca. 85%) was  
43 recovered from the skin surface for both whole skin and stripped skin when the receptor pH  
44 was adjusted at pH 7. 4. It appears that titanium has little tendency to permeate through  
45 the skin. The amount of titanium oxide recovered in the skin tissue may include the physical  
46 adsorption of titanium oxide to the skin surface, which was difficult to be rinsed off by  
47 methanol.

48 The effect of pH in the receptor fluid may play an important role towards the penetration of  
49 titanium oxide. The point of zero charge (pzc) of microfine titanium oxide (Hombifine S35)  
50 is 5.6. Therefore, the receptor fluid will provide better "sink" conditions if its pH is adjusted  
51 further away from 5. 6. Less titanium was found in the skin when the receptor pH was  
52 controlled at pH 5. This can be explained by the fact that pH 5 (0.6 pH unit away from  
53 pzc) is providing less "sink condition" compared to pH 7.4 (1.8 pH unit from zpc). It was  
54 concluded that the chance for titanium oxide to penetrate across human cadaver skin is  
55 slim.

#### 56 57 **SCCS Comments**

1 This is a special and limited study to investigate the influence of different pH conditions.  
 2 Reporting is very concise. Therefore this study provides some additional but limited  
 3 information for the risk assessment.

4  
 5  
 6 Guideline/method:  
 7 Species: human abdominal skin  
 8 Test substances: Sunscreen cream with 5% UV-Titan M160 formulation containing 5%  
 9 titanium dioxide Sunscreen cream without UV-Titan (ca 50 ml).  
 10 Particle size: not given  
 11 Group sizes: 3 donors, 1 male and 2 females; 17 samples from 3 donors were  
 12 treated with sunscreen cream with UV -titan M 160 formulation. A  
 13 total of 4 samples of epidermis taken from the 3 different donors  
 14 were treated with the control formulation  
 15 Dose applied: 2.06 mg/cm<sup>2</sup> of cream with a content of 5% micronized titanium  
 16 dioxide  
 17 Skin area: 0.32 cm<sup>2</sup>  
 18 Skin temperature: 30-32°C  
 19 Test chamber: flow through diffusion cells  
 20 Receptor fluid: 0.9% saline  
 21 Exposure period: 8 hours  
 22 GLP: yes  
 23 Published: no  
 24 Study period: 1996  
 25 Reference: Reference 30 submission 1

#### 26 Method

27 The amount applied to each cell was 2.06 mg/cm<sup>2</sup>. Skin integrity was checked. The  
 28 penetration through the skin membranes was determined over a period of 6 hours under  
 29 non-occluded conditions. The receptor fluid was delivered at a flow rate of about 1.5 mL/h  
 30 during the testing period. The perfusate from each cell was collected separately at an  
 31 ambient temperature for 0-8h post application. Eight hours post application the perfusate  
 32 sampling was terminated.

33  
 34  
 35 Results  
 36 The absorbed amount of Titanium Dioxide was below the detection limit of 5 ng (1ug/l in  
 37 ICP-MS) in all samples. The analyses of the samples did not indicate significant penetration  
 38 of Titanium Dioxide UV-TITAN within the detection limit of the method.

#### 39 SCCS Comments

40 The study shows lack of detectable dermal penetration of TiO<sub>2</sub> nanoparticles. The tested  
 41 material is S75-I (>99.5% Rutile, coated with 7% alumina 10% stearic acid).

42  
 43  
 44  
 45 Guideline/method:  
 46 Species: Human (4 females, mean age 26), upper arm  
 47 Test substances: A- Oil/Water lotion: 5% w/w TiO<sub>2</sub> from 12.5% Tioveil AQG  
 48 B- Water/Oil cream: 7.5% w/w TiO<sub>2</sub> from 18.75% Tioveil TG  
 49 C- Oil/Water lotion: 7.5% w/w TiO<sub>2</sub> from 18.75% Tioveil OP  
 50 Particle size: not reported  
 51 Group sizes: 4 volunteers, 3 different locations of the upper arm (for application A,  
 52 B and C)  
 53 Dose applied: 2.0 ul/cm<sup>2</sup> : 8 ul spread over 4 cm<sup>2</sup> area of skin.  
 54 Skin: Intact human skin  
 55 Skin temperature: 37 °C  
 56 Exposure period: 8h under occlusion  
 57 GLP: No

1 Study period: 1993  
 2 Reference: Reference 29 submission 1

3

## 4 Method

5 The three test products were randomly allocated to three of the four test sites on the  
 6 forearm. After the 8 hour occlusion, the dressings were removed. The sites were not wiped  
 7 prior to removal of stratum corneum by the skin surface biopsy (SSB) procedure.  
 8 Successive SSBs were taken from the same site such that a profile across the stratum  
 9 corneum was obtained. Four SSBs were taken from each of the treated sites.  
 10 The migration of titanium dioxide from sunscreen formulas into the skin was investigated  
 11 using a range of sunscreen formulas (A-C) and four subjects. Consecutive 4cm<sup>2</sup> skin  
 12 samples (biopsies) were taken from test areas. A maximum of 4 biopsies were taken from  
 13 any one skin area, providing 16-20 skin layers in total. Selected skin biopsies were then  
 14 analysed using X-ray microanalysis to determine the concentration of titanium dioxide in the  
 15 biopsy and to show the migration of the titanium dioxide through the skin.

16

## 17 Results

18 Emulsion A did not appear to have migrated past the first biopsies from subjects 1, 2 and 4  
 19 but had migrated to the second biopsy from subject 3.

20 Comparing the emulsions tested on subject 1, emulsions A and B showed little difference  
 21 with titanium only present in the first biopsy, but the titanium from emulsion C had  
 22 migrated to the second biopsy. These results have been confirmed by transmission electron  
 23 microscopy examination of these samples where titanium dioxide crystals were shown to be  
 24 present through the first biopsy and in the second skin biopsy of the area treated with  
 25 emulsion C but not in the second biopsies of the areas treated with any of the other  
 26 emulsions. Repeat analyses on selected samples showed that there was an error of  $\pm 0.2\%$   
 27 in the measurement of titanium in these samples. This indicates that there is some variation  
 28 across the samples possibly due to uneven migration of the sunscreen or uneven thickness  
 29 of the biopsies. The detection limit of the analyser was  $-0.1\%$  and though comparative  
 30 results were obtained by this method it is not as accurate as observations made by  
 31 transmission electron microscopy. Any measurements less than  $0.3\%$  were confirmed by  
 32 repeat analyses. It was concluded that in all cases no TiO<sub>2</sub> was detected beyond the top two  
 33 (out of four) skin surface biopsies. No evidence of penetration to the viable epidermis was  
 34 found.

35

36 **SCCS Comments**

37 The study shows some penetration of TiO<sub>2</sub> nanoparticles to the outer layers of skin, but not  
 38 to the viable epidermis. The tested material relates to S75-B (>99.5% Rutile, coated with  
 39 6% silica, 16% alumina).

40

41

## 42 Study Design:

43 Guideline/method: Comparative study according to an internal laboratory methodology  
 44 considering real use conditions and recommendation of US FDA and  
 45 COLIPA SPF requirements

46 Species: Human (25- to 65-year-old adults)

47 Test substances: Commercial products containing coated (Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub>) nano-sized  
 48 titanium dioxide. No information on size except for Eusolex T-2000

49 TiA: contained only TiO<sub>2</sub>

50 TiB: contained TiO<sub>2</sub> plus ZnO

51 TiHB: (Eusolex T-2000) contained coated rutile TiO<sub>2</sub> (average size of  
 52 20 nm)

53 Particle size: Nanoparticles of TiO<sub>2</sub> needle-shaped; dimension not given

54 Group sizes: TiA, TiHB: 8 volunteers (intact skin)

55 TiB: 9 volunteers (intact skin)

56 TiA, TiB, TiHB: 10 volunteers (stripped skin)

57 TiA: 4 psoriatic patients



1	Controls:	6 volunteers for basal elemental concentration in the skin
2	Dose applied:	0.5 – 1.0 mg/cm <sup>2</sup> on an area of 25 cm <sup>2</sup>
3	Skin:	Intact and tape stripped human skin
4	Skin temperature:	37 °C
5	Exposure period:	2 h (intact) or 48 h (stripped skin and psoriatic patients)
6	GLP:	No
7	Published:	Yes
8	Study period:	Before 2009
9	Reference:	Filipe et al., 2009 (54, 155)

10  
11 Method  
12 The localization and possible skin penetration of TiO<sub>2</sub> nanoparticles dispersed in three  
13 sunscreen formulations, in use under certain conditions were investigated in normal and  
14 altered skin. Commercial products containing nano-sized particles of coated TiO<sub>2</sub> and ZnO  
15 dispersed in hydrophobic emulsions were used. One product contained only TiO<sub>2</sub> (TiA),  
16 another TiO<sub>2</sub> plus ZnO (TiB) and a third material (TiHB) contained nanoparticles of coated  
17 rutile form TiO<sub>2</sub>.

18 The nanoparticles were dispersed in hydrophobic basis gel composed by high pressure  
19 polyethylene and viscous paraffin with Al<sub>2</sub>O<sub>3</sub> (8-11%) and SiO<sub>2</sub> (1-3%). The coated  
20 preparations contained 76 – 82% TiO<sub>2</sub>. The size and shape of nanoparticles in the three  
21 formulations were inspected with transmission electron microscopy and X-ray microanalysis.  
22 Nanoparticles were needle-shaped and similar in both commercial and test formulation. The  
23 application protocol consisted of an open test. The formulation was applied on the sacral  
24 region and buttocks for 2 h, using a sunscreen application of approximately 0.5-1.0 mg/cm<sup>2</sup>  
25 within an area of 25 cm<sup>2</sup>.

26 The 3 formulations used in the study were tested in normal skin: TiB was applied to 9  
27 individuals and both TiA and TiHB to 8 individuals. Nanoparticle penetration (TiA, TiB, TiHB)  
28 was also evaluated in normal skin in an independent group of 10 individuals under non-  
29 physiological conditions induced by tape stripping and occlusive patches (48-hour  
30 application). Tape stripping consisted of series of strips until the tapes were free of  
31 corneocytes. A TiA-containing commercial sunscreen was further tested in involved skin  
32 areas of 4 psoriatic patients. A matched control group constituted by 6 individuals was used  
33 for the determination of basal elemental concentrations in skin including Ti.

34 Skin punch biopsies of 3 mm diameter were taken after application, quench-frozen and kept  
35 in containers until processing. One biopsy was taken from each volunteer. Sections of 14  
36 µm thickness were cut from the frozen biopsy in a cryostat at -25 °C. Biopsies were  
37 mounted in mounting medium for microscopy. Sections were obtained from the non-  
38 immersed portion of the tissue, and sectioning performed from inside to outside to avoid  
39 tissue contamination. Tissue integrity and the efficacy of corneocyte removal after tape  
40 stripping were checked by preparing intercalary stained sections for optical microscopy  
41 purposes. Scanning Transmission Ion Microscopy technique and Particle Induced X-ray  
42 Emission technique were used for detection. The minimum detectable concentration of TiO<sub>2</sub>  
43 in the skin was 0.31 µmol/g (24.8 µg/g tissue or 25 ppm).

#### 44 Results

45  
46 For imaging and localizing TiO<sub>2</sub> and ZnO nanoparticles in intact skin, the coverage of the  
47 outer skin layer with the TiA and TiB sunscreen formulations was homogeneously  
48 distributed. The TiHB formulation showed a patchy distribution. Sunscreen formulations  
49 accumulated in skin wrinkles and depressions as well as infundibulum cavities. Exogenous Ti  
50 and Zn remained at the outer layers of the keratinized tissue that enfold the follicle i.e.  
51 outside the living skin.

52  
53 The nanoparticles penetration profiles obtained with the treated skin groups (TiA, TiB and  
54 TiHB) were all similar. The high levels of TiO<sub>2</sub> observed at the outer layers of stratum  
55 corneum sharply decreased within deeper layers to become undetectable (as Ti by x-ray  
56 emission technique). High Ti concentrations levels were only determined in the stratum  
57 corneum of skin treated with the three formulations. In the subcorneal regions Ti

1 concentration was below the minimum detectable concentration estimated for the analytical  
 2 technique. In non-treated skin Ti was below the minimum detection limit in all strata  
 3 inspected. For the depth positions, where TiO<sub>2</sub> nanoparticle penetration ended an estimated  
 4 error of 10% was obtained, which approximately corresponds to 0.5 µm. In occluded skin,  
 5 there was no significant difference in TiO<sub>2</sub> nanoparticles distribution and penetration depth  
 6 profiling.

#### 7 Nanoparticle localization in damaged skin

8 Parts of the outer layers of the stratum corneum were removed by tape stripping (at least  
 9 15 strips) before sunscreen application. Removal of the stratum corneum was confirmed by  
 10 histological examination and ultimately by nuclear microprobe examination. Under this  
 11 condition there was negligible adhesion of the formulation tested (TiA). The TiO<sub>2</sub> contents  
 12 determined on the skin outer layers were unimportant suggesting that, in normal skin, the  
 13 outer layers of stratum corneum trapped nanoparticles inside the desquamating corneocytes  
 14 network.

#### 16 Results

17 In psoriatic skin, where the horny layer is thicker and less compacted than in normal skin  
 18 showed that the sunscreen formulation remained only, in the first layers of the stratum  
 19 corneum. The Ti distribution was often non-uniform and in some "hot-spots" sunscreen was  
 20 deposited at the outer layers of stratum corneum partly even in the hair follicle  
 21 infundibulum region.

#### 23 Conclusion

24 The authors concluded that following 2 h exposure period of normal human skin to nano-  
 25 sized TiO<sub>2</sub>-containing sunscreens, detectable amounts of these physical UV filters were only  
 26 present at the skin surface and in the upper most stratum corneum regions. Layers deeper  
 27 than the stratum corneum were devoid of TiO<sub>2</sub>, even after 48 h exposure to the sunscreen  
 28 under occlusion. Deposition of TiO<sub>2</sub> and ZnO nanoparticles in the openings of the  
 29 pilosebaceous follicles was also observed. Penetration of nanoparticles into viable skin tissue  
 30 could not be detected.

#### 32 SCCS Comments

33 The study is of good quality. Although for the TiO<sub>2</sub> nanomaterial used in this study  
 34 information on surface area, number of particles per mass was not provided, the results  
 35 showed penetration of the nanoparticles only to the outer layers of Stratum corneum, but  
 36 not to the viable epidermis. The tested material relates to S75-N (>95% Rutile, <5%  
 37 anatase, coated with alumina 10% simethicone 2%, doped with 1000 ppm Fe).

#### 39 Study Design:

##### 40 Guideline/method:

41 Species: Porcine and human skin

42 Test substances: TiO<sub>2</sub> uncoated nanoparticles, mixture of rutile and anatase,  
 43 average primary particle size 21 nm, uncoated, approximately  
 44 spherical platelets (Degussa-P25)

45 TiO<sub>2</sub> coated nanoparticles, rutile, composition 76-82% TiO<sub>2</sub>, 8-  
 46 11% Al<sub>2</sub>O<sub>3</sub> and 1-3% SiO<sub>2</sub>, primary particle size about 20-100  
 47 nm, needle shaped (Eusolex T-2000, Merck KGaA)

48 Formulations: All formulations contained 5% TiO<sub>2</sub> nanoparticles.

49 1. TiO<sub>2</sub> uncoated: carbomergel, 20% propylenglycol, 0.5%  
 50 carbomer 500,000, 0.3% trometamol, and 79.2% purified  
 51 water.

52 2. TiO<sub>2</sub> coated: hydrophobic basisgel, 5% high pressure  
 53 polyethylene and 95% viscous paraffin

54 3. TiO<sub>2</sub> coated: polyacrylategel, 20% propylenglycol, 0.5%  
 55 carbopol 980, 0.3% trometamol, and 79.2% purified water.

56 Dose applied: 2 mg/cm<sup>2</sup>



1	Skin:	Porcine skin. The porcine skin specimens (n=12) were obtained
2		from domestic pigs. Specimens were sampled from the inner
3		parts of thighs in the form of punch biopsies.
4		Human skin. The human skin was obtained from the dorsal
5		region and buttocks of healthy adult volunteers (n=8).
6		Human grafted skin samples were produced from normal human
7		foreskins obtained from circumcision and grafted on a severe
8		combined immunodeficient (SCID) mouse model (n=4).
9	Skin temperature:	Not stated
10	Exposure period:	Porcine and human skin 2 h under semi-occlusive conditions,
11		human grafted skin 1 h, 24 h, and 48 h under occlusive
12		conditions.
13	GLP:	No
14	Published:	Yes
15	Study period:	Before 2008
16	Reference	Gontier et al., 2008 (158)

## 19 Method

20 All three formulations were topically applied at 2 mg/cm<sup>2</sup> and for 2 h to porcine and human  
 21 skin under semi-occlusive conditions, i.e., a breathable plaster protected the area. In a  
 22 previous pilot study with exposure times between 8 and 48 h no significant differences were  
 23 found for different exposure times. The sunscreen was applied to human skin grafted on  
 24 SCID mice for 1 h, 24 h, and 48 h under occlusive conditions. Untreated control samples  
 25 were also prepared for each analysis.

26 The skin biopsies (3 mm in diameter) were studied by Transmission Electron Microscopy  
 27 (HRTEM) and Scanning Transmission Ion Microscopy (STIM) combined with Rutherford  
 28 Backscattering Spectrometry (RBS) and Particle Induced X-Ray Emission (PIXE) on ultra-  
 29 thin and thin cross-sections, respectively.

## 31 Results

### 32 Porcine skin

33 TiO<sub>2</sub> uncoated. By superimposing the titanium distribution obtained by the PIXE map  
 34 on to the STIM map, it was possible to unambiguously determine the distribution of TiO<sub>2</sub>  
 35 particles via their chemical fingerprint with a close correlation to the epidermal layers. TiO<sub>2</sub>  
 36 particles were exclusively localized on the surface of the outermost SC layer. No titanium  
 37 could be found in the layers containing vital cells. The porcine skin after application of  
 38 hydrophobic basisgel exhibited a similar titanium distribution. To quantify the penetration  
 39 depth of TiO<sub>2</sub> particles, a region of interest was chosen to extract the titanium depth profile  
 40 displayed. The extent of the profile was about 30 µm. A clear titanium peak is visible at the  
 41 skin surface, the titanium being strictly limited to the SC. The nuclear microprobe  
 42 observations were cross-checked by the results obtained on the same type of samples  
 43 studied by HRTEM. Apart from corneocyte layers, nanoparticles and agglomerates on and in  
 44 between the corneocytes are clearly visible. Electron X-ray microanalysis on individual  
 45 nanoparticles proved that they contain Ti. In addition, morphological features of the TiO<sub>2</sub>  
 46 particles were examined. The TiO<sub>2</sub> particles sometimes appear as individual particles, but  
 47 more frequently agglomerated to clusters of different sizes.

49 TiO<sub>2</sub> coated. An average size of 12 nm in width and of 60 nm for the length was estimated  
 50 for the primary needle-shaped particles. The large amount of the titanium particles for  
 51 both test emulsions, carbomergel and hydrophobic basisgel, was strictly located at the  
 52 surface of the last corneocyte layer with the possible exception of agglomerates below the  
 53 first and third corneocyte layer.

### 55 Human skin

56 The STIM map exhibits a thick SC and a well delineated SS containing keratinocyte cell  
 57 bodies. The Ti PIXE-maps are superimposed onto the STIM image, demonstrating that the

1 particles were exclusively located on the outermost layers of the SC. This observation is  
 2 corroborated by the superimposition of the same titanium PIXE-map onto the RBS carbon  
 3 map. The depth profile of titanium extracted from the region of interest demonstrates that  
 4 the presence of this element is limited to a layer with a thickness of about 20 µm.

5  
 6 On the STIM map obtained for the commercial formulation, the SC is easily observable due  
 7 to its high density despite its unusually low thickness. In the titanium PIXE-map is  
 8 superimposed onto the STIM image. Ti is exclusively localized on the surface of the horny  
 9 layer. From the titanium depth profile, extracted from the region of interest, titanium was  
 10 found to penetrate into a 10 µm thickness layer of the SC only, but no titanium was  
 11 detected in the SS.

12  
 13 In the HRTEM micrograph, TiO<sub>2</sub> particles were identified by the presence of large  
 14 homogeneous electron dense objects on the surface of the horny layer. At low magnification  
 15 the particles appear to be spread in a very homogeneous thin layer. With a high  
 16 magnification, the particles occasionally appeared as needle-shaped individual particles, but  
 17 most frequently aggregated in clusters of different sizes. The primary particles have a width  
 18 of 12 nm and an average length of 60 nm. Some particles were seen four to five layers  
 19 deeper, apparently only when a passage exists due to the looseness of corneocytes.

#### 20 21 Human skin grafted to SCID-mice

22 The murine SCID model allows human skin to be grafted without any rejection. The  
 23 commercial product was applied for 2 h under occlusive conditions. Here, the STIM image  
 24 enables to delineate the SC from the large SS by its high density. In addition it shows the  
 25 papillary dermal-epidermal junction and the dermis. When the PIXE-titanium maps were  
 26 superimposed onto the STIM images obtained from the two different areas of interest a  
 27 microlesion, i.e., a partly detached horny layer, with Ti in the cleft was seen. The result  
 28 seemed to indicate that in some areas of the SC titanium penetrated more deeply compared  
 29 to other skin samples. The HRTEM micrographs revealed a thinner SC constituted by two or  
 30 three layers of corneocytes only. In fact, this sample was taken from the border between  
 31 mouse and human skin. The corneocytes are separated by larger spaces which have allowed  
 32 the product to penetrate down to the innermost corneocyte layer. The TiO<sub>2</sub> particles seem  
 33 to be attached to the corneocyte layers. Nevertheless no TiO<sub>2</sub> particles were observed in  
 34 the very close SG.

#### 35 36 Conclusion

37 The authors concluded that whereas the HRTEM and STIM/PIXE images reveal clear  
 38 differences – mainly related to the different thickness of the cross-sections – they  
 39 unambiguously show that penetration of TiO<sub>2</sub> nanoparticles is restricted to the topmost 3–5  
 40 corneocyte layers of the stratum corneum.

#### 41 42 **SCCS Comments**

43 The study is of good quality. Although for the TiO<sub>2</sub> nanomaterial used in this study  
 44 information on surface area, number of particles per mass was not provided, the results  
 45 showed penetration of the nanoparticles only to the outer layers of Stratum corneum, but  
 46 not to the viable epidermis. The tested material relates to S75-G (uncoated, anatase 85%,  
 47 rutile 15%), and S75-N (>95% Rutile, <5% anatase, coated with alumina 10% simethicone  
 48 2%, doped with 1000 ppm Fe).

#### 49 50 51 **Compromised skin**

##### 52 53 Study Design:

54 Guideline/method: Exploratory comparative percutaneous skin penetration study *in vitro*  
 55 after UVB radiation *in vivo* (sunburn simulation)

56 Test system: Skin of weanling Yorkshire pigs (approximately 20–30 kg)

57 Test substances: O/W and W/O sunscreen formulations

## Revision of the opinion on Titanium Dioxide, nano form

1	A: T-Lite SF (coated, 10% O/W formulation, CM 630)
2	B: T-Lite SF (coated, 10% W/O formulation, CM 634)
3	CM 630 and CM 634 consist of TiO <sub>2</sub> (rutile, crystallite of 14–16 nm)
4	coated with hydrated silica, dimethicone/methicone copolymer, and
5	aluminium hydroxide for a primary particle size of 10 x 50 nm and
6	specific surface area of 100 m <sup>2</sup> /g. The mean size of the agglomerates
7	was 200 nm with a range of ca. 90–460 nm
8	Batch: Not stated (source: BASF SE, Germany)
9	UVB exposure: A Fiber optic UVB lamp (Lightning cure 200 UV-Spot light) was used.
10	Reference: Monteiro-Riviere et al., (2011) (181, 182).
11	
12	Method
13	On day 1 a pig was sedated and the hair clipped. The minimal erythemic dose (MED) was
14	determined by sequential exposure to UVB light (30 – 110 mJ/cm <sup>2</sup> , - 22 sec.). On day 2 the
15	exposed sites were analyzed to determine the UVB dose required to produce 1 MED. The pig
16	was subsequently sedated and multiple sites (52 sites) on the back were exposed to the
17	UVB dose that caused a consistent +2 erythema, a pale red in a defined area of the skin.
18	Twenty-four hours after UVB exposure (Day 3), the pig was sedated, sites visually analyzed
19	for consistency, and the pig euthanatized. The UVB-exposed sites were dermatomed to a
20	thickness of approximately 400-500 µm and placed dermis side down on paper towels
21	saturated with physiological saline.
22	The skin prepared for the <i>in vitro</i> or <i>in vivo</i> studies.
23	UVB dose: 100, 110 and 120 mJ/cm <sup>2</sup> (pig 1, 2, 3 for MED of about 2.5)
24	
25	<i>In vitro</i> part:
26	Dose level: 50 µl of each formulation on 0.64 cm <sup>2</sup> dermatomed pig skin
27	Skin preparation: Exposed and unexposed skin sites were dermatomed to 400 µm.
28	Dermatomed skin, placed dermis side down on towels saturated with
29	physiological saline, was cut into with a 19 mm circular punch.
30	Cells: Formulation A and B: 4 with UVB exposed skin, 2 with unexposed
31	skin
32	Control: 2 with UVB exposed skin, 2 with unexposed skin
33	Skin temperature: 37 °C
34	Test chamber: Flow-through diffusion cells
35	Route: Topical application
36	Exposure time: 24 hours
37	Sampling time
38	points: Every 2 h for the first 12 h, every 4 h thereafter up to 24 h
39	Examinations: Light microscopy (LM). Transmission electron microscopy (TEM) plus
40	X-ray microanalysis (EDS) Scanning electron microscopy (SEM).
41	Time-of-flight secondary ion mass spectrometry (TOF-SIMS)
42	
43	<i>In vivo</i> part:
44	Dose level: 250 µl of each formulation on exposed sites (n = 3 per formulation)
45	on 2 pigs on 1.0 cm <sup>2</sup> pad Hill Top chamber
46	Controls: Normal pig skin (no UVB, no sunscreen, no Hill Top chamber (n = 2
47	per pig)
48	UV-B exposed: No sunscreen, dry chamber (n = 2 per pig)
49	Sunscreen in a Hill
50	Top chamber: No UVB (n = 2 per formulation)
51	Route: Topical application
52	Exposure time: 2x 24 h and termination after 48 h
53	Sampling: Skin was removed by 8-mm biopsy punch
54	Examinations: As <i>in vitro</i> part
55	GLP: No
56	Published: Yes
57	

## 1 Method

2 The purpose of the study was to determine whether skin damaged by UVB radiation  
3 inducing moderate sunburn with a +2 erythema reaction, enhanced the penetration of TiO<sub>2</sub>  
4 or ZnO nanoparticles (see Opinion on ZnO (nanoform)) present in sunscreen formulations.

5 Weanling Yorkshire pigs (approximately 20–30 kg) were sedated and multiple sites (about  
6 52) on the back were exposed to the UVB dose that caused a consistent +2 erythema (a  
7 pale red in a defined area of the skin).

8 Twenty-four hours after UVB exposure, the pig was sedated, sites visually analyzed for  
9 consistency, and the skin prepared for *in vivo* or *in vitro* studies.

10 For the *in vitro* studies, the UVB exposed and non exposed sites were dermatomed to a  
11 thickness of approximately 400–500 µm. The dermatomed skin was mounted in the flow-  
12 through diffusion cells with a dosing area of 0.64 cm<sup>2</sup> and maintained at 37°C. The skin was  
13 equilibrated in perfusate and a flow rate of 2 ml/h for 30 min prior to dosing. The skin was  
14 subsequently dosed with 50 µL of each formulation (CM 630: (n=4 UVB exposed skin, n=2  
15 unexposed skin; CM 634: n=4 UVB exposed skin, n=2 unexposed skin; and control: n=2  
16 UVB exposed skin, n=2 unexposed skin). After completion of dosing, the perfusion was  
17 resumed and the perfusate collected every 2 hours for the first 12 hours and every 4 hours  
18 thereafter up to 24 hours. After 24 hours, the perfusion was terminated and the skin was  
19 removed from the diffusion cells.

20  
21 The dose site was removed with an 8 mm biopsy punch and cut into thirds. One third was  
22 placed in Trump's fixative and stored at 4°C for later processing by light microscopy (LM;  
23 flow-through 1 and 2 only) and transmission electron microscopy (TEM). The remaining  
24 third of the skin was cut in half and immediately frozen and stored at -20°C for later  
25 elemental analysis. The vials containing perfusate from each timed collection were capped  
26 and the samples immediately stored at 4°C.

27  
28 For *in vivo* treatment exposed sites (n = 3 per formulation) on two pigs were treated with  
29 250 µl of each formulation; 200 µl was loaded onto the pad of the Hill Top chamber (1.0  
30 cm<sup>2</sup> area) and 50 µl was placed directly on the skin within a template. Controls included  
31 normal pig skin (no UVB, no sunscreen, no Hill Top chamber; n = 2 per pig), UVB-exposed  
32 (no sunscreen, dry chamber; n = 2 per pig), and sunscreen in a Hill Top chamber (no UVB;  
33 n = 2 per pig per formulation). Sites were redosed with new Hill Top chambers after 24 h,  
34 and the treatment was terminated after 48 h. Erythema was scored for each site, and the  
35 pigs were euthanatized as above. Skin from all of these sites was removed with an 8-mm  
36 biopsy punch for microscopy studies as stated above.

## 37 Results

38  
39 For the *in vitro* studies, light microscopy showed that UVB exposed skin showed focal  
40 intracellular epidermal oedema, sunburn cells, dermal inflammation and focal microblister  
41 and residual sunscreen containing TiO<sub>2</sub> limited to the stratum corneum. The morphology of  
42 the normal and the UVB-exposed skin was not affected by topical treatment with the  
43 sunscreen formulations. The TiO<sub>2</sub> in each formulation was confirmed by TEM and elemental  
44 analysis. EDS found the presence of Ti and Cu (copper grid) in CM 630 and CM 634. Si for  
45 the coating, Pb for lead citrate and U for uranyl acetate staining.

46 In the *in vitro* flow-through studies, TEM/EDS found penetration of Ti to a depth of 9 layers  
47 in the stratum corneum of normal skin and 17 layers in the stratum corneum of UVB-  
48 exposed skin. TEM/energy dispersive x-ray spectroscopy or inductively coupled plasma  
49 mass spectrometry detected no Ti or Zn, indicating minimal transdermal absorption.

50 For *in vivo* tests, skin was dosed at 24 h occluded with formulations and at 48 h. TiO<sub>2</sub> NP in  
51 o/w formulation penetrated 13 layers into UVB-damaged SC, whereas only 7 layers in  
52 normal skin; TiO<sub>2</sub> in w/o penetrated deeper in UVB-damaged SC. Coated and uncoated ZnO  
53 NP in o/w were localized to the upper one to two SC layers in all skin. TOF-SIMS showed Ti  
54 within epidermis and superficial dermis, whereas Zn was limited to SC and upper epidermis  
55 in both treatments. In summary, UVB-damaged skin slightly enhanced TiO<sub>2</sub> NP or ZnO NP  
56 penetration in sunscreen formulations but no transdermal absorption was detected.

57

## 1 Conclusion

2 In summary, UVB-sunburned skin slightly enhanced the *in vitro* or *in vivo* penetration of the  
3 TiO<sub>2</sub> or ZnO NPs present in the sunscreen formulations into the stratum corneum (SC).  
4 Although penetration of the two NPs into the SC was shown by TEM, and into the epidermis  
5 and dermis by TOF-SIMS, there was no definitive evidence that they penetrated the skin *in*  
6 *vitro* into the perfusate. In most cases, TiO<sub>2</sub> penetration into the SC was greater than ZnO.  
7 These results viewed together suggest minimal penetration of TiO<sub>2</sub> and ZnO NPs into the  
8 upper epidermal layers when applied topically in sunscreen formulations to normal and  
9 UVB-sunburned skin, with no evidence of systemic absorption.

## 10 11 12 **SCCS Comments**

13 The study is of a good quality. The test material relates to S75-K (>94% rutile, coated with  
14 6-8% aluminium hydroxide, 3.5-4.5% dimethicone/ methicone copolymer). The results of  
15 transmission electron microscopy indicated penetration of TiO<sub>2</sub> nanoparticles into stratum  
16 corneum, whereas TOF-SIMS analysis indicated penetration into the epidermis and dermis.  
17 However, analysis of perfusate by TEM/Energy Dispersive Analysis or ICP-MS did not detect  
18 Ti or Zn indicating nanoparticles did not penetrate the skin *in vitro*.

## 19 20 **In Vitro study (Senzui et al., 2010 - Ref 204)**

21 Study Design:

22 Guideline/method:

23 Species: Yucatan micropig skin

24 Test substances: All TiO<sub>2</sub> are rutile-type

25 T-35. size 35 nm, uncoated

26 TC-35, size 35 nm, coated alumina + silica + silicone

27 T-disp, size 10 x 100 nm, mixture of alumina coated and silicone  
28 coated

29 T-250, size 250 nm, uncoated

30 Formulations: All formulations contained 10% TiO<sub>2</sub> nanoparticles.  
31 Cyclopentasiloxane (silicone, KF-995) used as dispersing medium

32 Dose applied: 2 µl/cm<sup>2</sup>

33 Skin: Yucatan micropig skin removed the subdermal tissue and fat was  
34 used as full-thickness skin (intact skin). The SC was removed from  
35 intact skin with adhesive tape (Scotch 313, 3M) (stripped skin). Hair  
36 was removed from intact skin using tweezers (hair removed skin)

37 Skin temperature: Not stated

38 Exposure period: 24 h

39 GLP: No

40 Published: Yes

41 Study period: Before 2010

42 Reference: Senzui et al., 2010 (204)

## 43 44 45 **Method**

46 The TiO<sub>2</sub> was suspended in a volatile silicone fluid used for cosmetics, cyclopentasiloxane,  
47 at a concentration of 10%. The suspension was applied at a dose of 2 mg/cm<sup>2</sup> for 24 h.

48 The skin penetration was investigated *in vitro* with intact skin and with stripped skin (the SC  
49 removed from intact skin with adhesive tape) as a model of injured skin. In addition hair-  
50 removed skin (hair was removed from intact skin using tweezers) was used to represent  
51 skin damaged by hair-removal treatment.

52 Two µl of suspension were applied to an area of skin of approximately 1 cm<sup>2</sup>. Then the skin  
53 was placed on a modified Franz-type diffusion cell. After 24 h, the receptor phase (pH 7.1  
54 isotonic phosphate buffer solution) was collected, the skin was removed from the diffusion  
55 cell and cut off at the rim for mounting the cell. Residues on the skin surface were removed  
56 by two cyanoacrylate stripping and Ti in the skin was determined. For some samples, the  
57 epidermis and dermis were separated by heating after cyanoacrylate stripping.

1 Skin conditions after application of TiO<sub>2</sub> was observed using two methods. After application  
2 and drying, the skin surface was observed by digital fine scope microscopy. The epidermis  
3 of the skin prepared by a heat separation method was mounted on a scanning electron  
4 microscope (SEM) stage with adhesive tape.

5  
6



## 1 Results

2 The particle size distribution of TiO<sub>2</sub> in silicone was determined. The mean particle size of T-35 was 1700 nm, which was larger than that of T-250, 1200 nm. In contrast, suspensions of the coated TC-35 and T-disp contained nanoparticles with mean diameter of 80 and 130 nm, respectively.

6 Ti concentration in the receptor phase was similar in all skin conditions and formulation applied. For intact and stripped skin, no significant difference in Ti concentration was found between the control and suspension applied, which indicates TiO<sub>2</sub> did not penetrate into the skin regardless of particles size and even when the SC was removed. For hair-removed skin, Ti concentration in skin after application of TC-35 suspension was significantly higher than that of the control, and after application of T-disp suspension, tended to be high. The Ti concentration in the dermis was not different from the control.

13 Ti concentration in the epidermis after application of TiO<sub>2</sub> nanoparticles tended to be greater than that of the control, but the difference was not significant. The epidermis consists of SC, viable epidermis and hair follicles. Ti was detected in the hair follicle pockets of hair-removed skin, but not in the surrounding viable skin. The radius of a hair follicle is 0.05 – 0.2 mm which allow solvent to enter the hair shaft and sebum did not fill the follicle space. When fluid enters a small space by capillary action, small particles of Ti in fluid may be able to enter the follicle. Large particles cannot be moved by such small force, but TC-35 well dispersed in solvent might enter a follicle more easily than other types of TiO<sub>2</sub>. For T-disp, the dispersing agent had some effect, resulting in particles left in the skin after drying of the suspension

## 24 Conclusion of the Applicant

25 The authors concluded that TiO<sub>2</sub> does not penetrate into viable skin, even if the particle size is less than 100 nm and the SC is damaged. However, immediately after hair removal the concentration of Ti in skin was higher when TC-35 was applied, which was most probably caused by dispersion. SEM-EDS observation showed that Ti penetrated into vacant hair follicles but in any case did not penetrate into dermis or viable epidermis. It was noted that since this was an *in vitro* study, inflammation could affect the results and further *in vivo* studies on viable skin with hair removal are needed.

## 33 SCCS Comments

34 The quality of the study is difficult to evaluate. Moreover, the study was performed with skin from Yucatan micropigs and experience with this skin type in skin absorbance studies is limited.

## 39 ***In vitro* exploratory study - percutaneous skin penetration - pig skin (Ref 70)**

41 Study design.

42 Guideline/method: exploratory study

43 Species: pigs

44 Test substances: T805 (Degussa), hydrophobically coated with trimethyloctylsilane

45 Particle size: about 20 nm

46 Group sizes: n=2 skin samples

47 Dose applied: 0.8 mg total (20 mg with 4% TiO<sub>2</sub>), 0.16 mg TiO<sub>2</sub> per cm<sup>2</sup>

48 Skin: fresh skin obtained from pigs used within 3 h after collection

49 Skin area: 4.9 cm<sup>2</sup>

50 Skin temperature: 32°C

51 Test chamber: custom-made Franz-type diffusion cells

52 Receptor fluid: 0.9% w/v NaCl, 0.1% w/v gentamycin sulfate, 1% w/v bovine serum albumin in bi-distilled water

54 Exposure period: 24 h

55 GLP: no

56 Published: yes

57 Study period: 1999

1 Reference: Reference 70 submission III+IV Pflücker et al., 1999

2

3 Method

4 Fresh pig skin was obtained from the butcher, and used within 3 hours after collection. Skin  
5 samples were punched (5 cm in diameter). The dermal absorption study was performed  
6 with custom-made Franz-type diffusion cells. The lower cell was placed on a magnetic stirrer  
7 (Variomag, Germany) and connected by tygon tubes to a thermostat (Type CS-C6, Lauda,  
8 Germany) set at a temperature of 32°C (*in vivo* skin temperature). Magnetic stirring bars  
9 were placed in the lower cells, which were filled with the receptor fluid (0.9% w/v NaCl,  
10 0.1% w/v gentamycin sulfate, 1% w/v bovine serum albumin in bidistilled water). 20 mg of  
11 the test emulsion, which contained 4% titanium dioxide, were topically applied with a  
12 gloved finger to two excised pig skin discs (area 4.9 cm<sup>2</sup>, 2.5 cm in diameter, giving a  
13 concentration of 4 mg cm<sup>2</sup>). After 24 h incubation 2 mm punch biopsies were obtained for  
14 histological evaluation (TEM and SEM). SEM micrographs were recorded to evaluate the  
15 morphology of the freeze-dried skin samples and the stripped stratum corneum sheets.  
16 Freeze dried skin samples were investigated before and after 10-fold tape stripping.

17

18 Results

19 TiO<sub>2</sub> was found exclusively on the outermost SC layer. No titanium dioxide could be found in  
20 the living cell layers of the stratum granulosum. The surface deposit, as displayed by TEM,  
21 featured clearly distinguishable agglomerates as well as single particles with a characteristic  
22 cubic shape and a primary particle size of about 20–50 nm. Concurrently, SEM/EDXA  
23 micrographs first showed an even distribution of TiO<sub>2</sub> on the skin surface. After 10-fold  
24 stripping, however, TiO<sub>2</sub> was found to be localized only in the furrows and not on the  
25 partially removed ridges of the skin surface. In the upper part of the hair follicle TiO<sub>2</sub> was  
26 demonstrated.

27

28 **SCCS Comments**

29 The actual TiO<sub>2</sub> dose was 0.16 mg, and not 20 mg as mentioned in the paper. The study  
30 does not show quantitative results but demonstrates by electron microscopy that the TiO<sub>2</sub>  
31 nanoparticles are present on the skin mainly as aggregates. The study is of limited value  
32 with number of samples investigated was only 2, but can be considered as supporting  
33 evidence that TiO<sub>2</sub> nanoparticles do not penetrate to the viable cell layers of the dermis.

34

35 ***In vitro* exploratory study - percutaneous skin penetration and *in vivo* - human**  
36 **skin (Ref 78)**

37

38 Study design.

39 Guideline/method: exploratory study

40 Species: human healthy volunteers (female)

41 Test substances: Mixture of broad spectrum UV water-in -oil emulsions containing  
42 water, glycerin, dimethicone, ethylhexyl methoxycinnamate,  
43 isododecane, cyclomethicone, C12-15 alkyl benzoate, PEG-30  
44 dipolyhydroxystearate, decyl glucoside, dodecyl glycol copolymer,  
45 magnesium aluminium silicate, preservatives, zinc oxide, tocopheryl  
46 acetate, *o*-cymen-5-ol, fragrance, xanthan gum and 3% ultrafine  
47 TiO<sub>2</sub> (**T805**, Degussa, Germany) and 8% methylene bis-  
48 benzotriazolyl tetramethylbutylphenol (MBBT) in a dispersion of decyl  
49 glucoside.

50 TiO<sub>2</sub> was coated with trimethyloctylsilane.

51 Particle size: TiO<sub>2</sub> 20 nm

52 Group sizes: n=3

53 Dose applied: 2 mg/cm<sup>2</sup> of formulation, 60 µg TiO<sub>2</sub> / cm<sup>2</sup>

54 Skin: *in vitro* abdominal and face skin frozen until use,  
55 *in vivo* skin of upper arm

56 Skin area: *in vivo* 10 cm<sup>2</sup> (2x5 cm)

57 Teflon static diffusion cell 10 cm<sup>2</sup> (2x5 cm)

1		Franz diffusion cell 1.13 cm <sup>2</sup>
2	Test chamber:	Teflon® homemade static diffusion cell with a 10 cm <sup>2</sup> (5x2 cm)
3		surface and a receptor volume of 8 ml.
4		Franz diffusion cell with a 1.13-cm <sup>2</sup> surface and 5 ml of receptor fluid.
5	Receptor fluid:	0.9% NaCl water solution with 3% bovine serum albumin
6	Skin temperature:	32°C
7	Exposure period:	5 h
8	GLP:	no
9	Published:	yes
10	Study period:	2007
11	Reference:	Reference 78 Submission VII Mavon et al., 2007.

12  
13  
14 **Method**  
15 Samples of the mixture of broad spectrum UV water-in –oil emulsions were applied on skin  
16 of volunteers (10 cm<sup>2</sup>, 2x5 cm) and on two types of diffusion chambers, one Teflon®  
17 homemade static diffusion cell with a 10 cm<sup>2</sup> surface allowing tape stripping of the test  
18 system, and a Franz diffusion cell with a 1.13-cm<sup>2</sup> surface. The applied dose for the *in vitro*  
19 study was 60.6 ± 3.1 µg/cm<sup>2</sup>, and for the *in vivo* study 58.4 ± 1.9 µg/cm<sup>2</sup>.  
20 The distribution of the sunscreens in the skin was directly assessed by the tape stripping  
21 method, using adhesive tape (Scotch TM No. 6204, 3M Corp.). A total of 15 tape strippings  
22 were applied onto the surface of the skin, and each was pressed on the skin 10 times with a  
23 roller. Each strip was removed with 1 quick movement. No washing procedure was used.  
24 The titanium analysis in the tape strippings and skin samples (epidermis, dermis and  
25 receptor fluid) was based on a microwave assisted treatment, which digested the organic  
26 components in the presence of sulphuric and nitric acid. The samples were then analyzed by  
27 colorimetric assay, using diantipyrylmethane (0.5 g in 20 ml HCl 1 N). One ml of the colored  
28 solution and 1 ml of the solution to be tested were mixed. The absorbance was read at 390  
29 nm with a spectrophotometer (Anthelie Advanced, France) 30 min later. Using this  
30 technique, a LOD of 0.2 µg/ml was obtained.  
31 Transmission electron microscopy and particle-induced X-ray emission (PIXE) techniques  
32 were used to localize the TiO<sub>2</sub> in skin sections. Punch biopsies of 6 mm in diameter were  
33 made on skin samples, consecutively after 1, 8 and 15 tape strippings and were fixed with  
34 2% glutaraldehyde in a Sorensen buffer for TEM analysis.

35  
36 **Results**  
37 For the *in vitro* experiments with n=3 >94.2% of the recovered TiO<sub>2</sub> was found in the 15  
38 tape strippings and in the stratum corneum. In the epidermis 5.6% was found, and <0.1%  
39 was found in the dermal compartment. No TiO<sub>2</sub> was found in the receptor fluid (below LOD).  
40 The amount recovered accounted for 88.8% of the applied dose of TiO<sub>2</sub>. In the *in vivo* study  
41 (n=3) the recovery was 93% of the TiO<sub>2</sub> dose. Most of the recovered dose was in the first  
42 three tape strippings. After 15 tape strippings a few grains could be distinguished in the  
43 TEM samples (amplification x 15,000), attributed to TiO<sub>2</sub> nanoparticles, but they were very  
44 few and isolated in the stratum corneum (SC) layer. Deeper in the SC, no particles could be  
45 observed, which suggested an absence of penetration into the viable skin tissue.  
46 The 2-dimensional mapping of titanium using Micro-PIXE analysis of the skin showed that  
47 most of the Ti applied at the skin surface remained there or penetrated only into the opened  
48 infundibulum. Quantitative analysis revealed a concentration of Ti at the LOD, in the  
49 underlying layer of the epidermis, the dermis, the follicle and the sebaceous glands.  
50 It was concluded by the authors that the study confirms that TiO<sub>2</sub> accumulates in the  
51 uppermost layers of the SC and in the opened infundibulum only. No TiO<sub>2</sub> was detected in  
52 the viable skin layers through either transcorneal or transfollicular pathways. From these  
53 data the authors concluded that the amount of TiO<sub>2</sub> found in the *in vitro* 'epidermal'  
54 compartment is located mainly in the furrows or the opened infundibulum and does not  
55 represent actual transcorneal penetration.

56  
57

**SCCS Comments**

Both TiO<sub>2</sub> and MBBT were present in the broad spectrum UV water-in-oil emulsions. Lack of penetration of TiO<sub>2</sub> was supported by both *in vitro* and *in vivo* studies. Whether the detected particles were attributed to TiO<sub>2</sub> or not has not been identified by the study.

***In vitro* study - Percutaneous skin penetration pig skin (Ref 56)**

Study design.

Guideline/method: yes (OECD 428, SCCNFP/0750/03) skin absorption *in vitro* method

Species: pig

Test substances: T-Lite SF-S coated with silica (2%-5% wt%) and methicone (4.5%-6.5%)

T-Lite SF coated with methicone (3.5%-5.5%)

Particle size: T-Lite SF-S, needle like with a size of 30-60x10 nm

T-Lite SF, needle like with a size of 30-60x10 nm

Both TiO<sub>2</sub> materials were present including aggregates up to 200 nm and higher (1 µm)

Group sizes: skin from 3 pigs, and per sample 3 skin preparations (n=9)

Dose applied: 4mg/cm<sup>2</sup> corresponding to nominal doses of about 400 µg/cm<sup>2</sup> of titanium dioxide or to nominal doses of 240 µg/cm<sup>2</sup> of titanium,

Skin: full thickness skin samples from lateral abdominal region

Skin area about 1 cm<sup>2</sup>

Skin temperature: 32 ± 1°C

Test chamber: modified Franz static dermal penetration cells

Receptor fluid: physiological saline containing 5% bovine serum albumin

Exposure period: 24 h, sampling at various time intervals (3, 6, 12, and 24 h)

GLP: yes

Published: yes

Study period: 2007

Reference: Reference 56 Submission VII Gamer et al., 2007

**Method**

After removal of the receptor fluid the skin was removed from the diffusion cell and put onto parafilm. Titanium was removed from the skin preparations by washings with sponge pieces dipped into soap solution, and subsequent tape stripping was used to remove titanium together with the superficial layers of the stratum corneum. Ti was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) or ICP-mass spectrometry (ICP-MS).

**Results**

For the titanium dioxide formulations T-Lite SF-S and T-lite SF, mean total recoveries of Ti ranged from 98% to 100% and 86% to 93% of the total Ti applied, respectively. Virtually the total amount of applied Ti could be removed from the skin surface by washing. The amounts of titanium found in the tape strips and skin preparations were in the order of the analytical determination limit. No Ti was found in the receptor fluid at any sampling time.

**SCCS Comments**

This is a GLP study with three independent measurements indicating lack of TiO<sub>2</sub> penetration in an *in vitro* assay using pig skin. Although the number of measurements (n=3) per skin is limited, it was repeated in skin samples of three different pigs.

1 ***In vitro* exploratory study percutaneous skin penetration, and *in vivo* study on**  
 2 **human skin (Ref 130)**

3  
 4 Study design.

5 Guideline/method: exploratory study

6 Species: human (male and female)

7 Test substances: commercial microfine TiO<sub>2</sub> dispersion either in octyl palmitate or in  
 8 water (Tioxide Specialities Ltd)

9 Particle size: not reported

10 Group sizes: n=3

11 Dose applied: *in vitro* 150 µl/cm<sup>2</sup> of commercial preparation (5% TiO<sub>2</sub>, 7.5mg/cm<sup>2</sup>)  
 12 in aqueous or oily dispersion

13 *In vivo* 2 µl/cm<sup>2</sup> (5% TiO<sub>2</sub>, 0.1 mg/cm<sup>2</sup>) in aqueous or oily dispersion

14 Skin: *in vitro* human skin from abdominal area (samples stirred at -20°C),  
 15 and skin equivalents with cultivated human keratinocytes and  
 16 fibroblasts

17 *In vivo* ventral side of forearm of male and female volunteers

18 Skin area not reported

19 Skin temperature: 32°C

20 Test chamber: penetration cells identified with figure.

21 Receptor fluid: phosphate buffer pH 7.4

22 Exposure period: 24 h for *in vitro* studies

23 45 minutes for the *in vivo* studies

24 GLP: no

25 Published: yes

26 Study period: 2000

27 Reference: Reference 130 Submission VII Bennat and Müller-Goymann 2000

28  
 29 Method

30 A penetration cell was used for both skin samples and the human skin equivalent studies.  
 31 For *in vitro* test the amount added was 150 µl per skin sample, for the *in vivo* tests 2 µl per  
 32 skin area. This results in TiO<sub>2</sub> administrations of 7.5 mg/cm<sup>2</sup> and 0.1 mg/cm<sup>2</sup>, respectively.  
 33 All dispersions were removed after the exposure period (*in vitro* 24 h, *in vivo* 45 minutes)  
 34 with a paper towel. Both *in vivo* and *in vitro* Tesa® were used for collection of cell layers of  
 35 the skin treated with the TiO<sub>2</sub> formulations. Atomic absorption spectrometry (AAS) was used  
 36 for determination of the Ti content. Tests were performed in triplicate. The formulations  
 37 investigated were: an oil/water emulsion with carboxymethylcellulose (CMC), and  
 38 dimethicon and silicon oil; a liposomal formulation with phospholipid and water.

39  
 40 Results

41 The amounts of Ti observed after the *in vitro* and *in vivo* exposure of skin was in the µg  
 42 range. In the sequential tape strips starting at about 25-35 µg/cm<sup>2</sup> in the first tape strip  
 43 and declining just above the limit of detection (0.1 µg/cm<sup>2</sup>) level at tape strip #6-#12. For  
 44 the oily dispersion having the highest Ti levels were measured in the first tape strips. For  
 45 the *in vivo* exposure the Ti recovery started at about 7.5 µg/cm<sup>2</sup> and declined in the  
 46 following tape strips. Microfine TiO<sub>2</sub> was found to penetrate deeper in the human skin from  
 47 an oily dispersion than from an aqueous one.

48  
 49 **SCCS Comments**

50 No information was provided on the actual size of the used microfine TiO<sub>2</sub>, so the study can  
 51 only be considered as supporting evidence.

**In vitro exploratory study percutaneous skin penetration - human skin (Ref 142)**

1	
2	
3	Study design.
4	Guideline/method: exploratory study
5	Species: human (female Caucasian)
6	Test substances: Solaveil CT10W 3% W/Si emulsion, 3% W/O emulsion, both used as
7	sprayable product (Uniqema, UK)
8	Particle size: not reported in M&M section. Mentioned in Discussion to be between
9	20-70 nm
10	Group sizes: n=6 (experiments)
11	Dose applied: 2 mg/cm <sup>2</sup>
12	Skin: abdominal skin from plastic surgery stored at -25°C for maximally 6
13	months
14	Skin area 5.31 cm <sup>2</sup>
15	Skin temperature: 32 ± 1 °C
16	Test chamber: static Franz-type diffusion cell,
17	Receptor fluid: PBS with 4% bovine serum albumin
18	Exposure period: 1, 2, 4, 6, 8, 12 and 24 h
19	GLP: no
20	Published: yes
21	Study period: 2009
22	Reference: Reference 142 submission VII Durand et al., 2009

**Method**

25 The incubation in the diffusion cells was performed in wells covered with Parafilm paper to  
 26 avoid drying, and the whole system was protected from sunlight by opaque paper. Receptor  
 27 fluid was removed at several time points and replaced immediately with fresh solution. Ti  
 28 level was quantified and determined by Inductively Coupled Plasma-Optical Emission  
 29 Spectroscopy (ICP-OES). Samples were digested and dissolved before Ti determination.

30 At the end of the experiment, the skin samples were removed from the cell and rinsed with  
 31 PBS solution and tetrahydrofuran/ acetonitrile (THF/CAN, 80 : 20, v : v) until no product  
 32 was left on the skin. The skin was then ground and mixed with a THF/ACN (80 : 20, v : v)  
 33 solution and placed in an ultrasonic bath for 30 min. Each solution was then divided in two  
 34 parts: one part was kept at -25°C for the further analysis of the TiO<sub>2</sub> by spectrometric  
 35 methods. Three types of sample were taken and analysed:

36 1 The receptor fluid (5 mL).

37 2 A solution of the recovered product remaining on the skin (after evaporation of all liquid).

38 3 The mixture of ground skin (after evaporation of all the liquid).

39 The samples were heated at 450°C in a muffle furnace for 10–12 h. They were then fused  
 40 with 5 g of K<sub>2</sub>S<sub>2</sub>O<sub>7</sub> in a flame. The resulting substance was dissolved in 10 mL hot H<sub>2</sub>SO<sub>4</sub>  
 41 solution (1 : 1 v/v) and diluted with ultra-pure water to 100 mL.

42 The solutions obtained were then injected into the ICP-OES apparatus.

**Results**

45 The recovery of the TiO<sub>2</sub> from the emulsions and spiked PBS solution with 4% BSA was  
 46 92.5% (W/O emulsion), 92.4% (W/Si emulsion, and 96.8% for the BSA-PBS solution ,  
 47 respectively, demonstrating the validity of the method for determination of Ti. In each part  
 48 of the skin and in the receptor fluid for W/O and W/Si, respectively. the levels of recovery  
 49 were between 76% and 86% for Ti present in the skin and/or on the skin. The limits of  
 50 detection and of quantification are respectively 0.01 ppm (0.01 µg/g) and 0.1 ppm (0.1  
 51 µg/g) for the W/O and W/Si emulsion. Presence on skin after washing was about 40% and  
 52 50% for the W/O and W/Si emulsion, respectively. Approximately 20% (W/O) to 40%  
 53 (W/Si) of the TiO<sub>2</sub> was observed in the skin. After 24 h of experiment titanium levels were  
 54 below the limit of detection. So it was considered that no TiO<sub>2</sub> passed into the receptor fluid.  
 55 There was a loss in the recovery up to 25% of the administered dose.

56  
 57



**SCCS Comment**

No characterization data for TiO<sub>2</sub> is presented - only size has been indicated in discussion section of the paper. Presence of coating indicated in Table 4.2 of supplicant but not mentioned in the paper. Data on receptor fluid indicated in text but not shown in paper. Level of Ti at 24 h mentioned to be below limit of detection but data on recovery/determinations at various time points are not presented in the paper. Results are of limited value for the evaluation of skin penetration of TiO<sub>2</sub> as no data on the receptor fluid were presented. It was demonstrated that approximately 20% to 40% of the TiO<sub>2</sub> was observed in the skin. No further evaluation of localization was done.

***In vitro* exploratory study percutaneous skin penetration - human skin (Ref 143)**

Study design.

Guideline/method: exploratory study

Species: human

Test substances: Titanium dioxide T805, and Spectra veil MOTG, a 60% dispersion of zinc oxide in mineral oil/triglyceride.

Particle size: not reported

Group sizes: not reported

Dose applied: 1 mg/cm<sup>2</sup> *in vitro*

Skin: abdominal skin recovered from plastic surgery

Skin area: not reported

Skin temperature: room temperature

Test chamber: not reported

Receptor fluid: not reported

Exposure period: not reported

GLP: no

Published: yes

Study period: 1997

Reference: Reference 143 submission VII Dussert et al., 1997.

**Method**

The presence of TiO<sub>2</sub> in the skin was evaluated by TEM. At TEM characterization the TiO<sub>2</sub> was identified as a mixture of rutile and anatase crystal forms. The sunscreen formulation investigated was a mixture of both TiO<sub>2</sub> and ZnO. The test formulation was a w/o emulsion formulated with ultrafine titanium dioxide (11% wt), and zinc oxide (2.5% wt). The formulation was used as topical administration *in vitro* with a dose of 1 mg/cm<sup>2</sup>. Skin penetration was evaluated by TEM.

**Results**

Cross-sections of the horny layer of human epidermis, after topical application of the sunscreen emulsion, show an almost regular mineral-coating of the stratum corneum. The crystals appear to surround the desquamating corneocytes. However, neither intercellular nor intracellular penetration of crystallites is evident in transmission electron microscopy. The TEM evaluation shows the presence of particles above the stratum corneum and between desquamating stratum corneum cells.

**SCCS Comments**

Although this study provides some evidence that there is no penetration of the nanoparticles from the formulation into the skin, the information on the study itself is rather limited, e.g. time of incubation and surface area of treated skin were not indicated. A mixture of TiO<sub>2</sub> and ZnO nanoparticles was used in the formulation. In the TEM evaluations the TiO<sub>2</sub> and ZnO could not be identified separately. This study is of no value for the evaluation of skin penetration of TiO<sub>2</sub> nanoparticles. Presence of coating is indicated in Table 4.2 of supplicant but not mentioned in the paper.

1 **Kertesz et al. 2004, Ion-microscopic evaluation of porcine or human skin after**  
 2 **treatment with TiO<sub>2</sub> samples (Ref 66)**

3 Samples investigated by ion microscopy are 14-16  $\mu\text{m}$  thick porcine and human skin.

4 Quantitative elemental concentrations and distributions a new measurement setup and data  
 5 evaluation system has been developed.

6 The penetration studies using different formulations were started on domestic pig skin,  
 7 which resembles human skin closest. In a next step, human skin xenografts transplanted  
 8 into SCID mice were used.

9 22 pig skin, 11 transplanted human skin and 13 human skin samples were investigated.

10 Results

11 The results obtained by ion microscopy or electron microscopy show that in the case of  
 12 healthy skin the nanoparticles penetrate into the deepest corneocyte layer of the skin, but  
 13 never reach the vital layers.

14

15 Conclusion

16 No penetration of the test material into viable porcine or human skin

17

18 **Nanoderm - Quality of skin as a barrier to ultra-fine particles (ref 67)**

19 Penetration of TiO<sub>2</sub>-nanoparticles through the epidermis of human foreskin grafts  
 20 transplanted into SCID (Severe Combined Immune Deficiency) mice.

21 The skin grafts were treated with a hydrophobic emulsion (Antheil's XL F60) containing  
 22 micronized TiO<sub>2</sub>-nanoparticles in occlusion, for 1, 24 and 48 h.

23 Quantitative elemental concentrations and distributions have been determined in 14-16  $\mu\text{m}$   
 24 thick freeze-dried sections obtained from quick frozen punch biopsies using PIXE (Particle  
 25 Induced X-ray Emission), STIM (Scanning Transmission Ion Microscopy) and RBS  
 26 (Rutherford Backscattering) analytical methods.

27 Result

28 In most cases it was found that the remnant of the liposome crème together with the  
 29 outermost stratum corneum was removed during the sample preparation. When the crème  
 30 remained on the skin the Ti was quasi homogeneously distributed in the outermost layers,  
 31 and the penetration seemed to be limited to the outermost part of the stratum corneum.  
 32 However, in two cases, both after 48 h exposure, penetration through the stratum corneum  
 33 to the limit of the vital stratum granulosum was observed. The sample originates from the  
 34 entry of a sweat gland.

35

36 Conclusions

37 No penetration to the viable skin was reported except for some limited observations of  
 38 material entering sweat glands.

39

40 **Adachi et al., 2010, *In vivo* effect of industrial titanium dioxide nanoparticles**  
 41 **experimentally exposed to hairless rat skin (Ref 126)**

42 Guideline/method: No specific guidelines followed

43 Test system: Hairless Rat (Male Westar Yogi Rats) 8 weeks old, weighing 202–267  
 44 g, (Japan SLC, Hamamatsu)

45 Test items: Uncoated anatase TiO<sub>2</sub> nanoparticles (ST-01) from Ishihara Sangyo,  
 46 Ltd, Japan.

47 Formulation White water/oil (W/O) emulsion containing 10 wt% TiO<sub>2</sub>, 4 wt%  
 48 Nikko Nikkomulse WO (cyclopentasiloxane, PEG-10 dimethicone,  
 49 dosteardonium hectrite), 50.0 wt% decamethylcyclopentasiloxane

1 KF-995 and 0.55 wt% acetic acid, and purified water was added to a  
 2 final volume of 100 wt%

3 Concentrations: Four mg/cm<sup>2</sup> emulsion (0.4 mg/cm<sup>2</sup> TiO<sub>2</sub>) was applied to a 15 cm<sup>2</sup>  
 4 area on the rat dorsal skin in the absence of ultraviolet (UV)  
 5 radiation.

6 Exposure: Skin samples at 4 h after exposure were observed using light,  
 7 electron, and confocal laser scanning microscopy over 48 hrs. Time  
 8 course study for light microscopic evaluation in the other groups of  
 9 rats (10 TiO<sub>2</sub>-treated and five control rats) was carried out at 24, 72  
 10 and 168 h after exposure.

#### 11 Results

12 After 24 h, no particles were observed in keratinized layers of the follicular infundibulum,  
 13 but a small amount of particles remained in the superficial part of the stratum disjunctum.  
 14 After 72 h, the particles were still observed in upper keratinized layers of the infundibulum  
 15 but were not found in the interfollicular horny cell layer (Figure 3d). After 168 h, small crops  
 16 of particles were found in the uppermost keratinized layer of only a few follicular openings.  
 17

#### 18 Conclusion

19 The study shows no penetration of TiO<sub>2</sub> in water / oil emulsion into viable skin through  
 20 either the transcorneal or transfollicular pathway.  
 21

#### 22 **Gopee et al., 2009, Lack of dermal penetration following topical application of** 23 **coated and uncoated nano- and micron-sized titanium dioxide to intact and** 24 **dermabraded skin in mice (Ref 162 - poster presentation)**

25 Guideline/method: No

26 Test system: Mice (hairless)

27 Test item: TiO<sub>2</sub> (Unreported batch) roughly spherical uncoated particles,  
 28 with 25.1 ± 8.2 nm diameter (minimum particle size was 13 nm  
 29 and maximum particle size was 71 nm). Formulation consisted  
 30 of titanium dioxide suspended in polyglyceryl-3 distearate,  
 31 cetearyl alcohol, light mineral oil, propylene glycol, k-phosphate  
 32 buffer, methyl paraben, propyl paraben, and propylene  
 33 glycol:water (1:4, v:v).

34 Treatment: Mice (hairless) were treated with 5 uL of 5% uncoated anatase  
 35 TiO<sub>2</sub> (intact or dermabraded skin). At 6 and 24 hr post-  
 36 application, mice were sacrificed and skin, right regional lymph  
 37 nodes, blood, liver, kidney and spleen were collected and  
 38 analyzed for titanium (Ti) by ICP-MS. Tissues of one mouse was  
 39 analyzed microscopically.

#### 40 Result

41 No significant elevations in Ti levels were observed in any of the organs analyzed for Ti.  
 42

#### 43 Conclusion

44 The results suggest that both intact and compromised skin of hairless mice may be an  
 45 effective barrier for nano-sized TiO<sub>2</sub>.  
 46

#### 47 **Kiss et al. 2008, Investigation of micronized titanium dioxide penetration in** 48 **human skin xenografts and its effect on cellular functions of human skin-derived** 49 **cells (Ref 167)**

50 Guideline/method: No

1	Test system:	<i>In vivo</i> SCID mice, grafts area, 6-mm diameter human foreskin
2		punch biopsies were taken.
3	<i>In vitro</i> :	human immortalized HaCaT keratinocyte cells, human dermal
4		fibroblasts (HDFs) & human immortalized sebaceous gland cell
5		line SZ95.
6	Test items:	TiO <sub>2</sub> , 9 nm Anatase (gift from Prof. Z. Stachura, Krakow,
7		Poland)
8	Vehicle:	hydrophobic emulsion ('TiO <sub>2</sub> -emulsion') was used (Anthelios XL
9		SPF 60, La Roche Posay, La Roche Posay, France)
10	Concentrations:	2 mg / cm <sup>3</sup>
11	Exposure:	24 h

## 12 Result

13 TiO<sub>2</sub> particles did not penetrate through the stratum corneum of human skin transplants.  
 14 TiO<sub>2</sub> nanoparticles are internalized by *in vitro* cultured fibroblasts and melanocytes but not  
 15 by keratinocytes and sebocytes.

## 16 Conclusions

17 This type of TiO<sub>2</sub> (custom made, anatase) does not penetrate human foreskin grafts. *In*  
 18 *vitro* uptake is cell type dependent.

## 19 **Pinheiro et al. 2007, The influence of corneocyte structure on the interpretation of** 20 **permeation profiles of nanoparticles across skin (Ref 191)**

21	Guideline/method:	No
22	Test system:	Healthy and psoriatic human skin was collected by .punch
23		biopsy (3 mm diameter) at lumbar-sacral region,
24	Test material:	Commercial sunscreen formulation (unknown source),
25		containing nano TiO <sub>2</sub> .
26	Concentrations:	Unknown
27	Exposure:	2h

## 28 Results

29 The TiO<sub>2</sub> permeation in psoriatic skin reached deeper regions of the stratum corneum than  
 30 in healthy skin. However, for both cases TiO<sub>2</sub> nanoparticles did not reach the living layers of  
 31 the granulosa or spinosum strata.

## 32 Conclusion

33 Psoriasis seems to have only a limited effect on the permeation profile of TiO<sub>2</sub>  
 34 nanoparticles. It has to be mentioned that the source and concentration of the particles is  
 35 not specified in this study.

## 36 **Popov et al. 2005, 2005, 2010 (Ref 192, 193, 194)**

37	Test system:	human skin (volunteers). Sunscreen including rutile TiO <sub>2</sub>
38		particles (100 nm) was administered five times over a period of
39		4 days onto the surface area of flexor forearm skin. The tape-
40		stripping procedure started on the fourth day, 1 h after last
41		application. The surface density of TiO <sub>2</sub> particles on the tape
42		strips was analyzed by x-ray fluorescent measurements.
43	Test material:	Sunscreen including rutile TiO <sub>2</sub> particles (100 nm), this was not
44		further specified.

1 Concentration: 2 mg/cm<sup>2</sup> sunscreen. skin area of 10 X 8 cm (160 mg  
2 sunscreen).

### 3 Results

4 Approximately 14 µg/cm<sup>2</sup> of TiO<sub>2</sub> was found on the first tape strip and almost zero on those  
5 taken at the depth of 15 µm. The particles were mainly located at a depth range of 0 to 3  
6 µm.

### 8 Conclusions

9 No penetration into living layer of skin. The source and nature of TiO<sub>2</sub> is not well reported.  
10 Three different papers all presenting the same experiment as an original study.

## 12 **Sadrieh et al. 2010, Lack of significant dermal penetration of titanium dioxide 13 (TiO<sub>2</sub>) from sunscreen formulations containing nano- and sub-micron-size TiO<sub>2</sub> 14 particles (Ref 199)**

15 Test system: Female Yucatan minipigs (~4 months of age; n ¼ 12) from  
16 Sinclair Research Center (Auxvasse, MO, USA).

17 Test items: Uncoated nano titanium dioxide (Degussa Aeroxide P25, a  
18 mixture of anatase and rutile and known to be photocatalytic;

19 1. coated (aluminum hydroxide/dimethicone copolymer) nano  
20 titanium dioxide (BASF T-Lite SF obtained from BASF,  
21 Shreveport, LA; rutile; "coated nano")

22 2. uncoated submicron titanium dioxide (treated with  
23 aluminum hydroxide, Ishihara Tipaue CR-50 obtained from  
24 Ishihara Corporation, San Francisco, CA; rutile;  
25 "submicron")

26 Vehicle All used particles were added to the same sunscreen  
27 preparation, preparation without particles was used as control.

28 Concentrations: Approximately 5% preparations were achieved.

29 Exposure: Topical application four times daily, 5 days a week, for a total of  
30 22 days. Dose of 2 mg/cm<sup>2</sup>, each animal received a total of 176  
31 mg/cm<sup>2</sup> cream resulting in a average of ~1.32 l of cream per  
32 animal

33 Negative control: cream without TiO<sub>2</sub>

### 34 Result

35 The epidermis from minipigs treated with sunscreens containing TiO<sub>2</sub> showed elevated  
36 titanium levels. Increased titanium was detected in abdominal and neck dermis of minipigs  
37 treated with uncoated and coated nano TiO<sub>2</sub>. EM-energy dispersive x-ray analysis showed  
38 that TiO<sub>2</sub> particles were found in the stratum corneum and upper follicular lumens in all  
39 treated skin samples. Isolated titanium particles were present at various locations in the  
40 dermis of animals treated with any of the three types of TiO<sub>2</sub> sunscreens; however, there  
41 was no pattern of distribution or pathology.

### 43 Conclusion

44 These findings indicate that there is some, though probably not significant, penetration of  
45 TiO<sub>2</sub> nanoparticles through the intact normal epidermis in minipigs. The quantification of the  
46 concentration in the dermis is difficult since the removal of the epidermis is almost never  
47 perfect (resulting in possible false positive results).

## 49 **Exploratory study, dermal penetration and toxicity, hairless mice and porcine skin, 50 subchronic dermal exposure (Wu et al., 2009)**

51

1 The paper has its focus on the penetration of TiO<sub>2</sub> nanoparticles through the skin after  
2 dermal exposure.

- 3
- 4 • No penetration in *in vitro* porcine skin model of TiO<sub>2</sub> (4, 10, 25, 60 and 90nm). The  
5 amount of TiO<sub>2</sub> was below detection limit, but materials and methods stated that TiO<sub>2</sub>  
6 was not removed. Not clear whether the TiO<sub>2</sub> was removed before tape stripping.  
7 Results indicate that tape stripping most probably was done after removal of TiO<sub>2</sub>,  
8 hence there was a low levels in the tape strip pools.
  - 9 • Pig skin *in vivo*: TiO<sub>2</sub> present in stratum corneum, stratum granulosum, prickle cell layer  
10 and stratum basale of the epidermis but not in dermis. Only 4nm TiO<sub>2</sub> in basal cell  
11 layer. Figure 2 does NOT clearly show presence of TiO<sub>2</sub> nanoparticles in epidermis.
  - 12 • Hairless mice: Effect of TiO<sub>2</sub> on body weight observed. Decreased growth compared to  
13 control mice and mice treated with normal sized TiO<sub>2</sub>. 10-25- and 21 (P25) nm TO<sub>2</sub>  
14 induced growth retardation.
  - 15 • Biochemical parameters for skin and liver malondialdehyde (MDA) increase (10-25-  
16 21nm), superoxide dismutase (SOD) skin and liver decrease (10-21nm), skin  
17 hydroxyproline (HYP) decrease (10-25-21-60nm)
  - 18 • Organ distribution after 60 days skin exposure showed 10, 25, 21, 60nm TiO<sub>2</sub> in skin,  
19 sub muscles, heart, liver, spleen, 21, 60nm TiO<sub>2</sub> in lung, 21nm TiO<sub>2</sub> in brain, whereas  
20 TiO<sub>2</sub> in kidney was similar to control. However the differences were not significant.

## 21 Conclusion

22 Local effects on skin are demonstrated by biochemical parameters SOD, MDA, and HYP, and  
23 histopathology (keratinization). Systemic effects are not clearly identified because of  
24 possible alternative route of exposure by oral uptake. Also the lesions shown in various  
25 organs may be due to background lesions present in animal strain. This is not excluded by  
26 scoring of lesions in control versus treated animals. However, the treatment resulted in  
27 growth retardation of the animals.

## 28 Studies with limited information

29 *In vivo* study (Gottbrath et al., 2003; FitzGerald, 2005)

30 Penetration of nano-sized titanium dioxide (Tioveil AQ N; (rutile, coated with alumina/silica)  
31 into human stratum corneum after *in vivo* application of two formulations was studied.  
32 Penetration was measured by tape stripping of skin (10 strips). Tape strips from the  
33 titanium dioxide-treated skin sites were assayed for titanium by atomic absorption  
34 spectrometry. Tape strips from the vehicle control treated sites were viewed with an  
35 inverted microscope to estimate the amount of corneocyte aggregates. Titanium dioxide  
36 nanoparticles in the formulations and tape strips were visualized by transmission electron  
37 microscopy (TEM). The authors concluded that, after application of the liposomal  
38 formulation, a fraction of the TiO<sub>2</sub> nanoparticles penetrated into the stratum corneum and  
39 did not remain in shallow valleys formed by the corneocytes, explaining the water resistance  
40 of the liposomal formulation, i.e. the deposition of TiO<sub>2</sub> nanoparticles depends on the  
41 formulation used.

42 *In vivo* study (Tan et al., 1996; FitzGerald, 2005)

43 Review of recent literature on safety of nanomaterials in cosmetics with special references  
44 to skin absorption and resorption of ultrafine titanium dioxide and zinc oxide, prepared for  
45 Physical Sunscreens Manufacturers Association (PSMA), European Cosmetic, Toiletry and  
46 Perfumery Association and BASF AG, 28 September 2005.

47 A study with 10-50 nm TiO<sub>2</sub> particles was performed in order to evaluate if the particles  
48 could penetrate the stratum corneum to the dermis following repeated application in  
49 volunteers (13 patients with compromised skin scheduled to have surgery for skin lesions).



1 The patients received repeated application (twice a day for 2-6 weeks) of a sunscreen lotion  
2 containing 8% microfine TiO<sub>2</sub>. Chemical analysis (ICPMS) were performed on skin biopsies.  
3 The authors concluded that non-statistically significantly higher Ti levels in the dermis of  
4 treated subject vs. controls (cadaver skin) were found.  
5

6 *In vivo* study (Lademann et al., 1999)

7 The dermal penetration of 20 nm TiO<sub>2</sub> nanoparticles (Titan M 160, coated, rutile) (assumed  
8 particle size, based on description of product used) in a sunscreen formulation (o/w  
9 emulsion) was studied. The sunscreen was applied repeatedly (11 times) over 4 days to the  
10 forearm skin (2 mg/cm<sup>2</sup>) of human volunteers. UV/Vis spectroscopic evaluation, X-ray  
11 fluorescence measurements LIFM, SRLSM and Raman spectroscopy of skin tape strips and  
12 histological evaluation of skin biopsies were performed. The only significant finding  
13 concerning a potential penetration of TiO<sub>2</sub> beyond the upper skin layers was their deposition  
14 in single hair follicle openings, although there was no evidence that these residues were  
15 located within the living skin. The concentration of Ti in the hair follicle openings was two  
16 orders of magnitude lower than that in the upper skin layers. The authors concluded that  
17 that there was no penetration of TiO<sub>2</sub> particles in living skin and that the TiO<sub>2</sub> particles  
18 were mainly located in the outer layers of the SC.  
19

20 *In vivo* study (Schulz et al., 2002)

21 The influence of particle size on the dermal absorption of three TiO<sub>2</sub> preparations was  
22 investigated (T805 [20 nm, cubic, Ti/Si coating, rutile/anatase], Eusolex T-2000 [rutile, 10-  
23 15 nm NPs in 100 nm aggregates, needles, Ti/ Al<sub>2</sub>O<sub>3</sub>/SiO<sub>2</sub> coated] Tioveil AQ-1 OP [100  
24 nm, needles, Ti/Al/Si coated]). Each had a different primary particle size (10-15 nm, 20 nm  
25 and 100 nm), shape (cubic or needles) and hydrophobic/hydrophilic characteristics. The  
26 preparations were topically applied (4 mg/cm<sup>2</sup>) in an oil-in water emulsion containing 4%  
27 TiO<sub>2</sub> to the forearm skin of human volunteers for 6 hours. Skin biopsies were examined by  
28 scanning electron microscopy to visualize the distribution of particles within the skin layers.  
29 TiO<sub>2</sub> particles were only deposited on the outermost surface of the SC, and were not  
30 detected in deeper SC layers, the human epidermis and dermis. The authors concluded that  
31 none of the particles penetrated beyond the outer layer of the stratum corneum.  
32

33 Another study provided under dermal penetration (Reference 10, submission 1) seems to be  
34 an irritation study and has therefore not been reviewed.  
35

### 36 **SCCS Comments on Dermal/ Percutaneous Absorption**

37 The studies presented in the submission cover a range of nanomaterials of which some  
38 relate to the materials under assessment. The studies range from *in vitro* to *ex vivo* and *in*  
39 *vivo* experimental conditions, and intact and UV damaged skin. The results from these  
40 studies suggest that TiO<sub>2</sub> nanoparticles, when applied to skin in a sunscreen formulation,  
41 are likely to stay largely on the skin, whilst a small proportion of the particles may  
42 penetrate to the outer layers of stratum corneum. A few reports have suggested the  
43 possibility that TiO<sub>2</sub> nanoparticles may penetrate deeper to reach stratum granulosum –  
44 e.g. in human foreskin grafts transplanted onto SCID mice (Kertész et al., 2005) - or to  
45 dermis of minipigs treated with nano TiO<sub>2</sub> (Sadrieh et al., 2010 (Ref 199)). There is,  
46 however, a consistent and large body of evidence from the submitted studies, and other  
47 studies published in open literature (e.g. NANODERM, 2007; Nohynek et al., 2007), which  
48 shows that nanoparticles do not penetrate deep enough to reach the viable epidermis or  
49 dermis cells of healthy skin. In psoriatic skin, Pinheiro et al. (2007) showed that nano-TiO<sub>2</sub>  
50 in a sunscreen formulation penetrated into deeper areas of the stratum corneum than in  
51 healthy skin, but did not reach living cells in either psoriatic or healthy skin. Some *in vitro*  
52 test systems, however, lack a stratum corneum layer, which can block penetration of TiO<sub>2</sub>  
53 nanoparticles. Toxicological effects from such tests therefore need a careful consideration  
54 since they may be difficult to extrapolate to the effects *in vivo* (Nohynek et al., 2007).  
55  
56

1 A recent study by Bennett et al. (2012) investigated the penetration of TiO<sub>2</sub> particles  
 2 through isolated pig skin sections and found a small fraction of the total dose in the skin  
 3 sections. The study found nanoparticles, or small clusters, in the interstitial spaces of the  
 4 porcine dermis after irradiation up to 500 µm depth, in comparison to the control skin  
 5 samples (tested under dark) where TiO<sub>2</sub> was only found on the surface of the stratum  
 6 corneum. This study does raise questions over the possible disagglomeration of nanoparticle  
 7 clusters and enhanced penetration of TiO<sub>2</sub> nanoparticles into skin under use conditions. The  
 8 study used TiO<sub>2</sub> (anatase, non-coated) material, the type which is not recommended in this  
 9 opinion. Further studies will be needed on different crystalline forms and coated materials to  
 10 draw any conclusions on other TiO<sub>2</sub> nanomaterials.

11  
 12 Contrary to the strong evidence suggesting a lack of penetration of TiO<sub>2</sub> nanoparticles to  
 13 viable epidermis or dermis cells, there are a number of studies (in this submission and  
 14 published elsewhere), which indicate that nanoparticles can enter hair follicles. According to  
 15 SCCP opinion (2007) and NANODERM report (2007), adverse effects are not expected from  
 16 dermal exposure of healthy unflexed skin to photostable nano-TiO<sub>2</sub> in sunscreens. However,  
 17 if photocatalytic nano-TiO<sub>2</sub> is present in a sunscreen, it can potentially lead to generation of  
 18 reactive oxygen species (ROS) on exposure to UV light.

19  
 20 Most, if not all, studies provided in the submission were performed with nano TiO<sub>2</sub> as  
 21 present in sunscreen formulations depicting consumer use. The studies were not directed  
 22 towards hazard identification using either a dose response approach or a worst case  
 23 scenario (overdosing situation). It is also of note that currently there are certain knowledge  
 24 gaps in relation to the possible dermal penetration of nano TiO<sub>2</sub> on repeated or long term  
 25 use of cosmetic products, which may not only be used on flexed healthy skin but also on  
 26 skin that may have lesions or cuts. Studies provided in support of this submission have  
 27 shown that TiO<sub>2</sub> nanoparticles do not penetrate the (simulated) sunburnt skin, whereas  
 28 such information on flexed or damaged skin is currently not available.  
 29

### 30 **1.5.5 Repeated dose toxicity**

31

#### 32 **1.5.5.1 Repeated Dose (30 days) oral toxicity**

33

#### 34 **Exploratory subchronic oral study – Mice 30 day oral (gavage)**

35

36	Guideline:	No guideline
37	Species/strain:	Mice/CD-1
38	Group size:	20 females per group
39	Test substance:	TiO <sub>2</sub> (Anatase, prepared from hydrolysis of Ti-tetrabutoxide, Primary 40 particle size 5 nm)
41	Batch:	
42	Purity:	
43	Vehicle:	
44	Dose levels:	0, 62.5, 125 and 250 mg/kg bw/day
45	Dose volume:	
46	Route:	Oral
47	Administration:	Intragastric administration every other day for 30 days
48	GLP:	No
49	Study period:	2009
50	Reference:	SI-II-Duan et al., 2010, (140)

51

52 Results

- 1 Mice treated with doses  $\geq 125$  mg/kg bw/d showed body weight reduction, an increase in  
 2 coefficients of the liver and increased coefficients of the liver, kidney, spleen and thymus  
 3 and serious damage to liver function as shown by:
- 4 • A decrease in interleukin-2 activity, white blood cells, red blood cells, haemoglobin,  
 5 mean corpuscular haemoglobin concentration, thrombocytes, reticulocytes, T  
 6 lymphocytes (CD3+, CD4+, CD8+), NK lymphocytes, B lymphocytes, and the ratio of  
 7 CD4 to CD8 of mice.
  - 8 • An increase in NO level, mean corpuscular volume, mean corpuscular haemoglobin, red  
 9 (cell) distribution width, platelets, hematocrit, mean platelet volume of mice.
  - 10 • Disruption of the liver function in terms of enhanced activities of alanine  
 11 aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate  
 12 dehydrogenase and cholinesterase, increase of total protein, and reduction of albumin to  
 13 globulin ratio, total bilirubin, triglycerides, and the total cholesterol levels.
- 14 No such effects were seen at low dose, and the NOAEL appears to be 62.5 mg/kg bw/d.

#### 16 **SCCS Comment**

17 NOAEL derived from this study is 62.5 mg/kg bw/d.

#### 18 1.5.5.2 Sub-chronic (90 days) toxicity (oral, dermal)

19

#### 20 **Subchronic oral toxicity – Rat 90 day oral (diet)**

21

22 Guideline: No guideline  
 23 Species/strain: Rat/F344  
 24 Group size: 10 m, 10 f per group  
 25 Test substance: TiO<sub>2</sub> (uncoated, Unitane®, Anatase), CAS No. 13463-67-7  
 26 Batch: 402110C46  
 27 Purity: 98%  
 28 Vehicle:  
 29 Dose levels: 6250, 12500, 25000, 50000, 100000 ppm  
 30 Dose volume:  
 31 Route: Oral  
 32 Administration: Diet  
 33 GLP: No  
 34 Study period: 1978  
 35 Reference: I-NCI, 1979 (22); DHS-NCI, 1979 (9)

36

#### 37 Results

38 No deaths, no differences in body weight gains, no substance-related gross or microscopic  
 39 pathological finding, NOAEL: 100000 ppm.

40

#### 41 **SCCS Comment**

42 No information has been provided on the particle size profile of the material tested in this  
 43 study. The study is therefore of little value in relation to the current assessment for nano-  
 44 forms of TiO<sub>2</sub>.

45

#### 46 Note

47 The two references provided (I-NCI, 1979 (22) and DHS-NCI, 1979 (9)) are in fact the  
 48 same.

49

#### 51 Subchronic oral toxicity – Mouse 90 day oral (diet)

52

53 Guideline: No guideline  
 54 Species/strain: Mouse/B6C3Fi

1 Group size: 10 m, 10 f per group  
 2 Test substance: TiO<sub>2</sub> (uncoated, Unitane®, Anatase), CAS No. 13463-67-7  
 3 Batch: 402110C46  
 4 Purity: 98%  
 5 Vehicle:  
 6 Dose levels: 6250, 12500, 25000, 50000, 100000 ppm  
 7 Dose volume:  
 8 Route: Oral  
 9 Administration: Diet  
 10 GLP: No  
 11 Study period: 1978  
 12 Reference: I-NCI, 1979 (22); DHS-NCI, 1979 (9)

13  
 14 Results  
 15 No deaths, no differences in body weight gains, no substance-related gross or microscopic  
 16 pathological finding, NOAEL: 100000 ppm.  
 17

18  
 19 **SCCS Comment**

20 No information has been provided on the particle size profile of the material tested in this  
 21 study. The study is therefore of little value in relation to the current assessment for nano-  
 22 forms of TiO<sub>2</sub>.

23 Note: Two references provided (I-NCI, 1979 (22); DHS-NCI, 1979 (9)) are in fact the same.  
 24

25  
 26 Exploratory subchronic oral study – Mice 60 day oral (gavage)  
 27

28 Guideline: No guideline  
 29 Species/strain: Mice/CD-1  
 30 Group size: 20 females per group  
 31 Test substance: TiO<sub>2</sub> (Anatase, prepared from hydrolysis of Ti-tetrabutoxide, Primary  
 32 particle size 5 nm)  
 33 Batch:  
 34 Purity:  
 35 Vehicle:  
 36 Dose levels: 0, 5, 10, 50 mg/kg bw/d  
 37 Dose volume:  
 38 Route: Oral  
 39 Administration: Intragastric administration every day for 60 days  
 40 GLP: No  
 41 Study period: 2010  
 42 Reference: SI-II- Hu et al., 2010 (163)

43  
 44 Results  
 45 Potential effects on nervous system function, significant impairment of the behaviours of  
 46 spatial recognition memory. Indications for impaired neurofunction and behaviour at all  
 47 dose levels, indicated by:  
 48 • Significantly altered levels of Ca, Mg, Na, K, Fe and Zn in brain  
 49 • Inhibition of the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase,  
 50 acetylcholine esterase, and nitric oxide synthase;  
 51 • Disturbed function of the central cholinergic system – significantly decreased levels of  
 52 monoamines neurotransmitters such as norepinephrine, dopamine and its metabolite 3,  
 53 4- dihydroxyphenylacetic acid, 5-hydroxytryptamine and its metabolite 5-  
 54 hydroxyindoleacetic acid,  
 55 • Increased levels of acetylcholine, glutamate, and nitric oxide.  
 56

**SCCS Comment**

From the 60 day oral (gavage) study in mice, a LOAEL of 5 mg/kg bw/d may be derived.

**1.5.5.3 Chronic (> 12 months) toxicity**

No study provided

**SCCS Comment on Repeated Dose Toxicity:**

Two out of the 4 subchronic studies provided are of little value to the assessment of nano-forms of TiO<sub>2</sub> because particle size distribution of the tested materials is not provided. The other two studies used anatase nanomaterials. From the 60 day oral (gavage) study in mice, a LOAEL of 5 mg/kg bw/d may be derived.

**1.5.6 Mutagenicity / Genotoxicity**

There are a number of issues in regard to *in vitro* testing of nanomaterials for mutagenicity. Bacterial mutagenicity assays are considered to be less appropriate for the testing of nanoparticles compared to mammalian cell systems due to the lack of endocytosis by bacterial cells (EFSA, 2011). Therefore, for a negative outcome of such tests to be acceptable, it is essential that contact of the test materials with bacterial DNA (*i.e.* nanoparticle uptake by bacteria) is demonstrated. Furthermore, for testing of (conventional) chemical substances, generally accepted positive controls are used for the various *Salmonella* strains. The use of such chemical positive controls in testing nanomaterials would not provide a proof for a negative response of the nanomaterial. Currently, there is no accepted nanoparticle positive control that can demonstrate whether the assay is suitable for the mutagenicity testing of insoluble/poorly soluble nanoparticles.

It is of note that the following studies have not been reviewed as part of this assessment because they relate to test materials that are either not nanomaterials, or lack data on material characterisation to establish whether they were relevant nanomaterials for this assessment.

SI-Dunkel et al., 1985 (32); SI-Tennant et al. 1987 (33 (i, ii)); SI-Ivett et al., 1989 (35); SIII-Lu et al., 1998 (56c), Nohynek, 1999 (56), PSMA statement, 1999 (66); SI-II Warheit et al., 2007 (215); SIII-Lu et al., 1998 (56c), SI-Myhr, Caspary, 1991 (34); SI-Poole et al. 1986, (36); SI-Lemaire et al., 1982 (37); SI-II Msiska et al., 2010 (183); SI-Casto et al., 1979 (38); SI-Mikalsen et al., 1988 (39); SI-DiPole, Casto, 1979 (40); SI-Tripathy et al., 1990 (44); SI-Kitchin, Brown, 1989 (43); SI-II Pan et al., 2010 (189), SI-II DiVirgilio et al., 2010 (139), Osman et al. 2012 (188).

**1.5.6.1 Mutagenicity / Genotoxicity in vitro****Bacterial gene mutation test**

Guideline/method: OECD 471 (1997)

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2uvrA. Tests were performed in absence or presence of S9-mix

Replicates: Triplicate cultures in 2 independent experiments

Test items: T805 (coated, A/R, PSMA 1 type)

Batch: /

Solvent: Ethanol

1 Concentrations: 8, 40, 200, 1000 and 5000 µg/plate in 1st experiment (range findings  
 2 experiment); 312.5, 625, 1250, 2500 and 5000 µg/plate in 2nd  
 3 experiment  
 4 Exposure: 48 h using the direct plate incorporation method  
 5 Negative control: yes (vehicle)  
 6 Positive control: ENNG for WP2uvrA, TA100 and TA1535; 9AA for TA1537 and 4NQO for  
 7 TA98; 2AA in all strains in experiments with S9-mix.  
 8 GLP: in compliance  
 9 Date of report: 19 June 1994 – 25 August 1994  
 10 Reference: Submission DHS (11), II(67)

11  
 12 The test substance was tested for mutagenicity in bacterial gene mutation assays with and  
 13 without metabolic activation (S9-mix prepared from Arochlor 1254 induced male Sprague  
 14 Dawley rat livers) using the direct plate incorporation method. Test concentrations were  
 15 based on the results of a preliminary toxicity study. The *S. typhimurium* strains TA98,  
 16 TA100, TA1535 and TA1537, and the *E. coli* strain WP2uvrA<sup>-</sup> were exposed for 48 h to the  
 17 test substance (suspended in ethanol) in concentrations ranging from 8 - 5000 µg/plate (1<sup>st</sup>  
 18 experiment) and 312.5 - 5000 µg/plate (2<sup>nd</sup> experiment).  
 19

#### 20 Results

21 The test substance caused no visible growth reductions. Precipitation was observed at  
 22 concentrations of 625 µg/plate and above. All positive controls showed marked effects on  
 23 revertant colony numbers and the ethanol vehicle tested negative. Exposure to the test  
 24 substance did not result in biologically relevant increases in revertant colony numbers.  
 25

#### 26 Conclusion

27 Under the experimental conditions used T805 was not mutagenic in this gene mutation tests  
 28 in bacteria.  
 29

#### 30 SCCS Comment

31 See comments under 3.3.6 on the issues relating to the suitability of bacterial mutagenicity  
 32 assays for nanomaterials.  
 33  
 34

#### 35 **Bacterial gene mutation test**

36 Guideline/method: OECD 471 (1983)  
 37 Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537.  
 38 Tests were performed in absence or presence of S9-mix  
 39 Replicates: Triplicate cultures in 2 independent experiments  
 40 Test items: T817 (coated, A/R, PSMA 1 type)  
 41 Batch: 04095  
 42 Solvent: Ethanol  
 43 Concentrations: 33.3, 100, 333.3, 1000, 2500 and 5000 µg/plate  
 44 Exposure: 48 h using the direct plate incorporation method  
 45 Negative control: vehicle  
 46 Positive control: NaN<sub>3</sub> for TA100 and TA1535; 4-NOPD for TA1537 and TA98; 2AA in all  
 47 strains in experiments with S9-mix.  
 48 GLP: in compliance  
 49 Date of report: 1997  
 50 Reference: Submission DHS (12), II(67)

51  
 52 The test substance was tested for mutagenicity in a bacterial gene mutation test with and  
 53 without metabolic activation (S9-mix was prepared from phenobarbital/β-naphthoflavone  
 54 induced male Wistar Rat livers). The *S. typhimurium* strains TA98, TA100, TA1535 and  
 55 TA1537 were exposed for 48 h to the test substance (suspended in ethanol) at  
 56 concentrations ranging from 33.3 to 5000 µg/plate.  
 57



## 1 Results

2 Normal background growth was observed up to 5000 µg/plate. All positive controls showed  
3 distinct increases in revertant colony numbers. Exposure to the test substance did not result  
4 in biologically relevant increases in revertant colony numbers.

## 6 Conclusion

7 Under the experimental conditions used T817 was not mutagenic in this gene mutation tests  
8 in bacteria.

## 10 SCCS Comment

11 The study is on T817 (coated, A/R, 95%, PSMA 1 type) which relates to Eusolex T in the  
12 dossier. This study is relevant to the nanomaterial group (anatase).

13 See comments under 3.3.6 on the issues relating to the suitability of bacterial mutagenicity  
14 assays for nanomaterials.

16 **Bacterial Gene Mutation Test**

17 Guideline/method: OECD 471 (1997)

18 Test system: *Salmonella typhimurium* strains T 98, T 100, T 102, T 1535 and TA1537,  
19 in presence or absence of S9-mix

20 Replicates: Triplicate plates

21 Test items: T-Lite™ SF, pure rutile, primary particle size 10 x 50 nm, mean  
22 agglomerates approximately 200 nm (d10: 90 nm, d90: 460 nm);  
23 coating consisting of aluminium hydroxide and dimethicone/methicone  
24 copolymer

25 T-Lite™ MAX, pure rutile, primary particle size 10 x 50 nm, mean  
26 agglomerates approximately 200 nm (d10: 90 nm, d90: 460 nm);  
27 coating consisting of dimethoxydiphenylsilane, triethoxycaprylylsilane  
28 crosspolymer, hydrated silica and aluminium hydroxide

29 Batch: /

30 Solvent: DMSO (SPT), FCS (PIT)

31 Concentrations: 0, 20, 100, 500, 2500, 5000 µg/plate

32 Exposure: Standard plate test and or preincubation test

33 GLP: in compliance

34 Reference: Landsiedel *et al.*, 2010

36 The test substances were tested for mutagenicity in the reverse mutation assay in bacteria  
37 with and without metabolic activation. The S9 fraction was prepared from phenobarbital/β-  
38 naphthoflavone induced male Wistar rat liver. Both the standard plate test (SPT) and the  
39 plate incorporation test (PIT) were used. The *S/ typhimurium* strains TA98, TA100, TA102,  
40 TA1535 and TA1537 were exposed to the test substance (dissolved in DMSO (SPT) or fetal  
41 calf serum (PIT)) at concentrations ranging from 20–5000 µg/plate. For control purposes,  
42 DMSO) as negative control and the positive controls (NOPD, MNNG, AAC, MIT.C, 2-AA) were  
43 also investigated.

## 44 Results

45 With the T-Lite™ SF a weak bacteriotoxicity was occasionally observed from 2500 µg/plate  
46 onward in the presence of S9-mix only. With T-Lite™ MAX no bacteriotoxicity was noted.  
47 Precipitation of the test substance was recorded from 100 µg/plate onward for T-Lite™ SF  
48 and from 2500 µg/plate with T-Lite™ MAX.

49 The test substances did not induce a biologically relevant increase in revertant colony  
50 numbers in the bacterial strains at any concentration tested in the presence or absence of  
51 metabolic activation.

## 53 Conclusion

54 Under the experimental conditions used T-Lite™ SF and T-Lite™ MAX were not mutagenic in  
55 this gene mutation tests in bacteria.

56

## 1 SCCS Comment

2 The tested materials relate to S75-K (94% rutile, coated with aluminium hydroxide,  
3 dimethicone/methicone copolymer). See comments under 3.3.6 on the issues relating to the  
4 suitability of bacterial mutagenicity assays for nanomaterials.

5  
6

7 **Chromosome aberration test in mammalian cells**

8 Guideline/method: OECD 473 (1997)  
9 Test system: CHO cells. Tests were performed in absence or presence of S9-mix  
10 Replicates: Duplicate cultures in 2 independent experiments  
11 Test items: T805 (coated A/R, PSMA 1 type)  
12 Batch: 0510067  
13 Solvent: Ethanol  
14 Concentrations: Experiment 1: 86.72, 209.7 and 800 µg/ml without S9 mix  
15 167.8, 640 and 800 µg/ml with S9-mix  
16 Experiment 2: 167.8, 512 and 800 µg/ml  
17 Exposure: Experiment 1: 20 h treatment without S9 mix  
18 3 h treatment and 17 h recovery with S9-mix  
19 Experiment 2: 3 h treatment and 17 h recovery with S9-mix  
20 Negative control: Vehicle  
21 Positive control: NQO (without S9), CPA (with S9)  
22 GLP: yes  
23 Date of report: 17 November 1998 – 11 January 1999  
24 Reference: Submission DHS (13), II(67)

25  
26 The test substance was evaluated for potential cytogenetic effects in Chinese hamster ovary  
27 (CHO) cells in the absence or presence of S9-mix. The S9 fraction was prepared from livers  
28 of rats treated with Arochlor 1254 (experiment 1) or phenobarbital/β-naphtoflavone  
29 (experiment 2). Cytotoxicity was measured as a reduction in cell number compared to the  
30 solvent control. In the absence of S9-mix only one experiment was performed. 4-  
31 nitroquilonine 1-oxide and cyclophosphamide were used as positive controls in the  
32 experiments without and with S9-mix respectively. For each culture cells with structural  
33 aberrations excluding gaps, and polyploidy, endoreduplication or hyperdiploidy were  
34 categorized.

35

36 **Results**

37 The number of cells with structural aberrations in the negative control cultures were within  
38 normal range. A biologically relevant increase in the number of cells with chromosome  
39 aberrations was not observed due to exposure to T805 both without and with S9-mix. The  
40 positive controls NQO and CPA induced statistically significant increases in the number of  
41 cells with structural aberrations in the absence or presence of S9 mix respectively.

42

43 **Conclusion**

44 Under the experimental conditions used T805 was not genotoxic (clastogenic) in this  
45 chromosome aberration test in mammalian cells.

46

47 **SCCS Comment**

48 The experiment in the absence of S9-mix was performed only once.

49

50

51 **Chromosome aberration test in mammalian cells**

52 Guideline/method: OECD 473 (1997)  
53 Test system: CHO cells. Tests were performed in absence or presence of S9-mix  
54 Replicates: Duplicate cultures in 2 independent experiments  
55 Test items: T817 (coated A/R, PSMA 1 type)  
56 Batch: 04095  
57 Solvent: Ethanol

1	Concentrations:	Experiment 1:	85.9, 640 and 800 µg/ml without S9-mix
2			167.8, 512 and 800 µg/ml with S9-mix
3		Experiment 2:	209.7, 512 and 800 µg/ml with S9-mix
4	Exposure:	Experiment 1:	20 h treatment without S9 mix
5			3 h treatment and 17 h recovery with S9-mix
6		Experiment 2:	3 h treatment and 17 h recovery with S9-mix
7	Negative control:		Vehicle
8	Positive control:		NQO (without S9), CPA (with S9)
9	GLP:		yes
10	Date of report:		June 1999
11	Reference:		Submission DHS (14), II(67)

12  
13 The test substance was evaluated for potential cytogenetic effects in Chinese hamster ovary  
14 (CHO) cells in the absence or presence of S9-mix. The S9-mix was prepared from livers of  
15 rats treated with Arochlor 1254 (experiment 1) or phenobarbital/β-naphtoflavone  
16 (experiment 2). In the absence of S9-mix only one experiment was performed. Cytotoxicity  
17 was measured as a reduction in cell number compared to the solvent control. 4-  
18 nitroquilonine 1-oxide and cyclophosphamide were used as positive controls in the  
19 experiments without and with S9-mix respectively. For each culture cells with structural  
20 aberrations excluding gaps, and polyploidy, endoreduplication or hyperdiploidy were  
21 categorized.

22  
23 **Results**  
24 The number of cells with structural aberrations in the negative control cultures was within  
25 normal range. In the experiment without S9-mix, a slight but not statistically significant  
26 increase in the number of cells with chromosomal aberrations was observed. In the  
27 experiments with S9-mix no biologically relevant increase in the number of cells with  
28 chromosomal aberrations was observed. The positive controls NQO and CPA induced  
29 statistically significant increases in the number of cells with structural aberrations in the  
30 absence or presence of S9-mix, respectively.

31  
32 **Conclusion**  
33 Under the experimental conditions used T805 was not genotoxic (clastogenic) in this  
34 chromosome aberration test in mammalian cells.

35  
36 **SCCS Comment**  
37 The experiment in the absence of S9 mix was performed only once. A tendency of an  
38 increasing number of cells with structural aberrations was noted in the experiment without  
39 S9-mix.

40  
41  
42 **In vitro micronucleus test in human epidermal cells**  
43 Guideline/method: According to an generally accepted published protocol  
44 Test system: Human epidermal cell line, A431  
45 Replicates: 3 independent experiments  
46 Test item: TiO<sub>2</sub> NP (Anatase, 99.7%), commercial  
47 Batch: /  
48 Solvent: DMEM with 10% FBS  
49 Concentrations: 0.008, 0.08, 0.8, 8, 80 µg/ml  
50 Exposure: 6 h treatment without S9-mix, harvest time 24 h after the start of  
51 treatment  
52 Negative control: Vehicle  
53 Positive control: Ethyl methanesulfonate (6 mM)  
54 GLP: Not in compliance  
55 Published: Shukla *et al.*, 2011  
56 Reference: Submission SI-II, (205)  
57

1 The cytokinesis-block micronucleus (CBMN) assay was carried out to determine the  
2 potential genotoxicity of TiO<sub>2</sub> NP in the human epidermal cell line A431. The cells were  
3 treated for 6 h with different concentrations of TiO<sub>2</sub> NP (0, 0.008, 0.08, 0.8, 8, and 80  
4 µg/ml). Ethyl methanesulfonate was used as positive control. After the 6 h exposure, the  
5 NPs were removed by washing with medium and cells were grown for additional 18 h in  
6 fresh DMEM medium containing Cytochalasin-B (3 µg/ml medium). Cytospin preparations  
7 were examined for the presence of micronuclei in binucleate cells. From each concentration  
8 2000 binucleate cells were scored; the cytokinesis block proliferation index (CBPI) was  
9 calculated from 500 cells/concentration as recommended in OECD Guideline 487.  
10 Transmission electron microscopy (TEM) was used to evaluate uptake of the TiO<sub>2</sub> NP into  
11 the cells.

12  
13 **Results**  
14 CBPI was not significantly different from the control treatments. TEM analysis showed that  
15 NPs were taken up by the cells. The NPs were found to be distributed mostly in cytoplasm,  
16 some NP were also localised in the nucleus. A statistically significant induction in the  
17 number of cells with micronuclei was observed after 6 h exposure to TiO<sub>2</sub> NP.  
18 The particles were also found to induce oxidative stress in the cells indicated by a significant  
19 depletion of glutathione, induction of lipid peroxidation and reactive oxygen species  
20 generation.

21  
22 **Conclusion**  
23 Under the experimental conditions used TiO<sub>2</sub> NPs induced an increase in the number of cells  
24 with micronuclei and, consequently, TiO<sub>2</sub> NPs is genotoxic (clastogenic and/or aneugenic) in  
25 the human epidermal cell line A431.

26  
27  
28 **Fpg modified Comet assay in human epidermal cells**  
29 Guideline/method: According to an generally accepted published protocol  
30 Test system: Human epidermal cell line A431  
31 Replicates: 2 cultures  
32 Test item: TiO<sub>2</sub> NP (Anatase, 99.7%), commercial  
33 Batch: /  
34 Solvent: DMEM with 10% FBS  
35 Concentrations: 0.008, 0.08, 0.8, 8, 80 µg/ml  
36 Exposure: 6 h treatment  
37 Negative control: Vehicle  
38 Positive control: 25 µM hydrogen peroxide  
39 GLP: Not in compliance  
40 Published: Shukla et al., 2011  
41 Reference: Submission SI-II, (205)

42  
43 TiO<sub>2</sub> NP was assayed for DNA damage in the human epidermal cell line A431 with the Comet  
44 assay. The cells were treated for 6 hours with TiO<sub>2</sub> NP in a concentration range up to 80  
45 µg/ml. DNA damage was evaluated by formamidopyrimidine DNA glycosylase (fpg) modified  
46 Comet assay. The fpg allows for detection of oxidative DNA base damage lesions, in  
47 particular, 8-OH guanine. Hydrogen peroxide was included as a positive control and  
48 cytotoxicity was evaluated by MTT and NRU assay.

49  
50 **Results**  
51 The TiO<sub>2</sub> NP caused a significant concentration-dependent induction of DNA damage. Effects  
52 were statistically significant at the two highest testing concentrations. These concentrations  
53 were not cytotoxic after 6 or 24 h treatment in the MTT or NRU assay. Significant  
54 cytotoxicity for both concentrations was found in these assays after 48 h treatment. Uptake  
55 of NP into the A431 cells was shown by TEM analysis. Particles were observed mostly in the  
56 cytoplasm, but occasionally also in the nucleus. Oxidative stress in the cells was indicated

1 from the significant depletion of glutathione, induction of lipid peroxidation and reactive  
2 oxygen species generation.

#### 3 Conclusion

4 Under the experimental conditions used the results of the study indicate that TiO<sub>2</sub> NPs  
5 possess DNA damaging potential in human epidermal cells.

#### 6 Comet assay in human lymphocytes

7  
8  
9  
10 Guideline/method: According to an generally accepted published protocol for the alkaline  
11 Comet assay  
12 Test system: Human lymphocytes  
13 Replicates: triplicate culture in 2 independent experiments  
14 Test items: TiO<sub>2</sub> NP commercial, declared size of 100 nm and surface area of 14.0  
15 m<sup>2</sup>/g  
16 Batch: /  
17 Solvent: RPMI-1640  
18 Concentrations: 0, 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2 mM  
19 Exposure: 3 hour treatment  
20 GLP: Not in compliance  
21 Published: Ghosh et al., 2010  
22 Reference: Submission SI-II, (157)  
23

24 The DNA damaging potential of TiO<sub>2</sub> NP was evaluated using the Comet assay in human  
25 lymphocytes obtained by venipuncture from peripheral blood of healthy volunteers. Cells  
26 were isolated by gradient centrifugation using Histopaque and resuspended in RPMI-1640  
27 culture medium. Cells were treated for 3 hours with the TiO<sub>2</sub> NP at a concentration range of  
28 0 to 2mM. The Comet assay was performed according to published methods. DNA damage  
29 was reported as % tail DNA in treated lymphocytes. Slides were prepared in triplicates per  
30 concentration and each experiment was repeated twice. Viability was determined by trypan  
31 blue dye exclusion, MTT assay and WST-1 assay in the same concentration range as used  
32 for the Comet assay.

#### 33 Results

34 Trypan blue indicated viability above 80% at the highest treatment concentrations. MTT and  
35 WST-1 assay showed increased toxicity, with an LC50 in the range of 1.0 to 1.25 mM. A  
36 statistically significant increase in DNA damage was observed in lymphocytes treated with  
37 the TiO<sub>2</sub> NP at a concentration of 0.25 mM. No concentration dependent effect and no  
38 statistically significant effects were found at any of the other testing concentrations.  
39

#### 40 Conclusion:

41 Under the experimental conditions used, the results of the study indicate that TiO<sub>2</sub> NPs  
42 possess DNA damaging potential in human epidermal cells.  
43

#### 44 SCCS Comment

45 The authors of the paper conclude that TiO<sub>2</sub> NP were genotoxic to human lymphocytes.  
46 They propose that the absence of a dose-dependent effect on DNA damage may be due to  
47 the agglomeration behaviour of the nanoparticles.

48 SCCS concludes that in view of the absence of a dose-dependent effect, this study does not  
49 provide evidence for the genotoxicity of TiO<sub>2</sub> in human lymphocytes.  
50

#### 51 ***In vitro* mammalian cell gene mutation test**

52 Guideline/method: /  
53 Test system: *gpt* delta transgenic mouse primary embryo fibroblasts  
54  
55

---

1	Replicates:	/
2	Test items:	TiO <sub>2</sub> NP anatase (5 nm, 114m <sup>2</sup> /g), Sigma Aldrich,
3		TiO <sub>2</sub> NP anatase (40 nm, 38 m <sup>2</sup> /g), Inframat Advanced Materials LLC
4		Fine TiO <sub>2</sub> (325 mesh, 8.9m <sup>2</sup> /g), Sigma-Aldrich
5	Batch:	/
6	Solvent:	/
7	Concentrations:	0, 0.1, 1, 10 and 30 µg/ml
8	Exposure:	24 h treatment
9	Solvent:	Distilled water (sonicated and then further diluted in culture medium)
10	GLP:	not in compliance
11	Reference:	Xu <i>et al.</i> , 2009
12		

13 Mutant frequencies in *red/gam* loci by *Spi*- detection by two nano-sized and one fine TiO<sub>2</sub>  
 14 materials were evaluated in *gpt* delta transgenic mouse primary embryo fibroblasts. The  
 15 samples were suspended in distilled water, subsequently sonicated for 30 min, sonicated on  
 16 ice, and diluted in medium before addition to the cells. S9-mix was not included in the  
 17 assay.

#### 18 19 Results

20 Concurrent cytotoxicity of the samples was evaluated by the MTT assay and uptake of the  
 21 NP in the cells was assessed by flow-cytometry. Increased mutants frequencies were  
 22 observed with both the nanosize TiO<sub>2</sub> samples, but not with the fine TiO<sub>2</sub>, demonstrating  
 23 that these nanomaterials can cause kilo-base pair deletion mutations. These effects could be  
 24 abrogated by co-treatment of the endocytosis inhibitor (lipid raft/caveolae disrupting agent)  
 25 Nystatin, the nitric oxide synthase inhibitor, NG-methyl-L-arginine (L-NMMA) and the  
 26 cyclooxygenase-2 activity inhibitor NS-398.

#### 27 28 Conclusions

29 TiO<sub>2</sub> NP were taken up by the cells and induced kilo-base pair deletion mutations in a  
 30 transgenic mouse mutation system.

31 It was suggested that induction of [ONOO]-, triggered by the signalling events associated  
 32 with the transporting of nanoparticles into the cells, rather than the chemical  
 33 composition/surface area combination of the nanoparticles may be a critical event for the  
 34 observed genotoxicity.

#### 35 36 SCCS Comment

37 Translocation and contact of the test items with the fibroblast DNA has not been  
 38 demonstrated in the tests. The effects of the applied inhibitors suggest an indirect effect  
 39 mediated by TiO<sub>2</sub> triggered formation of reactive oxidants. Uptake cannot be verified by  
 40 flow-cytometry on the basis of side-scattering, as particles may merely be adhered to the  
 41 cell membranes.

42  
43

#### 44 **In vitro micronucleus test in mammalian cells**

45	Guideline/method:	Draft OECD 487
46	Test system:	V79 cells
47	Replicates:	Quadruplicate cultures
48	Test item:	T-Lite™ SF, pure rutile, primary particle size 10 x 50 nm, mean 49 agglomerates approximately 200 nm (d10: 90nm, d90: 460 nm); 50 coating consisting of aluminium hydroxide and dimethicone/methicone 51 copolymer
52	Batch:	/
53	Solvent:	FCS
54	Concentrations:	75, 150 and 300 µg/ml with 4 h exposure 55 18.8, 37.5 and 75 µg/ml with 24 h exposure
56	Exposure:	4 h treatment and harvest 24 h after start of the treatment 57 24 h treatment and harvest immediately after the end of treatment



1 Positive control: Ethyl methanesulfonate 500 µg/ml  
 2 GLP: not in compliance  
 3 Reference: Landsiedel *et al.*, 2010  
 4

5 Micronucleus formation was evaluated in V79 cells after treatment with TiO<sub>2</sub> rutile NP coated  
 6 with aluminium hydroxide and dimethicone/methicone copolymer (T-Lite™ SF rutile). The  
 7 study was performed without S9 mix which was considered scientifically justified because of  
 8 the nanoparticulate nature of the material. Cells were either treated for 4 hours with 75,  
 9 150 and 300 µg/ml, followed by 24 h recovery, or for 24 h at concentrations of 18.8, 37.5  
 10 and 75 µg/ml without recovery. The concentrations were selected on the basis of pilot  
 11 experiment on cytotoxicity. Ethyl methanesulfonate was used as positive control.  
 12

### 13 Results

14 The occurrence of precipitation at higher concentrations influenced the toxicity assessment.  
 15 Concurrent evaluation of cytotoxicity by analysis of proliferation index (PI) demonstrated  
 16 the absence of cytotoxicity up to highest scorable concentrations. A biologically relevant  
 17 increase in the number of cells with micronuclei was not observed after exposure to T-Lite™  
 18 SF.  
 19

### 20 Conclusions

21 Under the experimental conditions used T-Lite™ SF did not induce an increase in the  
 22 number of cells with micronuclei and, consequently, T-Lite™ SF is not genotoxic (clastogenic  
 23 and/or aneugenic) in V79 cells.  
 24

### 25 SCCS Comment

26 The test material relates to S75-K (94% rutile, coated with aluminium hydroxide and  
 27 dimethicone/methicone copolymer). Translocation and contact of the test material with the  
 28 V79 cells and its possible translocation into the nucleus and interaction with DNA has not  
 29 been demonstrated.  
 30  
 31

### 32 Alkaline Comet assay in mammalian lung cells

33 Test system: A549 human lung carcinoma cells  
 34 Replicates: Triplicate cultures  
 35 Test items: TiO<sub>2</sub> synthesized by laser pyrolysis (spherical, 12 nm, 92 m<sup>2</sup>/g, 95%  
 36 anatase, PZC (point of zero charge) = 6.4)  
 37 TiO<sub>2</sub> synthesized by laser pyrolysis (spherical, 21 nm, 73 m<sup>2</sup>/g, 90%  
 38 rutile)  
 39 TiO<sub>2</sub>-A25 AEROXIDE-P25 (spherical, 24 nm, 46 m<sup>2</sup>/g, 86% anatase, PZC  
 40 = 7.0) uncoated  
 41 TiO<sub>2</sub> ref. 637262 from Sigma-Aldrich (Elongated, L: 68 nm, d: 9nm, 118  
 42 m<sup>2</sup>/g, 100% rutile)  
 43 TiO<sub>2</sub> ref. T8141 from Sigma-Aldrich (spherical, 142 nm, 10 m<sup>2</sup>/g, 100%  
 44 anatase, PZC = 5.2)  
 45 Batch: /  
 46 Solvent: Ultrapure sterile water (pH5.5), suspended at 10 mg/ml, further diluted  
 47 in cell culture medium  
 48 Concentrations: 0 and 100 µg/ml  
 49 Exposure: 4 h, 24h and 48 h after start of the exposure  
 50 GLP: not in compliance  
 51 Reference: Jugan *et al.*, 2011  
 52

53 DNA damage of five different types of TiO<sub>2</sub> particles (which included AEROXIDE-P25) was  
 54 evaluated by alkaline Comet assay in A549 cells. No S9-mix was added to the test system.  
 55 Cells were treated with one concentration (100 µg/ml) for 4 h, 24 h and 48 h. Cytotoxicity  
 56 was evaluated by the MTT-assay. Electron microscopy was performed to evaluate uptake of  
 57 the test samples into the A549 cells after 4 h.

1  
2 **Results**  
3 Electron microscopic evaluation demonstrated a rapid uptake of the various test materials  
4 into the cytoplasm of the A549 cells. Samples were tested only at one concentration.  
5 Cytotoxicity, evaluated by MTT-test, revealed that cell death was less than 25% for all  
6 samples after 48 h of exposure.  
7 DNA damage was significantly increased with all samples at 4 h, with three out of the five  
8 samples at 24 h. After 48 h no significant increase was detected with the exception of one  
9 sample (i.e. laser pyrolysis synthesized rutile, 21 nm). The uncoated sample (AEROXIDE-  
10 P25) caused a significant increase in DNA single strand breakage at treatment times of 4  
11 and 24 h. For all smallest, including the uncoated sample cellular internalization and  
12 accumulation into cytoplasm was reported. For one sample (i.e. 12 nm laser pyrolysis  
13 synthesized), nanoparticles were found located in the nucleus.  
14 It was concluded that several types of TiO<sub>2</sub> can cause DNA single strand breaks. In parallel  
15 investigations, they also showed capacity of TiO<sub>2</sub> to cause formation of the oxidative DNA  
16 damage lesion 8-OHdG as well as an inhibition of DNA (base excision) repair activity. In  
17 contrast, they did not detect double strand breaks evaluated by  $\gamma$ H2AX  
18 immunohistochemistry or clastogenic/aneugenic effects evaluated by micronucleus assay in  
19 the same cells.

20  
21 **Conclusions**  
22 Under the experimental conditions used it was concluded that TiO<sub>2</sub> nanoparticles have a  
23 genotoxic potential in this alkaline Comet assay in mammalian lung cells.  
24

25

#### 26 **Alkaline Comet assay in mammalian liver cells**

27 Guideline/method: According to generally accepted and published protocols

28 Test system: Human hepatoblastoma cell line C3A

29 Replicates: Triplicate cultures

30 Test items: NM101 Anatase 9 nm (XRD), 4-8/50-100 nm; two different particle  
31 types (TEM), 322 m<sup>2</sup>/g

32 NRCWE001 Rutile 10 nm (XRD), 80-400 (TEM), 99 m<sup>2</sup>/g

33 NRCWE002 Rutile 10 nm (XRD), 80-400 (TEM), 84 m<sup>2</sup>/g, negative  
34 charged

35 NRCWE003 Rutile 10 nm (XRD), 80-400 (TEM), 84 m<sup>2</sup>/g, positive  
36 charged

37 NRCWE004 Rutile approx. 100 nm (XRD), 1-4/10-100/100-200/1000-  
38 2000; five different types of particles (TEM)

39 Batch: /

40 Solvent: Distilled water with FCS

41 Concentrations: Three concentrations, i.e. LC<sub>20</sub>, 1/2 of LC<sub>20</sub> and 2x LC<sub>20</sub>

42 Exposure: 4 h treatment

43 Positive controls: H<sub>2</sub>O<sub>2</sub>

44 GLP: not in compliance

45 Reference: Kermanizadeh *et al.*, 2012  
46

47 DNA damage in human hepatoblastoma C3A cell line was evaluated by the alkaline Comet  
48 assay (evaluated as % tail DNA), with inclusion of fpg enzyme to detect oxidative DNA  
49 damage. A total of five different types of TiO<sub>2</sub> were tested at a concentration that caused  
50 20% viability loss (LC<sub>20</sub>), as well as twice or half of this concentration. The toxicity was  
51 evaluated by WST-1 assay (24 h treatment), the treatment time for the Comet assay was 4  
52 hours. S9 mix was not included in the assays.  
53

#### 54 **Results**

55 Biologically relevant and small but statistically significant increases in DNA damage were  
56 found with several of the samples. The most pronounced effects were seen with NM101 and  
57 RWCE001. No biologically relevant increase in DNA damage was observed with the

1 negatively charged RCWE003. In view of the observed effects in the presence of fpg (as well  
2 as based on further analysis of oxidative stress markers in the study), the authors suggest  
3 that the DNA damage effects are mediated by reactive oxygen species (ROS).

#### 4 5 Conclusions

6 Under the experimental conditions used, it was concluded that short term exposure of liver  
7 cells to some TiO<sub>2</sub> particles caused small but significant increases in DNA damage.

#### 8 9 SCCS Comments

10 Translocation and contact of the test material with the hepatoblastoma cells and its' possible  
11 translocation into the nucleus and interaction with DNA have not been demonstrated. Some  
12 of the effects are minor but are concentration dependent, this might become significant at a  
13 certain exposure level.

14  
15 Further mutagenicity/genotoxicity in vitro studies (open literature):

16 The *in vitro* mutagenicity genotoxicity studies on TiO<sub>2</sub> nanomaterials have been recently  
17 reviewed by Magdolenova et al. (2013). In many of these studies, particle size (and  
18 chemistry) is not, or poorly specified in the publications. As such, these studies do not allow  
19 for evaluation of the potential effects of the nanosize aspect of the potential genotoxicity of  
20 TiO<sub>2</sub> (Le Boeuf et al., 1996; Endo-Capron et al., 1993; Pelin et al., 1995; Miller et al., 1995;  
21 Lu et al., 1998; Kamp et al., 1995; Dunford et al., 1997; Wamer et al., 1997). In several  
22 studies only fine TiO<sub>2</sub> was used (e.g. Driscoll et al., 1997; Van Maanen et al., 1999, in both  
23 these studies TiO<sub>2</sub> anatase 180nm with a BET value of 8.8 m<sup>2</sup>/g was used; Notably  
24 however, one may argue that this sample contains a particle distribution "tail" in the  
25 nanosize range).

26 Nagakawa *et al.* (1997) tested four TiO<sub>2</sub> samples, *i.e.* 21 nm and 255 nm anatase and 255  
27 nm and 420 nm rutile for DNA strand breaks by alkaline Comet assay in the mouse  
28 lymphoma cell clone L5178Y/*tk*<sup>+/-</sup>. In the presence of UV/light all samples showed enhanced  
29 DNA strand breaks at concentrations which also elicited cell death. Without irradiation only  
30 the 255 nm anatase showed enhanced strand breakage. The 21 nm anatase sample was  
31 also evaluated for the induction of chromosomal aberrations in the Chinese Hamster cell line  
32 CHL/IU, for mutagenicity in the *Salmonella typhimurium* strains TA100, TA98 and TA102,  
33 and colony formation in the L5178Y/*tk*<sup>+/-</sup> cells. Chromosomal aberrations (mainly polyploidy,  
34 chromatid breaks and chromatid exchanges) were found only in the presence of UV/visible  
35 light, and occurred at cytotoxic concentrations. In the absence of light the 21 nm anatase did  
36 not elicit chromosomal aberrations in contrast to the positive control (ofloxacin).  
37 Irrespective of UV/light irradiation, the 21 nm anatase failed to enhance the frequencies of  
38 revertant *Salmonella* colonies or mutant L5178Y colonies, in contrast to the positive control  
39 methyl methanesulfonate (MMS).

40  
41 Linnainmaa *et al.* (1997) investigated micronucleus formation in rat liver epithelial cells  
42 after treatment with various TiO<sub>2</sub> samples in the presence or absence of UV light. Mitomycin  
43 C was used as positive control. TiO<sub>2</sub> samples were a 170 nm and a 20 nm anatase sample,  
44 and a 20 nm coated rutile sample. The coated sample was prepared with aluminium  
45 hydroxide and stearic acid. The sample was ethanol washed to remove the stearic acid  
46 before treatment of the cells. In contrast to the positive control, none of the samples  
47 induced an increase in cells with micronuclei.

48  
49 Rahman *et al.* (2002) studied micronucleus formation in SHE fibroblasts after treatment  
50 with fine TiO<sub>2</sub> (>200nm) and nanosize TiO<sub>2</sub> (20nm). Apart from size, no further details of  
51 the samples were provided. Increased micronuclei were found only with the ultrafine TiO<sub>2</sub>.  
52 The authors reported (but did not show in the manuscript) that further kinetochore-staining  
53 experiments revealed indications for chromosomal non-disjunction during mitosis. The  
54 nanosize TiO<sub>2</sub> also elicited apoptosis shown by DNA fragmentation analysis and the  
55 appearance of apoptotic bodies (transmission electron microscopy evaluation).

56

1 Gurr *et al.* (2005) tested a variety of TiO<sub>2</sub> samples for micronucleus formation as well as the  
 2 induction of oxidative DNA damage using the Fpg-modified Comet assay in BEAS-2B human  
 3 bronchial epithelial cells. The samples used were four different anatase samples, with  
 4 respective sizes of 10, 20, 200 and >200 nm, and one rutile sample with the size of 200  
 5 nm. Micronucleus induction was found with the 10 and 200 nm anatase sample, but not  
 6 with the >200 nm anatase and the 200 nm rutile samples. For the 20 nm anatase sample  
 7 no data were provided. Enhanced oxidative DNA damage (fpg-Comet assay) was observed  
 8 with the 10 and 20 nm anatase samples and with the 200 nm rutile. All other samples were  
 9 negative. Finally, the authors showed that a 1:1 mixture of 200 nm anatase and 200 nm  
 10 rutile caused stronger oxidative DNA damage than the 200 nm anatase or 200 nm rutile  
 11 alone.

12  
 13 Bhattacharya *et al.* (2009) investigated the genotoxicity of anatase TiO<sub>2</sub> in BEAS-2B human  
 14 bronchial epithelial cells and IMR-90 human lung fibroblasts. The TiO<sub>2</sub> nanoparticles caused  
 15 induction of the oxidative DNA adduct 8-OHdG in IMR-90 cells (measured by an ELISA  
 16 method), but did not cause increased strand breaks (measured by Comet assay) in the IMR-  
 17 90 and BEAS-2B cells. Electron microscopy demonstrated that both particles translocated  
 18 near to nucleus, but were not found inside the nucleus, mitochondria or ribosomes.

19  
 20 Falck *et al.* (2009) investigated the genotoxicity of three TiO<sub>2</sub> samples in BEAS-2B human  
 21 bronchial epithelial cells by the alkaline Comet assay and the micronucleus test. The  
 22 samples were a nanosize rutile sample coated with <5 SiO<sub>2</sub> (10x40nm needle shaped, BET  
 23 132 m<sup>2</sup>/g), a fine rutile sample (<5 µm, 2 m<sup>2</sup>/g), and a nanosize anatase sample (<25 nm,  
 24 222 m<sup>2</sup>/g). Hydrogen peroxide and mitomycin-C were used as respective positive controls.  
 25 All samples showed mild but significant DNA damaging effects. The effects of the nanosize  
 26 rutile were much weaker than those of the nanosize anatase and fine rutile sample. The  
 27 nanosize anatase, in contrast to both other samples, also caused increased micronuclei. For  
 28 the observed DNA damaging and micronucleus effects mostly no clear dose-dependency  
 29 could be observed. It was also reported that the micronucleus scoring was difficult due to  
 30 the presence of the particles during microscopy.

31  
 32 Magdolenova *et al.* (2012a) showed in human TK6, EUE and Cos-1 cells that genotoxicity of  
 33 TiO<sub>2</sub> (DNA damage and oxidised DNA lesions) measured by the Comet assay (with and  
 34 without fpg) depends on the stock dispersion protocol. The same TiO<sub>2</sub> (Aeroxide P25,  
 35 primary particle size 21 nm, mixture of anatase /rutile), but prepared with different stock  
 36 dispersion protocol, following further with the same media and exposure conditions resulted  
 37 in differed state of agglomeration and gave different results. Larger agglomerates gave  
 38 positive results. Thus differences in stock dispersion preparation could explain contradictory  
 39 results published on the same nanoparticles. Magdolenova *et al.* (2012b) studied the  
 40 possible interference of TiO<sub>2</sub> and other nanoparticles with the fpg enzyme in the Comet  
 41 assay but did not find this to cause any artefacts.

42

#### 43 1.5.6.2 Mutagenicity/Genotoxicity in vivo

44

#### 45 **Open literature studies**

46

#### 47 **Micronuclei in peripheral blood erythrocytes after oral uptake**

48 Guideline/method: /

49 Species/strain: C57Bl/6Jp<sub>un</sub>/p<sub>un</sub> mice.

50 Group size: 5 mice/treatment group

51 Test substance: Aeroxide P25, Degussa/Evonik, primary particle size 21 nm, BET surface  
52 area 50 m<sup>2</sup>/g, DLS in water: 21-1446 nm)

53 Batch: /

54 Vehicle: water

55 Dose levels: 0, 50, 100, 250, and 500 mg/kg bw (estimated dose)

56 Treatment: /

1 GLP: not in compliance  
2 Reference: Trouiller et al., 2009

#### 3 4 Methods

5 C57Bl/6Jp<sub>un</sub>/p<sub>un</sub> mice, containing naturally occurring 70-kb internal duplication in the *pink-*  
6 *eyed dilution* (p) gene, were exposed via drinking water to the TiO<sub>2</sub> NP. The suspensions  
7 were ultrasonicated for 15 min before providing to animals. Water (with/without the NP)  
8 was provided *ad libitum* during 5 days. Peripheral blood was collected and erythrocytes  
9 were evaluated for the presence of micronuclei. The estimated exposures were 0, 50, 100,  
10 250 and 500 mg/kg bw. The doses were estimated on the basis of estimated drinking water  
11 consumption (set at 5 ml) and the average weight of the animals. The authors also  
12 evaluated DNA damage, measured as 8-hydroxy-2'-deoxyguanosine in liver tissue by  
13 HPLC/ECD analysis, and alkaline Comet assay in blood cells, but these were tested only at  
14 one concentration (500 mg/kg bw). Moreover, DNA deletions were evaluated in the  
15 offspring of pregnant C57Bl/6Jp<sub>un</sub>/p<sub>un</sub> mice treated for 10 days at 500 mg/kg bw/day, to  
16 evaluate in utero effects.

#### 17 18 Results

19 A biologically relevant increase in the number of peripheral blood erythrocytes after oral  
20 administration of TiO<sub>2</sub> NP was found in mice at the highest treatment dose only (500  
21 mg/kg). This concentration also caused increased DNA strand breakage in white blood cells  
22 (Comet assay),  $\gamma$ -H2AX foci in bone marrow cells, and 8-hydroxy-2'-deoxyguanosine  
23 formation in liver cells. A 10-day exposure in pregnant mice also led to DNA deletions in  
24 offspring. The TiO<sub>2</sub> NP exposure also caused a mild but statistically significant increase in  
25 systemic inflammation, as shown by qRT-PCR analysis of the mRNA expression of  
26 proinflammatory genes (*TNFalpha*, *IFNgamma*, *KC/IL-8*) in peripheral blood. It was  
27 concluded that oral TiO<sub>2</sub> NP exposure causes genotoxicity in mice, possibly caused by a  
28 secondary genotoxic mechanism associated with inflammation and/or oxidative stress.

#### 29 30 Conclusions:

31 Under the experimental conditions used, Aeroxide P25 was genotoxic (clastogenic and/or  
32 aneugenic) in human lymphocytes *in vitro*.

#### 33 34 SCCS Comments

35 The test material relates to S75-G (anatase/rutile, not coated). However, the description of  
36 the test material given in the paper suggests a different proportion of anatase and rutile  
37 (75%:25%) than the proportion specified for S75-G. Data indicate genotoxic effects of TiO<sub>2</sub>  
38 NP after oral exposure in mice in organs/tissues other than those that are in direct contact  
39 via the exposure route (*i.e.* effects in blood, bone marrow, liver and foetuses). Insufficient  
40 details have been provided in the article regarding methodology. This makes the findings of  
41 the study of limited value to this risk assessment.

42 Further limitations of the study are:

- 43 - The work does not contain biokinetics, *i.e.* dosimetry cannot be accurately  
44 determined. Actual intake of the NP is not measured, only indirect by calculation of  
45 the amount of drinking water. Translocation of particles and accumulation in different  
46 organs was also not determined.
- 47 - Potential local effects (histopathology, genotoxicity assays) in gastrointestinal tract  
48 target cells are not provided, and thus do not allow for assessment of potential  
49 effects on epithelial barrier integrity, inflammation and local mutagenicity.
- 50 - The effects were observed at a rather high dose (calculated cumulative oral dose of  
51 500 mg/kg). The authors do not report whether these concentrations affect intestinal  
52 physiology. The high surface burden of TiO<sub>2</sub> NP in the G.I. tract may have significant  
53 impact on the adsorption and transport of nutrients.

#### 54 55 56 **DNA double strand breakage in bone marrow cells after oral uptake**

57 Guideline/method: According to published protocols



1	Species/strain:	C57Bl/6Jp <sub>un</sub> /p <sub>un</sub> mice.
2	Group size:	5 / treatment group
3	Test substance:	Aeroxide P25, Degussa/Evonik, primary particle size 21nm, BET surface area 50m <sup>2</sup> /g, DLS in water: 21-1446nm)
4		
5	Batch:	/
6	Vehicle:	water
7	Dose levels:	0, 50, 100, 250, and 500 mg/kg bw (estimated dose)
8	Treatment:	/
9	GLP:	not in compliance
10	Reference:	Trouiller et al., 2009

## 11 12 Methods

13 DNA double strand breaks were analysed by immunohistochemical detection of  $\gamma$ -H2AX foci  
14 in C57Bl/6Jp<sub>un</sub>/p<sub>un</sub> mice exposed to TiO<sub>2</sub> NP via drinking water. Bone marrow smears were  
15 analysed after 5 exposure days for  $\gamma$ -H2AX foci, at estimated exposure of the mice to 0, 50,  
16 100, 250, and 500 mg/kg bw TiO<sub>2</sub> NP.

## 17 18 Results

19 Oral TiO<sub>2</sub> NP caused increased  $\gamma$ -H2AX foci in a clear dose dependent manner, being  
20 significant from the lowest dose (50 mg/kg bw) onwards. DNA double-strand break was  
21 considered the most sensitive parameter among a variety of genotoxicity endpoints. It was  
22 therefore concluded that oral TiO<sub>2</sub> NP exposure causes DNA double strand breaks in bone  
23 marrow of the mice and suggest that this may be caused by a secondary genotoxic  
24 mechanism associated with inflammation and/or oxidative stress.

25 The TiO<sub>2</sub> NP exposure also caused mild but significantly increased systemic inflammation, as  
26 shown by qRT-PCR analysis of the mRNA expression of proinflammatory genes (TNF $\alpha$ ,  
27 IFN $\gamma$ , KC/IL-8) in peripheral blood.

## 28 29 Conclusions:

30 Under the experimental conditions used, Aeroxide P25 was genotoxic rats causing DNA  
31 double strand breaks in bone marrow cells.

## 32 33 SCCS Comments

34  
35 The test material relates to S75-G (anatase/rutile, not coated). Marked dose dependent  
36 effects are observed, suggesting that the bone marrow may be a sensitive target for TiO<sub>2</sub>  
37 nanoparticle (after oral uptake). Whether the nanoparticles actually reached this target is  
38 not shown in the study. The effects were observed at high concentrations. Other limitations  
39 of the study are:

- 40 - The work does not contain biokinetics, *i.e.* dosimetry cannot be accurately  
41 determined. Actual intake of the NP is not measured, only indirect by calculation of  
42 the amount of drinking water. Translocation of particles and accumulation in different  
43 organs was also not determined.
- 44 - Potential local effects (histopathology, genotoxicity assays) in gastrointestinal tract  
45 target cells are not provided, and thus do not allow for assessment potential effects  
46 on epithelial barrier integrity, inflammation and local mutagenicity.
- 47 - The effects were observed at a rather high dose (calculated cumulative oral dose of  
48 500 mg/kg bw). The authors have not reported whether these concentrations affect  
49 intestinal physiology. The high surface burden of TiO<sub>2</sub> NP in the G.I. tract may have  
50 significant impact on the adsorption and transport of nutrients.

## 51 52 53 **Comet assay *in vivo* in rat lungs (five day inhalation study)**

54 Guideline/method: According to generally accepted and published protocols  
55 Species/strain: Male Wistar Crl:W1 Han rats  
56 Group size: 3 animals per group



1 Test substance: T-Lite™ SF, pure rutile, primary particle size 10 x 50 nm, mean  
2 agglomerates approximately 200 nm (d10: 90 nm, d90: 460 nm);  
3 coating consisting of aluminium hydroxide and dimethicone/methicone  
4 copolymer  
5 Batch: /  
6 Vehicle: /  
7 Dose levels: 0 and 10 mg/m<sup>3</sup>/treatment/day  
8 Treatment: 6 h/day for 5 consecutive days  
9 GLP: not in compliance  
10 Reference: Landsiedel et al., 2010

11  
12 Rats were exposed by inhalation (head-nose exposure) for 6 hours on five consecutive days  
13 to 0 or 10 mg/m<sup>3</sup>/treatment/day. DNA damage was evaluated by alkaline Comet assay in  
14 the rat lung cells (isolated by *in situ* perfusion) from three animals per group. Viability of  
15 the isolated cells was determined by trypan blue dye exclusion. Further parameters  
16 evaluated included body weight, and bronchoalveolar lavage levels of LDH and ALP.

17  
18 Results  
19 The treated animals showed significantly increased LDH and ALP concentrations in BAL.  
20 Average viability of the cells isolated for the Comet assay were 95% and 88.7% respectively  
21 for air and TiO<sub>2</sub> exposed animals. A biologically relevant increase in DNA damage was not  
22 detected by the Comet assay.

23  
24 Conclusion  
25 Under the experimental conditions used it was concluded that T-Lite™ SF has a genotoxic  
26 potential in this alkaline Comet assay in lung cells.

27  
28 SCCS Comment  
29 The test material relates to S75-K (94% rutile, coated with aluminium hydroxide and  
30 dimethicone/methicone copolymer). The applied method is not yet validated, but  
31 represents tissue that at least in part is directly exposed to the testing material. The  
32 isolation procedure may have affected the background damage in the cells from the  
33 animals.

34  
35  
36 **Further mutagenicity/genotoxicity studies *in vivo* (open literature)**  
37 In specific animal studies no information is provided on the size of the particles used, or  
38 only non-ultrafine samples were used for effects of nano-sized TiO<sub>2</sub> (Shelby, 1993; Driscoll  
39 *et al.*, 1997).

40  
41 Rehn *et al.* (2003) investigated oxidative DNA damage induction by two samples of TiO<sub>2</sub> in  
42 rat lungs after intratracheal instillation at the dosages of 0, 0.15, 0.3, 0.6 and 1.2 mg/kg  
43 bw/day. The samples used were an untreated TiO<sub>2</sub> and a trimethoxyoctylsilane-treated TiO<sub>2</sub>  
44 sample, both approximately 20 nm. DQ12 crystalline silica was used as a positive control at  
45 0.6 mg/kg. Oxidative damage induction was determined after 90 days by  
46 immunohistochemical analysis of lung sections using an 8-oxoguanine antibody. Enhanced  
47 oxidative DNA damage was not observed with the untreated or silanised TiO<sub>2</sub> nanoparticles,  
48 in contrast to the DQ12 crystalline silica. Analysis of markers of pulmonary inflammation  
49 and toxicity at 3, 21, and 90 days indicated a strong progressing inflammation with DQ12  
50 crystalline silica, whereas for both TiO<sub>2</sub> samples only mild inflammatory effects were  
51 noticed. Proliferation in lung tissue, as determined using Ki-67 staining, showed only minor  
52 differences between control and TiO<sub>2</sub> treated rats in contrast to DQ12 treated rats which  
53 showed strong increase in % Ki-67 positive cells after 90 days. The contrasting observations  
54 with regard to oxidative DNA damage induction and proliferation were considered to be due  
55 to the marked contrasts in severity and persistence of pulmonary inflammation.

56

1 Similar to these observations, Driscoll *et al.* (1997) have demonstrated the likely role of  
 2 pulmonary inflammation in driving mutagenesis in rat lungs after *in vivo* instillation of  
 3 different particles. These included a fine crystalline silica sample, a nano-sized carbon black  
 4 sample and a fine anatase TiO<sub>2</sub> sample (180 nm median diameter, 8.8 m<sup>2</sup>/g). Mutagenicity  
 5 was studied by *hprt*-analysis of lung epithelial cells isolated from the lungs of female SPF  
 6 F334 Fischer rats, 15 months after intratracheal instillation of each of the particles at 10  
 7 mg/kg or 100 mg/kg. For the fine TiO<sub>2</sub> sample, enhanced *hprt*-mutagenesis was observed  
 8 with 100 mg/kg, the dose which also elicited persistent lung inflammation, but not with the  
 9 10 mg/kg dose. Similar for the other particles used (carbon black, silica) *in vivo*  
 10 mutagenicity was only observed at doses that also caused persistent inflammation. The  
 11 inflammatory cells obtained by bronchoalveolar lavage from the particle-treated animals  
 12 were found to induce *hprt*-mutagenesis in a rat lung epithelia cell line *in vitro*.

13  
14

### 15 **SCCS Comments on Mutagenicity/Genotoxicity**

16 From the studies discussed above, the potential to cause DNA damage has been clearly  
 17 demonstrated for some TiO<sub>2</sub> nanomaterials. However, it is not clear how this relates to the  
 18 other nanomaterials presented in the submission.

19

## 20 **1.5.7 Carcinogenicity**

21

### 22 **Two stage skin painting carcinogenicity studies**

23

24	<i>Study Design:</i>	Two stage mouse skin carcinogenicity (Initiator: DMBA)
25	Date of publication:	Available online 30 November 2010.
26	Guideline/method:	Two stage mouse skin carcinogenicity test. Coated and uncoated titanium dioxide nanoparticles were used as promoter with 7,12-dimethylbenz[a]anthracene (DMBA) as initiator.
27		
28	Test system:	CD1 (ICR) female mice.
29	Test substance:	Industrial material-grades of coated (alumina and stearic acid) titanium dioxide nanoparticles (CTDN, titanium dioxide content: 79.2%, spindle shape, long axis of 50–100 nm, short axis of 10–20 nm) and uncoated titanium dioxide nanoparticles (UCTDN, titanium dioxide content: 96.0%, spindle shape, long axis of 50–100 nm, short axis of 10–20 nm) from Ishihara Sangyo Kaisha, Ltd., Osaka, Japan.
30		
31	Batch:	No data
32	Concentrations:	CTDN and UCTDN dispersed in Pentalan 408 (pentaerythrityl tetraethylhexanoate) at concentrations of 5 mg/0.1 g, 10 mg/0.1 g and 20 mg/0.1 g on ultra sonic cleaner.
33		
34	Exposure:	Twice weekly for 19 weeks
35	Solvent:	Pentalan 408 (pentaerythrityl tetraethylhexanoate)
36	Negative control:	Solvent
37	Positive Controls:	12-o-tetradecanoylphorbol 13-acetate (TPA)
38	GLP:	No
39	Reference:	Furukawa <i>et al.</i> , 2011

40

41 This study was conducted to examine the promoter potential of coated and uncoated  
 42 titanium dioxide nanoparticles (CTDN and UCTDN) in a two-stage mouse skin carcinogenesis  
 43 model using 7 week old CD1 (ICR) female mice. Initiation treatment: 0.1 ml (0.1 mg) DMBA  
 44 or vehicle alone was applied to furclipped back skin one time, using a micropipetter with  
 45 disposable tips. Starting 1 week after the initiation treatment, aliquots of 5, 10 and 20 mg  
 46 of CTDN or UCTDN in 0.1–0.09 ml of Pentalan were applied using a disposable syringe and  
 47 glass spreader daily, or 0.2 ml (4 µg) of TPA were applied using a micropipetter twice  
 48 weekly for 19 weeks to the animals as post-initiation treatments. TPA was used as a

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54

1 positive control promoter. Pentalan 408 served as a vehicle control as well as negative  
2 control.

3  
4 No changes in survival rate, general condition and body weight related to the test materials  
5 were observed. On macroscopic observation, 1–2 nodules/group on the skin were observed  
6 in each group applied CTDN and UCTDN as well as the control group after DMBA initiation.  
7 The nodules were histopathologically diagnosed as squamous cell hyperplasia, sebaceous  
8 gland hyperplasia, squamous cell papilloma and keratoacanthoma. While in CTDN and  
9 UCTDN experiments enlargement of the mandibular, pancreatic, lumbar region and  
10 inguinofemoral lymph nodes, spleen and thymus was observed in mice given 5 and 10 mg  
11 but not 20 mg, the lack of dose-dependence suggests no biological significance.

12  
13 The study authors concluded that CTDN and UCTDN applied as promoter at doses of up to  
14 20 mg/mouse did not increase the development of nodules. There were no significant  
15 differences between the number of nodules in the negative control (no initiator) and the  
16 experiments with TiO<sub>2</sub> as promoter. In the positive control, DMBA as initiator and TPA as  
17 promoter, 100% of the animals developed nodules. The authors concluded that titanium  
18 dioxide nanoparticles do not possess promoter activity for mouse skin carcinogenesis.

#### 19 20 **SCCS Comment**

21 The test material used in this study might be comparable to one type of materials included  
22 in this dossier. It was a good experiment with a procedure that is generally accepted for  
23 studying initiation and promoter activity. SCCS agree that under the experimental  
24 conditions uncoated and alumina- and stearic acid- coated nano TiO<sub>2</sub> do not show any  
25 carcinogenic promoter activity.

26  
27 *Study Design:* Two stage mouse skin carcinogenicity  
28 *Date of publication:* Published 2012  
29 *Guideline/method:* Two stage mouse skin carcinogenicity test. Coated and uncoated  
30 titanium dioxide nanoparticles were used as promoter with 7,12-  
31 dimethylbenz[a]anthracene (DMBA) as initiator.  
32 *Test system:* Female rash2 mice and their wild-type counterparts CB6F1 mice and  
33 CD1 mice.  
34 *Test substance:* sTiO<sub>2</sub> particles (rutile type, silicone coated, mean particulate diameter  
35 35 nm) and ncTiO<sub>2</sub> rutile type mean particulate diameter 20 nm)  
36 were provided by Japan Cosmetics Association, Tokyo.  
37 *Batch:* No data  
38 *Concentrations:* 0, 50 and 100 mg/ml  
39 *Exposure:* sTiO<sub>2</sub> rash2 mice 5 times a week for 8 weeks, CB6F1 mice 5 times a  
40 week for 40 weeks.  
41 ncTiO<sub>2</sub> CD1 mice 2 times a week for 52 weeks  
42 *Solvent:* sTiO<sub>2</sub> silicon oil, ncTiO<sub>2</sub> Pentalan 408 (pentaerythryl  
43 tetraethylhexanoate)  
44 *Negative control:* Solvent  
45 *Positive Controls:* sTiO<sub>2</sub> no positive control, ncTiO<sub>2</sub>, 12-o-tetradecanoylphorbol 13-  
46 acetate (TPA)  
47 *GLP:* No  
48 *Reference:* Sagawa et al., 2012

49  
50 TEM analysis showed that the shape of sTiO<sub>2</sub> particles was generally round to oval while  
51 ncTiO<sub>2</sub> particles were more club shaped. The mean length of sTiO<sub>2</sub> particles suspended in  
52 silicone was 0.28±0.22 µm. The mean length of ncTiO<sub>2</sub> particles suspended in Pentalan 408  
53 was 4.97±0.50 µm.

#### 54 55 sTiO<sub>2</sub> nano particles

56 The skin on the backs of 7-week old female rash2 mice and wild type CB6F1 mice was  
57 shaved and the animals received a single topical application of 0.1 ml DMBA (0.2 mg). Two

1 weeks later the animals were divided into 3 groups. Group 1 (control, only initiation than vehicle) (15 mice of each strain) were painted with 0.2 ml silicone oil. Group 2 (15 mice of each strain) were painted with 0.2 ml of 50 mg/ml sTiO<sub>2</sub> suspended in silicone oil. Group 3 (15 mice of each strain) were painted with 0.2 ml of 100 mg/ml sTiO<sub>2</sub> suspended in silicone oil. Group 4 (control, no initiation)(15 mice of each strain) were painted with 0.2 ml of 100 mg/ml sTiO<sub>2</sub> suspended in silicone oil without prior DMBA treatment. The mice were painted 5 times a week. The rasH2 mice were killed after 8 weeks and the wild-type CB6F1 mice after 40 weeks.

#### 9 rasH2 mice

10 The incidence of squamous cell papillomas was 100% in all groups (Group 1 – 3) of rasH2 mice treated with DMBA. No skin tumours were found in the group (Group 4) which was only treated with sTiO<sub>2</sub>. The incidence of squamous cell carcinomas was 33% in Group 1 (only DMBA and silicone oil), 60% in Group 2 (DMBA + 10 mg TiO<sub>2</sub>), and 53% in Group 3 (DMBA + 20 mg TiO<sub>2</sub>). The difference in carcinomas was not significant. No difference was found in the multiplicity of tumours.

#### 17 CB6F1 mice

18 The incidence of squamous cell papillomas was 7% (1 mouse) in Group 1 (only DMBA and silicone oil) and 13% (2 mice) in Group 2 and 3 (DMBA + 10 and 20 mg TiO<sub>2</sub>). No skin tumours were found in the group (Group 4) which was only treated with sTiO<sub>2</sub>. The incidence of squamous cell carcinomas was 7% (1 mouse in Group 1 (only DMBA and silicone oil)). No squamous cell carcinomas were found in any of the other groups.

#### 24 ncTiO<sub>2</sub> nano particles

25 The skin on the backs of 10-week old female CD1 mice was shaved and the animals received a single topical application of 0.1 ml DMBA (0.2 mg). Two weeks later the animals were divided into 4 groups. Group 1 (control, only initiation than vehicle) (16 mice) were painted with 0.2 ml Pentalan 408. Group 2 (16 mice) were painted with 0.2 ml of 50 mg/ml ncTiO<sub>2</sub> suspended in Pentalan 408. Group 3 (15 mice) were painted with 0.2 ml of 100 mg/ml ncTiO<sub>2</sub> suspended in Pentalan 408. Group 4 (positive control)(15 mice) were painted with 0.2 ml of TPA 200 nmol/ml in acetone. Groups 1 – 3 were painted 2 times a week and killed after 52 weeks. Group 4 was painted 4 times a week and killed after 40 weeks.

#### 34 CD1 mice

35 The incidence of squamous cell papillomas was 19% (3 mice) in Group 1 (only DMBA and silicone oil), 6% (1 mice) in Group 2 (DMBA + 10 mg TiO<sub>2</sub>) and 13% (2 mice) in Group 3 (DMBA + 20 mg TiO<sub>2</sub>). None of the mice in Groups 1 – 3 had developed squamous cell carcinomas. In the positive control (DMBA + TPA), 87% (13 mice) had developed squamous cell papillomas and 13% (2 mice) had squamous cell carcinomas.

#### 41 **SCCS Comment**

42 The results indicate that ncTiO<sub>2</sub> does not promote skin tumours in mice. With sTiO<sub>2</sub> an increase in the number of tumours was found among mice initiated with DMBA. The increase was not significant and no conclusion can be drawn.

#### 46 ***Two stage rat skin carcinogenicity***

##### 47 *Study Design*

48 Date of publication: Published 2012

49 Guideline/method: Two stage rat skin carcinogenicity test. Uncoated titanium dioxide nanoparticles (ncTiO<sub>2</sub>) was used as promoter with 7,12-dimethylbenz[a]anthracene (DMBA) as initiator.

50 Test system: Male Hras128 rats and their wild-type counterparts SD rats.

51 Test substance: ncTiO<sub>2</sub> rutile type mean particulate diameter 20 nm) were provided by Japan Cosmetics Association, Tokyo.

1	Batch:	No data
2	Concentrations:	0, 100 and 200 mg/ml
3	Exposure:	ncTiO <sub>2</sub> Hras128 rats 2 times a week for 28 weeks and SD rats 2 times
4		a week for 40 weeks.
5	Solvent:	Pentalan 408 (pentaerythrityl tetraethylhexanoate)
6	Negative control:	Solvent
7	Positive Controls:	None
8	GLP:	No
9	Reference:	Sagawa et al., 2012

10  
11 TEM analysis showed that the shape ncTiO<sub>2</sub> particles were clubbed shaped. The mean length  
12 of the ncTiO<sub>2</sub> particles suspended in Pentalan 408 was 4.97±0.50 µm.

#### 13 14 ncTiO<sub>2</sub> nano particles

15 The skin on the backs of 10-week old male Hras128 rats and wild type SD rats was shaved  
16 and the animals received a single topical application of 0.5 ml DMBA (2.5 mg). Two weeks  
17 later the animals were divided into 3 groups. Group 1 (control, only initiation than vehicle)  
18 (17 Hras128 rats and 12 SD rats) was painted with 0.5 ml Pentalan 408. Group 2 (16  
19 Hras128 rats and 12 SD rats) was painted with 0.5 ml (50 mg) ncTiO<sub>2</sub> suspended in  
20 Pentalan 408. Group 3 (17 Hras128 rats and 12 SD rats) was painted with 0.5 ml (100 mg)  
21 ncTiO<sub>2</sub> suspended in Pentalan 408. The rats were painted twice a week. The Hras128 rats  
22 were killed after 28 weeks and the SD rats after painting for 40 weeks.

#### 23 24 Hras128 rats

25 The incidence of squamous cell papillomas was 94% (16 rats) in Group 1 (only DMBA and  
26 Pentalan 408), 88% (14 rats) in Group 2 (DMBA + 50 mg TiO<sub>2</sub>) and 94% (16 rats) in Group  
27 3 (DMBA + 100 mg TiO<sub>2</sub>). None of the rats Groups 1 had developed squamous cell  
28 carcinomas, while 13% (2 rats) in both Group 2 and Group 3 had developed squamous cell  
29 carcinomas.

#### 30 31 SD rats

32 The incidence of squamous cell papillomas was 25% (3 rats) in Group 1 (only DMBA and  
33 Pentalan 408), 17% (2 rats) in Group 2 (DMBA + 50 mg TiO<sub>2</sub>) and 8% (1 rat) in Group 3  
34 (DMBA + 100 mg TiO<sub>2</sub>). None of the rats in Groups 1 and 3 had developed squamous cell  
35 carcinomas, while 17% (2 rats) in both Group 2 had developed squamous cell carcinomas.

#### 36 37 **SCCS Comment**

38 This rat model is less developed than the mouse two-stage carcinogenicity model. Since  
39 94% of the Hras rats treated with DMBA only developed tumours, the model is not adequate  
40 and no conclusion can be drawn from the study.

41		
42	<i>Study Design:</i>	Two stage rat skin carcinogenicity (Initiator: UV-B irradiation)
43	Date of publication:	2011
44	Guideline/method:	Exploratory Dermal UV-B initiated skin carcinogenesis promotion
45		study.
46	Test system:	Rat/Sprague-Dawley (wild-type and transgenic Hras128). 10 weeks
47		old
48	Group size:	5 – 8 male and 5 – 8 female per group.
49	Test substance:	TiO <sub>2</sub> NP (uncoated, rutile type, R, PPS: 20 nm, Ishihara Sangyo
50		Kaisha, Japan)
51	Batch:	No data
52	Concentrations:	0, 100 mg/ml per rat (0.5 ml on 9 cm <sup>2</sup> )
53	Route:	Topical application
54	Exposure:	42 weeks with/without pre-irradiation with UV-B for 10 weeks
55	Source of UV-light:	UV-B radiation unit, Dermaray 100, Eisai-Toshiba, Tokyo, Japan
56	Irradiation: UV-B:	800 mJ/cm <sup>2</sup> P, 2x/week for 10 weeks
57	Solvent:	Pentalan 408 (pentaerythrityl tetraethylhexanoate)



1 Negative control: Solvent  
 2 Positive Controls: None  
 3 GLP: No  
 4 Reference: Xu *et al.*, 2011  
 5

6 The potential of TiO<sub>2</sub> NPs (uncoated, R, PPS: 20 nm) to promote skin tumours after dermal  
 7 application after UV-B irradiation was studied in transgenic rats carrying the human c-Ha-  
 8 ras proto-oncogene (Hras128 rats), known to be sensitive to chemically induced skin  
 9 carcinogenesis in males and mammary carcinogenesis in females, and their wild-type  
 10 counterparts. A total of 80 Hras128 rats and their wild-type siblings were investigated.  
 11

12 The size of TiO<sub>2</sub> particles suspended in Pentalan 408 ranged from 10 nm to 300 µm (mean  
 13 size of 5.0 µm, median size of 4.6 µm) indicating that a large majority of the particles  
 14 formed aggregates in the Pentalan 408 suspension.  
 15

16 Group 1 (initiation and promotion) received ultraviolet B (UV-B) radiation (UV-B radiation  
 17 unit, Dermaray 100, Eisai-Toshiba, Tokyo, Japan) 2 times per week for 10 weeks at 800  
 18 mJ/cm<sup>2</sup>, on the shaved target skin, followed by painting with 0.5 ml of TiO<sub>2</sub> suspended in  
 19 Pentalan 408 at 100 mg/ml on the shaved (9 cm<sup>2</sup>) area twice a week until sacrifice. Group  
 20 2 (negative control, initiation + vehicle) received UV-B radiation and painting with the  
 21 vehicle Pentalan 408 on the shaved area twice a week until sacrifice, and Group 3 (no  
 22 initiation, only TiO<sub>2</sub> as promoter) received painting with 0.5 ml of TiO<sub>2</sub> suspension as in  
 23 Group1 but without prior UV-B radiation.  
 24

25 Any grossly visible papilloma lesions were carefully examined every day. All the animals  
 26 were sacrificed at week 52 (after 42 weeks painting) except for the female Hras128 rats,  
 27 which were terminated at week 16 (after 6 weeks painting) due to early mammary tumour  
 28 development. The skin, brain, lung, liver, mammary gland, mesenteric lymph nodes, spleen  
 29 and kidney, were excised, fixed and processed for light microscopic examination.  
 30

31 In male Hras128 rats, papillomas on the back skin developed from week 32 and the  
 32 incidence of skin papillomas was 12.5% (1/8) in Groups 1 and Group 3. No skin tumours  
 33 were observed on the targeted back skin in female Hras128 rats or wild-type rats of either  
 34 sex. Eye lid squamous cell papillomas were found in wild type female rats exposed to UVB  
 35 (Groups 1 and 2) with incidences of 12.5% (1/8) and 14.3% (1/7). No statistically  
 36 significant inter-group differences in incidence, multiplicity or weight were found. Mammary  
 37 tumours (adenocarcinomas) were induced with high incidence in Hras128 rats of both  
 38 sexes. Wild-type female rats also had an increased incidence of mammary tumours but no  
 39 statistically significant inter-group differences in incidence, multiplicity or weight were  
 40 observed.  
 41

#### 42 Conclusions by the authors

43 TiO<sub>2</sub> particles were detected in the upper *stratum corneum* but not in the underlying skin  
 44 tissue layers. TiO<sub>2</sub> did not induce or promote skin carcinogenesis in transgenic (Hras128)  
 45 and wild-type Sprague-Dawley rats under the conditions of this study. The data suggest  
 46 that TiO<sub>2</sub> does not cause skin carcinogenesis, probably due to its inability to penetrate  
 47 through the epidermis and reach underlying skin structures.  
 48

#### 49 **SCCS Comment**

50 This is not a generally accepted model for studying initiation and promotion of skin tumours.  
 51 Since no positive control was included it is not possible to make any conclusion with regard  
 52 to potential carcinogenic properties of TiO<sub>2</sub> from the study.  
 53

54 *Study Design:* Intra-pulmonary spraying

55 Date of publication: Advance Access publication February 25, 2010.

56 Guideline/method: Two stage rat skin carcinogenicity test. Uncoated titanium dioxide  
 57 nanoparticles (nTiO<sub>2</sub>) were used as promoter with DHPN as initiator.



1	Test system:	Female transgenic rats carrying the human c-Ha-ras gen (Hras128
2		rats) and female wild-type SD rats were obtained from CLEA Japan
3		Co., Ltd (Tokyo, Japan)
4	Test substance:	ncTiO <sub>2</sub> rutile type mean particulate diameter 20 nm) were provided
5		by Japan Cosmetics Association, Tokyo.
6	Batch:	No data
7	Concentrations:	TiO <sub>2</sub> particles were suspended in saline at 250 µg/ml or 500 µg/ml.
8	Exposure:	Initiation: 0.2% DHPN (N-nitrosobis(2-hydroxypropyl)amine), (Wako
9		Chemicals Co., Ltd Osaka, Japan) in the drinking water for 2 weeks.
10		Promotion: Two weeks after DHPN treatment, the rats were exposed
11		intratracheally every second week to TiO <sub>2</sub> suspensions under
12		isoflurane anesthesia for a total of 7 times. The rats were killed 3
13		days after the last exposure.
14	Solvent:	Saline
15	Negative control:	Only DHPN in drinking water
16	Positive Controls:	None
17	GLP:	No
18	Reference:	Xu <i>et al.</i> , 2011

19  
20 Female transgenic Hras128 rats and female wild-type SD rats were used in the study. TiO<sub>2</sub>  
21 particles were suspended in saline at 250 µg/ml or 500 µg/ml. The TiO<sub>2</sub> suspension was  
22 intratracheally administered to animals under isoflurane anesthesia using a Microsprayer  
23 (Series IA-1B Intratracheal Aerosolizer, Penn-Century, Philadelphia, PA) connected to a 1 ml  
24 syringe; the nozzle of the sprayer was inserted into the trachea through the larynx and a  
25 total of 0.5 ml suspension was sprayed into the lungs synchronizing with spontaneous  
26 respiratory inhalation (IPS).

#### 27 *IPS-initiation-promotion protocol*

28  
29 Female Hras128 rats aged 6 weeks were given 0.2% DHPN, in the drinking water for 2  
30 weeks. Two weeks later, the rats were divided into four groups. Group 1 (9 rats). DHPN  
31 alone. Group 2 (10 rats). DHPN followed by 250 µg/ml TiO<sub>2</sub>. Group 3 (11 rats). DHPN  
32 followed by 500 µg/ml TiO<sub>2</sub>. Group 4 (9 rats). 500 µg/ml TiO<sub>2</sub> without DHPN initiation.

33  
34 The TiO<sub>2</sub> particle preparations were administered by IPS once every 2 weeks from the end  
35 of week 4 to week 16 (a total of seven exposures). The total amount of TiO<sub>2</sub> administered to  
36 Groups 1, 2, 3 and 4 were 0, 0.875, 1.75 and 1.75 mg per rat, respectively. Three days  
37 after the last treatment, animals were killed and the organs (brain, lung, liver, spleen,  
38 kidney, mammary gland, ovaries, uterus and neck lymph nodes) were excised

39  
40 TiO<sub>2</sub> was distributed primarily to the lung, but minor amounts of TiO<sub>2</sub> were also found in  
41 other organs. Various sizes of TiO<sub>2</sub> aggregates were observed in alveolar macrophages. The  
42 TiO<sub>2</sub>-laden macrophages were evenly scattered throughout the lung alveoli. Of 452 particle  
43 aggregates examined, 362 (80.1%) were nanosized, i.e.100 nm. Overall, the average size  
44 was 84.9 nm and the median size was 44.4 nm.

45  
46 The author concluded that TiO<sub>2</sub> treatment significantly increased the multiplicity of DHPN-  
47 induced alveolar cell hyperplasias and adenomas in the lung. In the rats, which received  
48 TiO<sub>2</sub> treatment without prior DHPN treatment, alveolar proliferative lesions were not  
49 observed although slight inflammatory lesions were observed. TiO<sub>2</sub> aggregates were  
50 localized exclusively in alveolar macrophages and had a mean diameter of 107.4 nm.

51  
52 In the mammary gland, TiO<sub>2</sub> treatment significantly increased the multiplicity of  
53 adenocarcinomas from about 3 tumours per rat in Group 1 to about 6 tumours per rat in  
54 Group 2 and 3. The treatment did also tend to increase the weight of the mammary tumors  
55 from about 6 g per tumour in Group 1 to about 12 – 15 g per tumour in Group 2 and 3 (only  
56 shown in Figure with no Table).

57

### 1 *IPS 9 day protocol*

2 Twenty female SD rats (wild-type counterpart of Hras128) aged 10 weeks were treated by  
3 IPS with 0.5 ml suspension of 500 µg/ml TiO<sub>2</sub> particles in saline five times over a 9 day  
4 period. The total amount of TiO<sub>2</sub> administered was 1.25 mg per rat. Six hours after the last  
5 dose, animals were killed and the lungs and inguinal mammary glands were excised. Fatty  
6 tissue surrounding the mammary gland was removed as much as possible. The left lungs  
7 and inguinal mammary glands were used for biochemical analysis, and the right lungs were  
8 fixed in 4% paraformaldehyde solution in PBS adjusted at pH 7.3 and processed for  
9 histopathological examination and immunohistochemistry.

10  
11 Morphologically, TiO<sub>2</sub> particles were observed as yellowish, polygonal bodies in the  
12 cytoplasm of cells. These cells are morphologically distinct from neutrophils and strongly  
13 positive for CD68, indicating that the TiO<sub>2</sub> engulfing cells were macrophages. TiO<sub>2</sub>  
14 aggregates of various sizes were found in macrophages, and aggregates larger than a single  
15 macrophage were surrounded by multiple macrophages. Of 2571 particle aggregates  
16 examined, 1970 (76.6%) were <100 nm and five particles were >4000 nm in size. Overall,  
17 the average size was 107.4 nm and the median size was 48.1 nm.

18  
19 TiO<sub>2</sub> treatment significantly increased 8-hydroxydeoxy guanosine level, superoxide  
20 dismutase activity and macrophage inflammatory protein 1a (MIP1a) expression in the lung  
21

### 22 Comment by the authors

23 TiO<sub>2</sub> treatment significantly increased 8-hydroxydeoxy guanosine level, superoxide  
24 dismutase activity, and macrophage inflammatory protein 1a (MIP1a) expression in the  
25 lung. MIP1a, detected in the cytoplasm of TiO<sub>2</sub>-laden alveolar macrophages *in vivo* and in  
26 the media of rat primary alveolar macrophages treated with TiO<sub>2</sub> *in vitro*, enhanced  
27 proliferation of human lung cancer cells. Furthermore, MIP1a, also detected in the sera and  
28 mammary adenocarcinomas of TiO<sub>2</sub>-treated Hras128 rats, enhanced proliferation of rat  
29 mammary carcinoma cells. These data indicate that secreted MIP1a from TiO<sub>2</sub>-laden  
30 alveolar macrophages can cause cell proliferation in the alveoli and mammary gland and  
31 suggest that TiO<sub>2</sub> tumor promotion is mediated by MIP1a acting locally in the alveoli and  
32 distantly in the mammary gland after transport via the circulation.  
33

### 34 **SCCS Comment**

35 TiO<sub>2</sub> treatment significantly increased the multiplicity of DHPN-induced alveolar cell  
36 hyperplasias and adenomas in the lung, and the multiplicity of mammary adenocarcinomas.  
37 Thus, non-coated TiO<sub>2</sub> administered intratracheally had tumour promoter activity.  
38  
39

### 40 ***Oral carcinogenicity studies in non-nano TiO<sub>2</sub>***

41  
42 Oral study with F344 rats. Each groups consisted of 60 male and 60 female rats. The control  
43 diet contained 1% corn oil, while experimental diets contained 1.0, 2.0, or 5.0% titanium  
44 dioxide-coated mica and 1% corn oil.  
45

46 The article states: "TiO<sub>2</sub>-coated mica is a nonfibrous, naturally occurring silicate, which,  
47 when coated with TiO<sub>2</sub> is used as a pearlescent pigment in plastics, industrial coatings,  
48 simulated leather, and cosmetic preparations. Annual worldwide production of TiO<sub>2</sub>-coated  
49 mica exceeds 1 million pounds and the potential for human exposure is great."  
50

51 The test material consisted of a 1:1 blend of two samples of titanium dioxide-coated mica.  
52 The material was in the form of flat platelet with the longest dimension ranging from 10 to  
53 35 µm. The final blend of test material contained 28% TiO<sub>2</sub> and 72% mica. A purity of  
54 100% was assumed for purposes of diet formulations.  
55

1 The rats (6 week old) received the TiO<sub>2</sub> containing for up to 130 weeks. The study authors  
2 stated that "there was no evidence that TiO<sub>2</sub>-coated mica produced either toxicologic or  
3 carcinogenic effects at dietary concentrations as high as 5.0%.

4 Ref.: Bernard *et al.*, 1990  
5

6 Groups of 50 male and 50 female B6C3F1 mice, 5 weeks of age, were fed diets containing  
7 0, 2.5 or 5% titanium dioxide (size unspecified; anatase; purity, ≥98%) daily for 103  
8 weeks. Mice were killed at 109 weeks of age, at which time no significant difference in  
9 survival was observed between treated and control males. In females, a dose-related trend  
10 in decreased survival was noted. No significant differences in body weights or incidence of  
11 tumours were observed between treated and control groups.  
12

13 Groups of 50 male and 50 female Fischer rats, 9 weeks of age, were fed diets containing 0,  
14 2.5 or 5% titanium dioxide (size unspecified; anatase; purity, ≥98%) daily for 103 weeks.  
15 The rats were killed at 113 weeks of age, at which time no significant difference in survival  
16 was observed between treated and control groups of either sex. No significant differences in  
17 body weights or incidence of tumours were observed between treated and control groups.  
18

19 Ref.: National Cancer Institute, 1979  
20

### 20 **SCCS Comment**

21 From the studies, exposure to non-nano titanium dioxide via the oral route does not appear  
22 to lead to carcinogenic effects.  
23

## 24 **1.5.8 Photo-carcinogenicity**

### 25 ***Photo-carcinogenicity studies in non-nano TiO<sub>2</sub>***

26 The ability of MTD (titanium dioxide, not further specified) and 2-EHMC (2-ethylhexyl-p-  
27 methoxycinnamate) to protect mice from the "promotion phase" of tumorigenesis was  
28 studied.  
29

30 The dorsal trunks of inbred female C3H/HeJ mice (10 – 12 weeks old) were shaved and the  
31 relevant groups (15 mice) initiated with 10 nmol DMBA. Five days later UV-irradiation  
32 and/or sunscreen treatment was commenced and this was continued for 32 weeks. The  
33 mice were monitored for a further 14 weeks after cessation of irradiation.  
34

35 The sunscreens were in oil-in-water emulsion and contained MTD (7.2%) or 2-EHMC (8%).  
36 The MTD was a broad-spectrum-reflecting physical sunscreen with an SPF of 7, while the 2-  
37 EHMC was shown to be a UVB-absorbing sunscreen with an SPF of 4. The sunscreens or  
38 base lotion (BL) were applied at least 10 min prior to UV exposure at approximately 2  
39 mg/cm<sup>2</sup>. The integrated irradiance was 1.7 W/m<sup>2</sup> for UVB and 34 W/m<sup>2</sup> for UVA.  
40

41 The mice were irradiated 5 days per week for 32 weeks, i.e. until 50% of the DMBA plus UV  
42 irradiated groups had tumors. The average cumulative dose was 571 kJ/m<sup>2</sup> for UVB and  
43 11.4 mJ/m<sup>2</sup>.  
44

45 The DMBA-initiation alone and DMBA-initiated sunscreen-treated groups did not develop  
46 tumours. UV alone induced tumours in 46% of the mice at week 48. Initiation with DMBA  
47 prior to UV irradiation enhanced tumour formation such that 87% had tumours at week 48.  
48 Both MTD and 2-EHMC completely protected the mice from UV-induced tumour formation.  
49

50 Ref.: Bestak and Haliday, 1996.  
51

52 Groups of female inbred mice (hr/hr, strain Skh:HR-1) treated with an SPF 15 sunscreen  
53 formulated with MT100T microfine titanium dioxide coated with aluminium stearate (not  
54 further specified) were exposed daily to minimally skin reddening UV radiation over 12  
55

1 weeks. Throughout a 200 day observation period substantial protection was afforded from  
2 the induction of skin cancer compared to unprotected controls.

3  
4 Two groups of sunscreen protected mice were treated immediately following the radiation  
5 regime with the tumour promoter croton oil. UV + croton oil induced tumours in 100% of  
6 the mice. The mice protected by a sunscreen showed only 3.7% with tumours, which was  
7 less than with treatment with croton oil alone. However, where sunscreen protected mice  
8 were exposed to croton oil about 25% proved to have been initiated.

9  
10 The authors concluded that the superfine titanium dioxide sunscreen provided a high level  
11 of protection similar to that by conventional sunscreen formulations.

12 Ref.: Greenoak *et al.*, 1993.

### 13 14 **SCCS Comment**

15 The studies above are of little value because size and specifications of the titanium dioxide  
16 particles are unknown.

### 17 18 19 **SCCS Comments on Carcinogenicity**

20 Pigmentary and ultrafine titanium dioxide has been tested for carcinogenicity by oral  
21 administration in mice and rats, by inhalation exposure in rats and female mice, by  
22 intratracheal administration in hamsters and female rats and mice, by subcutaneous  
23 injection in rats, and by intraperitoneal administration in male mice and female rats.

- 24  
25 - According to the evaluation of titanium dioxide by IARC (2010), induction of lung  
26 tumours was observed in two inhalation studies with rats while two inhalation studies in  
27 rats and one in female mice gave negative results.
- 28 - Intratracheally instilled female rats showed an increased incidence of lung tumours  
29 following treatment with two types of titanium dioxide. Tumour incidence was not  
30 increased in intratracheally instilled hamsters and female mice.
- 31 - Oral, subcutaneous and intraperitoneal administration did not produce a significant  
32 increase in the frequency of any type of tumour in mice or rats.
- 33 - IARC concluded that there is *inadequate evidence* in humans for the carcinogenicity of  
34 titanium dioxide but *sufficient evidence* in experimental animals for the carcinogenicity  
35 of titanium dioxide. Titanium dioxide was classified as a Group 2B carcinogen (*Possibly  
36 carcinogenic to humans*).
- 37 - In their recent evaluation of TiO<sub>2</sub> NIOSH has determined that ultrafine TiO<sub>2</sub> with equal  
38 nano-sized TiO<sub>2</sub> is a potential occupational carcinogen and, that there is insufficient data  
39 to classify fine TiO<sub>2</sub> as potential occupational carcinogen after inhalation (NIOSH 2011).
- 40 - Nano titanium dioxide has been studied in 2 two-stage skin carcinogenicity studies with  
41 mice, 2 two-stage skin carcinogenicity studies with rats, and one two-stage lung study  
42 with rats.
- 43 - Both non-coated (ncTiO<sub>2</sub>) and coated titanium dioxide have been studied in the two-  
44 stage mouse skin carcinogenicity studies with CD1 mice and a transgenic mouse strain  
45 (rasH2). In one well performed study with non-coated and alumina and stearic acid  
46 coated titanium dioxide, no promoter activity was found (Furukawa *et al.*, 2011).  
47 Promoter activity was also not found for ncTiO<sub>2</sub> in the other study (Sagawa *et al.*, 2012).  
48 However, it is difficult to draw a firm conclusion from this study with silica coated  
49 titanium dioxide due to lack of positive controls and very high tumour activity in the  
50 "initiated" mice.
- 51 - Non-coated titanium dioxide was studied in 2 two-stage rat skin carcinogenicity studies.  
52 Although, no tumour promoter activity was observed, it is difficult to draw any

1 conclusion since little experience with the model used is available and no positive  
2 controls have been used in the studies.

- 3 - One two-stage rat lung carcinogenicity study has been carried out with non-coated  
4 titanium dioxide. The rats were "initiated" by DHPN in the drinking water prior to intra-  
5 pulmonary spraying with nTiO<sub>2</sub>. The experiment demonstrated promoter activity of  
6 nTiO<sub>2</sub> (Xu *et al.*, 2011).

7 Since TiO<sub>2</sub> particles have shown carcinogenic activity and since nano nTiO<sub>2</sub> also showed  
8 promoter activity after intra-pulmonary spraying, the use of nano TiO<sub>2</sub> in sprayable  
9 applications needs specific considerations.

10

### 11 **1.5.9 Reproductive toxicity**

12  
13 In the submission, no studies have been provided with reproductive toxicity data relevant to  
14 the nanomaterials under assessment. A review of reproductive and developmental toxicity  
15 studies of manufactured nanomaterials (including TiO<sub>2</sub>) has been provided (Ema *et al.*,  
16 2010 - Reference 146). The two TiO<sub>2</sub> materials referred to include a TiO<sub>2</sub> material with  
17 particle size <10µm (no further information), and a TiO<sub>2</sub> nanomaterial with primary particle  
18 size 25-70 nm (20-25m<sup>2</sup>/g surface area, anatase). Relevant studies in the review by Ema  
19 *et al.* (2010) showed that:

- 20 - Pregnant BALB/c mice administered on gestational day 14 with <10 µm TiO<sub>2</sub>  
21 suspended in phosphate-buffered saline at 50 µg/mouse by a single intranasal  
22 insufflation had higher serum levels of cytokines, including interleukin-1β, tumor  
23 necrosis factor-α, interleukin-6 and chemokine, 48 h after exposure compared with  
24 nonpregnant mice. The offspring of the dams exposed to TiO<sub>2</sub> showed increased  
25 airway hyperresponsiveness, increased percentage of eosinophils, and pulmonary  
26 inflammation. These findings showed that TiO<sub>2</sub> caused acute cellular inflammation in  
27 pregnant mice and increased allergic susceptibility in their pups.
- 28 - Pregnant Slc:ICR mice administered on gestational days 6, 9, 12 and 15 with TiO<sub>2</sub>  
29 nanomaterial suspended in saline with 0.05% Tween 80 via subcutaneous injection  
30 at 100µg/mouse/day caused changes in the expression of genes associated with  
31 brain development, cell death, response to oxidative stress, and mitochondria in the  
32 brain during the prenatal period, and genes associated with inflammation and  
33 neurotransmitters in the later stages of the offsprings.
- 34 - *In vitro* exposure of testis-constituent cells (mouse Leydig cell line TM3) to nano-  
35 TiO<sub>2</sub> showed uptake of the nanoparticles after incubation of cells at 30µg/mL for  
36 48h, and a remarkable inhibition of viability and transient reduction in proliferation of  
37 the cells at 100µg/mL after 24 h.

38 The article is, however, a review of exploratory studies, and as such is of a limited  
39 usefulness to this assessment.

40 Other studies in open literature, including some of those reviewed by Ema *et al.* (2010)  
41 have demonstrated the possibility of placental transport of different manufactured  
42 nanomaterials in pregnant animals into the fetus, or found effects in the offspring.  
43 Yamashita *et al.* (2011) reported on the presence of nano-TiO<sub>2</sub> in fetuses after the  
44 intravenous administration of nano-TiO<sub>2</sub> in pregnant mice. Nano-TiO<sub>2</sub> was detected by TEM  
45 in the placenta, fetal liver and fetal brain, and induced a decrease in uterine weight and  
46 higher fetal absorption. A limitation of the study was that relatively high doses (about 32  
47 mg/kg body weight on gestation days 16 and 17) were used. In addition, the chemical  
48 nature of the nanomaterials observed in the organs was not confirmed. For the silica  
49 nanoparticles investigated in the same paper a size dependency of transplacental migration  
50 was demonstrated as 70 nm nanoparticles did show placental transport while 300 nm and  
51 1000 nm silica nanoparticles did not (Yamashita *et al.*, 2011).



1 After subcutaneous administration to dams (Slc:ICR mice) on gestation days 3, 7, 10 and  
2 14 at 100µg/mouse/day, Takeda et al. (2009) observed TiO<sub>2</sub> particle aggregates (identified  
3 by Energy Dispersive X-ray Spectroscopy, EDS) in the testis of male offsprings at day 4 and  
4 week 6 after birth. Also histopathological alterations were observed in the testis. In  
5 addition, nano-TiO<sub>2</sub> particles were demonstrated in the brain of offspring mice (Takeda et  
6 al., 2009), suggesting that nano-TiO<sub>2</sub> might have passed through undeveloped or  
7 developing Blood Brain Barrier (BBB) in embryos of the young mice. However, since mice  
8 were tested at 4 days or 6 weeks of age, it is not clear whether exposure to nano-TiO<sub>2</sub>  
9 occurs in utero via the placenta or through milk. A previous study of the same research  
10 group observed alterations in gene expression in the brain (Shimizu et al., 2009). The gene  
11 expression alterations were already observed in 16 days old embryos. As only the mother  
12 animals were exposed to nano-TiO<sub>2</sub> it seems likely that the offspring received the Ti via the  
13 mother either during pregnancy or in the weaning period via the milk (Takeda et al., 2009).  
14 For some effects, like reduced pup weight and gene alterations, indirect mechanisms due to  
15 effects on the pregnant animals themselves could not be excluded.

16 After inhalation exposure to nano-TiO<sub>2</sub> during gestation days 8-18 moderate behavioural  
17 effects were observed in the offspring (Hougaard et al., 2010). Time to first litter was  
18 prolonged after mating the exposed male offspring to unexposed mice but did not reach  
19 statistical significance. For females there was no difference. After inhalation of a surface  
20 coated nano-TiO<sub>2</sub> by pregnant mice, no effects were seen on DNA damage in  
21 bronchoalveolar lavage fluid (BALF) cells and liver cells (Jackson et al., 2011), nor in  
22 offspring that had been prenatally exposed. Some changes were noted in liver gene  
23 expression profiles of female offspring. However, as in general the exposure of the fetuses  
24 would be rather low, the observed alterations might have been caused as a secondary  
25 response to the maternal inflammation in the lungs.

26 Shimizu et al. (2009), from the same research group as Takeda et al. (2009) performed a  
27 similar study in which pregnant mice were injected subcutaneously (100 µl of 1 mg/ml TiO<sub>2</sub>  
28 solution) with nano-TiO<sub>2</sub> (25-70 nm, anatase) on gestational days 6, 9, 12, and 15. This  
29 study also investigated the effects of maternal exposure to nano-TiO<sub>2</sub> on gene expression in  
30 brain during the developmental period using cDNA analysis. Expression levels of the genes  
31 associated with apoptosis were altered in the brain of newborn pups, whereas genes  
32 associated with brain development were altered in early age. The genes associated with  
33 response to oxidative stress were changed in the brains of 2 and 3 weeks old mice. Using  
34 Medical Subject Headings (MeSH) terms information, the changes of the expression of  
35 genes was found to be associated with neurotransmitters and psychiatric diseases.

36 In conclusion, although after inhalation or subcutaneous exposure of pregnant mice the  
37 exposure of offspring in the uterus has been reported, exposure through this route is likely  
38 to be low and some of the effects might be secondary to maternal toxicity induced by the  
39 nanomaterials. The reported fetal effects were observed after high doses of intravenously  
40 administered nano-TiO<sub>2</sub>, which are unlikely to occur in real life with the use of sunscreen  
41 products.

42

#### 43 **SCCS Comment**

44 No relevant study on reproductive toxicity is provided. One review article covering  
45 exploratory studies has been provided (SI-II, Ema et al., 2010 (146)). Overall information  
46 on this endpoint is as yet patchy and inconclusive.

47

#### 48 **1.5.9.1 Two generation reproduction toxicity**

#### 49 **SCCS Comment**

50 No data on two-generation reproductive toxicity is provided

51

52



### 1.5.9.2 Teratogenicity

#### SCCS Comment

No data on teratogenicity is provided

### 1.5.10 Toxicokinetics

The following studies on toxicokinetics and metabolism have been provided:

#### Exploratory distribution, excretion study in rat

Reference: Fabian et al., Arch Toxicol. 2007 ref. No. 28 + 53; and  
Fabian E. + Landsiedel R. ref. No. 28)

Guideline: Study considered a number of guidelines: EC Commission Directive 87/302/EEC (EC Commission Directive 1988), OECD Guidelines for Testing of Chemicals (Method No. 417) (OECD Guidelines 1984), U.S. EPA, Health Effects Guidelines, OPPTS 870.7485 (U.S. EPA 1998), and the Japan/MAFF: Guidelines on the Compiling of Test Results on Toxicity (Japan/MAFF2001).

Species/strain: male Wistar rat, 7–12 weeks old and weighed 200– 300g

Group size: 12 rats; 3 rats per group

Test substance: TiO<sub>2</sub>; 06/0489; P25 consisted of both anatase and rutile forms (70/30), had no surface coating, the TiO<sub>2</sub> primary particles were in the size range 20–30 nm; approximately 10 wt.% of the particle agglomerates/aggregates are found in the nano-size range. BET specific surface area of 48.6 m<sup>2</sup>/g.

Batch: 4165012298 (FI);

CAS No. 13463-67-7

Purity: unknown

Dose levels: 5 mg/kg body weight, TiO<sub>2</sub> particles suspended in serum

Route: A single intravenous injection followed by biokinetics study

GLP: not applied

Study period:

#### Results

Analysis was performed on ICP-AES. According to the analytical method there were no detectable levels of TiO<sub>2</sub> in blood cells, plasma, brain, or lymph nodes. There were no changes in the cytokines and enzymes measured in blood samples. Highest Ti retention was observed in the liver at about 100-150 µg/g of organ with a limited clearance during the next four weeks. Ti concentrations in spleen were only slightly lower than in the liver, but Ti concentrations in kidneys and in lungs were about one order of magnitude lower with rather remarkable clearance of about 66% during the next 14 days.

#### SCCS Comments

It is not clear which of the numerous noted guidelines were followed. Ti contents of the organs were not corrected for background levels but untreated rats were analysed as well. This means only 3 rats per group were analysed. Questions arise where the rest of the administered TiO<sub>2</sub> particles went, since an estimated dose of about 1.25 mg per rat were injected and liver, spleen, lungs and kidneys amounted only to 600-700 µg per rat providing no information on the remainder 500 µg.

#### Exploratory distribution, excretion study in rat

---

1		
2	Reference:	Sugibayashi K., Todo H., Kimura E Safety evaluation of titanium dioxide
3		nanoparticles by their absorption and elimination profiles. <i>Journal of</i>
4		<i>Toxicological Sciences</i> <b>33</b> (3), 293-8 (2008).
5		
6	Guideline:	not specified
7	Species/strain:	mouse of unspecified strain
8	Group size:	not clearly identified, probably 3-5 mice at each time point
9	Test substance:	rod-shaped TiO <sub>2</sub> rutile surface-coated with silica; (primary particle
10		diameter: 15 nm; agglomerated particle size: 220 nm);
11	Batch:	HD-AW-150 from a Japanese company
12	Purity:	rutile analysis by XRD, 27.5% silica content from surface modification, no
13		further analysis on impurities
14		
15	Dose levels:	no dose levels specified
16	Route:	intravenous injection followed by biokinetics study in mice;
17	Administration:	intravenous injection of titanium dioxide nanoparticles, single intravenous
18		injection; biokinetics after 5 min, 72 h and 30 d
19	GLP:	not specified
20	Study period:	

## 21 Results

22 Distribution of TiO<sub>2</sub> (measured as Ti) was in blood and several tissues (primarily liver) but  
23 not in brain. A slow decrease of TiO<sub>2</sub> in liver was observed over time (~30% decrease in  
24 one month). Observation of substantial amounts of Ti found in untreated mice prior to any  
25 treatment due to significant natural food contamination; this led to an estimated dose of 90  
26 µg/ Ti per day. After i.v. injection the Ti level was significantly increased in blood and  
27 tissues. Ti concentrations per organs are provided but it is not clear whether or not these  
28 were corrected for background Ti in all organs nor is the administered dose given.

29  
30  
31 **SCCS Comment**  
32 Neither the strain nor the number of mice is clearly identified. The i.v. injected dose of TiO<sub>2</sub>  
33 NP is also not specified. This study is therefore of no use to the current assessment.

## 34 35 36 Open literature

37  
38 There are other toxicokinetic data of inhaled agglomerated TiO<sub>2</sub> nanoparticles (Ma Hock et  
39 al. 2008, 2009) showing oxidative stress and inflammatory reactions similar to previously  
40 described 90-days exposure investigations. As far as toxicokinetic parameters were  
41 evaluated, due to the detection limits, extrapulmonary TiO<sub>2</sub> particles were not detected.

42  
43 There are also toxicokinetic studies in which TiO<sub>2</sub> NP were intravenously injected into the  
44 vein of rodents (Fabian et al., 2007, and other papers). Retention was highest in the liver  
45 followed by spleen, lungs, kidneys and it was highest at the first day compared to days 14  
46 and 28. Cytokine levels remained unchanged indicating no detectable toxicity.

47  
48 There are no new toxicokinetic data on the absorption of TiO<sub>2</sub> NP after administration to the  
49 gastrointestinal-tract (GIT). The most recent study from Wang et al. 2008 used  
50 unrealistically high doses of 5 g/kg BW in rats such that their findings are not useful and  
51 may even be modulated by uncontrolled other forms of intake like inhalation of aspiration.  
52 Their biodistribution data showed the highest retention in the liver followed by spleen,  
53 kidneys and lungs. Thus toxicokinetics data after GIT administration still rely on the studies  
54 of the group of Alexander Florence, in the 1990ies. These suggest that about 5-7% of the  
55 administered 500nm TiO<sub>2</sub> particles were absorbed and retained in the body, mainly in the  
56 liver.

57

1 Applicant's conclusions

2 Intravenous administration of large doses of nano TiO<sub>2</sub> did not result in adverse effects or  
 3 signs of toxicity in rodents. A non-specific and expected tissue distribution of TiO<sub>2</sub> was  
 4 observed. No TiO<sub>2</sub> was detected in brain, and the levels in other organs decreased over  
 5 time.

6  
7 **SCCS Comment**

8 The limited available evidence suggests that if TiO<sub>2</sub> nanoparticles become systemically-  
 9 available, they may accumulate mainly in liver with a very slow clearance.

12 **1.5.11 Photo-induced toxicity**14 **1.5.11.1 Phototoxicity / photoirritation and photosensitisation**15  
16 **Photo- irritation**

17 Guidelines: OECD good laboratory principles  
 18 Product tested: TiO<sub>2</sub> T805 (1992 batch 030492)  
 19 Species: SPF NZ white rabbits (Ch. River), Female  
 20 Groups: 3 animals/group  
 21 Dosing: 3, 10, 30% in ethanol 96% during 100 min  
 22 Exposure area: 15 – 7.5 cm total, each exposure side spot approximately 2 cm  
 23 diameter  
 24 UVA-light: 310-420 nm peak 365nm total dose 10J/cm<sup>2</sup> (approx. 50 min dosing)  
 25 Readings: 30 min, 24h, 48h, 72 h after UV-exposure  
 26 Observations: No irritation found, neither non-irradiated as irradiated TiO<sub>2</sub> treated  
 27 animals.  
 28 Reference: 15  
 29 Conclusion: TiO<sub>2</sub> (T805) is not photo-irritating for rabbit skin under the assay  
 30 conditions after UVA irradiation up to 10 J/cm<sup>2</sup>.

31  
32  
33 Guidelines: OECD good laboratory principles  
 34 Product tested: TiO<sub>2</sub> T805 (1992 batch 030492)  
 35 Species: SPF albino guinea pigs (Ch. River)  
 36 Sex: Males & Female  
 37 Experimental protocol: Following Ichikawa, Armstrong & Harber 1981, Induction treatment  
 38 followed by challenge 12 days later  
 39 Groups: Test groups 5 animals of each sex  
 40 Dosing: 30% TiO<sub>2</sub> in ethanol (96%) at induction treatment & challenge  
 41 treatment (day 12)  
 42 Induction protocol:  
 43 - 6-8 cm area cleared from fur

- 1 - Area is subcutaneously pre-treated with Freund adjuvant and exposed to 0.2 ml of  
 2 suspension followed by UVA-light: 310-420 nm (peak 365 nm) total dose 10 J/cm<sup>2</sup>  
 3 - In total 5 treatment over 2 weeks (only first time Freund adjuvant was used).  
 4 - Skin was not cleared after treatment  
 5 - Reading after each treatment

6 **Challenge protocol:**

- 7 - 12 days after last induction  
 8 - 5-10 cm area cleared from fur  
 9 - Exposed to 0.5 ml of 30% TiO<sub>2</sub> (T805) suspension direct followed by light: 310-420 nm  
 10 (peak 365 nm) total dose 10 J/cm<sup>2</sup> - 37 min

11 **Observations:** No irritation found, neither during induction phase or challenge  
 12 phase, in both non-irradiated as irradiated TiO<sub>2</sub> treated animals.

13 **Conclusions:** TiO<sub>2</sub> (T805) is not photo-sensitizer for guinea pigs under the assay  
 14 conditions after UVA irradiation up to 10 J/cm<sup>2</sup>.

15 **Reference:** 17

16

17 **Human data:**

18 **Product tested:** 0685115 (No other info in the document)

19 **Species:** 60 volunteers (19-77 y) of which 50 completed the study

20 **Sex:** Males & Female

21 Protocol:

- 22 - Induction: 3 patches per week (Mon, Wed, Fri) during 3 weeks (0.2 ml TiO<sub>2</sub> suspension  
 23 per patch – no concentration reported). Patches remain at place 24 h (removal by  
 24 volunteers). If reaction, next patch was moved to adjacent area (testing was  
 25 discontinued if severe reaction was noted)  
 26 - Challenge: 2 weeks after last induction at different spot

27 **Result:** No effects observed, in any of the volunteers

28 **Conclusion:** Product 0685115 is not a sensitizer for humans under the assay  
 29 conditions

30 **Reference:** 27

31

32 **SCCS Comment**

33 The study is not a photosensitisation study but is only sensitization study.  
 34  
 35

36 **Product tested:** 0685115 (No other information in the document)

37 **Species:** 29 human volunteers (18-60 y) of which 25 finished the whole study  
 38 (drop-out were not related to the test)

39 **Sex:** Males & Female

40 **Pre-testing:** MED (Minimal Erythral Dose) of unprotected skin of each volunteer  
 41 was assessed. [MED = time interval or dose of UV sufficient to  
 42 produce minimal perceptive erythema]

43 **Light source:** UV A (320-400 nm), 3 min(approximately 10.08 Joules)

44 Protocol:

- 1 - Induction: 2 spot prepared for exposure to compound 0685115, of which one is  
 2 irradiated while the other is not be irradiated.  
 3 The areas cleared from hair of 1 inch/ 1 inch, and 0.2 ml (no concentration of TiO<sub>2</sub>  
 4 suspension reported) of test material is placed on the spots. Exposed side is kept under  
 5 patch during 24 h.  
 6 2 applications applied per week for 3 weeks (total 6 applications).  
 7 After removal of patch spots irradiated with a dose of 2x MED of the volunteer  
 8 - Challenge: 2 weeks after last induction at different spots on the back.  
 9 Spots are under patch for 24 h, then irradiated for 3 min (non erythemogenic dose).  
 10 Reading after 24, 48 & 72 h
- 11 Result: No effects observed, in any of the volunteers
- 12 Conclusion: Product 0685115 is not a photo-sensitizer for humans under the assay  
 13 conditions
- 14 Reference: 28

15

**SCCS Comments**

16 Ref 16 and 18 could not be found. The given references are not correct, as they do not  
 17 report photo-irritation (Sonnenschutzformulierungen: Lotions und Cremes)  
 18  
 19

20 1.5.11.2 Phototoxicity / photomutagenicity / photoclastogenicity
---

21

22 A number of studies has not been reviewed as part of this assessment, because the  
 23 experiments were performed with bacterial cells. As discussed in section 3.3.6, bacterial  
 24 mutagenicity assays are not considered to be appropriate for the testing of nanoparticles  
 25 compared to mammalian cell systems. Other studies were not reviewed because they are  
 26 related to test materials that are either not nanomaterials, or they lack data on material  
 27 characterisation to establish whether they were relevant nanomaterials to this assessment.  
 28

29

**Phototoxicity test *in vitro***

30 Guideline/method: OECD TG432  
 31 Test system: Balb/c 3T3 fibroblasts, neutral red uptake (NRU)  
 32 Replicates: no replicates  
 33 Test item: T805 (coated, A/R, PSMA 1 type), T817 (coated, A/R, PSMA 1 type),  
 34 TiO<sub>2</sub> P25 (non coated)  
 35 Batch: 05 10067 (T805), 04095 (T817), P1S-3087 (p25)  
 36 Vehicle: EBSS wit 1% ethanol  
 37 Concentrations: 0.78 to 1—mg/L UV-A: 5.0 J/cm<sup>2</sup>  
 38 Exposure: 0, 0.79, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L  
 39 Negative control: vehicle  
 40 Positive control: not included  
 41 GLP: no  
 42 Date of report: 1999  
 43 Reference: Submission DHS, 24 and 25

44 Balb/c 3T3 cells were pre-incubated with eight different concentrations (0.79, 1.56, 3.13,  
 45 6.25, 12.5, 25, 50 and 100) of the nanoparticles in two 96-well plates, one plate was  
 46 subsequently exposed to 5 J/cm<sup>2</sup> UVA while the other plate was kept in the dark. Medium  
 47 was then replaced and after 24 h cell viability was determined by spectrophotometrical  
 48 evaluation of neutral red dye uptake (3 h incubation of neutral red). The phototoxic  
 49 potential was determined by calculation of the ratio of the nanoparticle concentration that  
 50 reduced viability by 50% (NR50) in presence versus absence of UV irradiation.

51 Results

1 T805 and T817 showed neither cytotoxicity nor phototoxicity up to a concentration of  
 2 100mg/L. The p25 (non coated NP) sample also was not cytotoxic up to the highest  
 3 concentrations, but in the presence of irradiation a viability reduction of 82 % (at 50 mg/L)  
 4 and 44% (at 100 mg/ml) was observed.  
 5

## 6 Conclusion

7 p25 sample is phototoxic towards Balb/c 3T3 cells, while T805 is not phototoxic.  
 8

## 9 SCCS Comment

10 This study is indicative of the importance of coating on the phototoxic properties of TiO<sub>2</sub>  
 11 nanoparticles.  
 12

## 14 Photoclastogenicity test *in vitro*

15 Guideline/method: Chromosomal aberration test in presence or absence of UV treatment

16 Test system: CHO-WBL cells

17 Replicates: Duplicate

18 Test item: See table

19 Batch: -

20 Vehicle: Ethanol (sample A), PBS (samples B and C), DMSO (D, E,F,G and H)

21 Concentrations: Three concentrations for each sample with as highest concentration either  
 22 5000 µg/ml or a dose that resulted in less than 50% cytotoxicity

23 Exposure: 3 h followed by 17 h recovery

24 UV dose: 750 mJ/cm<sup>2</sup> (provided 15 min after NP treatment initiation)

25 Negative control: Vehicle

26 Positive control: 8-methoxypsoralen (8-MOP), 4-nitroquinoline-1-oxide (NQO)

27 GLP: -

28 Published: yes

29 Reference: Theogaraj et al., 2007  
 30

## 31 Test items used:

Table 1  
 Description of ultrafine titanium dioxide particles tested

Sample code	Crystal type	Inorganic coating	Organic coating	Particle size
A	Anatase (80%), rutile (20%)	None	Trimethoxy caprylylsilane	Approximately 21 nm <sup>a</sup>
B	Anatase (80%), rutile (20%)	None, doped di-iron trioxide (2 ± 1%)	None	Approximately 21 nm <sup>a</sup>
C	Anatase (80%), rutile (20%)	None	None	Approximately 21 nm <sup>a</sup>
D	Rutile (100%)	Alumina (8–11%)	Simethicone (1–3%)	14 nm <sup>b</sup>
E	Anatase (100%)	Alumina (37%), silica (12–18%)	None	60 nm <sup>c</sup>
F	Rutile (100%)	Alumina (5–6.5%)	Dimethicone (1–4%)	20 nm <sup>b</sup>
G	Rutile (100%)	Alumina (3–8%)	Stearic acid (5–11%)	15 nm <sup>a</sup>
H	Rutile (100%)	Alumina (10.5–12.5%), silica (3.5–5%)	None	20–22 nm <sup>b</sup>

<sup>a</sup> Primary particle size determined by transmission electron microscopy (TEM).

<sup>b</sup> Primary particle size determined by X-ray diffraction.

<sup>c</sup> Characterisation by X-ray disc centrifugation (XDC) giving an aggregate rather than particle size.

32  
 33 The photoclastogenicity of TiO<sub>2</sub> was determined in CHO cells. S9 mix was not included in  
 34 the protocol. Cells were treated in the dark for 15 min and then UV radiated. After  
 35 irradiation the cultures were incubated in the dark, after which the medium was removed.  
 36 Cultures were washed and fresh medium was added for a further 17 h. Cells were then  
 37 harvested and stained slides were then evaluated for the presence of chromosomal  
 38 aberrations.  
 39

## 40 Results



1 No increases in chromosomal aberration frequencies were found either in the presence or  
2 absence of UV up to the highest treatment concentrations.

3  
4 Conclusion

5 No photogenotoxicity was observed under the applied testing conditions.

#### 6 7 **SCCS Comment**

8 Uptake of the NP into the cells was not evaluated. The UV treatment was performed shortly  
9 after initial exposure to the particles (15min). At this time uptake may have been limited.

### 12 **1.5.12 Human data**

13 A number of human studies have been quoted on different versions of skin patch test. Some  
14 of the studies have used TiO<sub>2</sub> materials for which no information on material  
15 characterisation has been provided, whilst others have been reviewed in relevant sections.

### 17 **1.5.13 Special investigations**

18 A number of studies have been provided, relating to cytotoxicity, coating stability and  
19 photostability of TiO<sub>2</sub> materials. Many of these studies have used TiO<sub>2</sub> materials for which  
20 information on material characterisation has not been provided.

### 22 **1.5.14 Human safety evaluation (including calculation of MoS)**

23 Given the very low, if any, dermal penetration of nano-TiO<sub>2</sub> when applied on skin, and in  
24 consideration of the low toxicity observed, the calculation of a margin of safety (MoS) is not  
25 relevant for this assessment.

26  
27 Any exposure to nano-TiO<sub>2</sub> via oral route from a dermally applied product is also likely to  
28 be insignificantly low. Again in consideration of the low toxicity observed, the calculation of  
29 a margin of safety (MoS) for the oral route is not relevant.

30  
31 In view of the concerns over safety of nano-TiO<sub>2</sub> via inhalation route, its use in applications  
32 that might lead to inhalation exposure (such as powders or sprayable products) is not  
33 recommended and therefore has not been considered in the calculation of MoS.

### 36 **1.5.15 Discussion**

#### 37 38 General considerations:

39 The submission consists of fifteen (15) TiO<sub>2</sub> nanomaterials that vary in terms of various  
40 physicochemical parameters. The studies provided in support of the submission range from  
41 old to recent ones. A major proportion of the (old) studies are on materials for which little  
42 or no information on characterisation has been provided, which makes it difficult to relate  
43 many of them to the nanomaterials under current assessment.

44  
45 The evaluation by the SCCS of these and other studies provided in this submission has  
46 shown that many of them are not relevant to the nanomaterials in the submission.  
47 Therefore the relevance and usefulness of the data provided for this evaluation is poor and  
48 patchy. It is difficult (in some cases impossible) to relate the studies to the types of  
49 nanomaterials under evaluation. It would have been more productive if a complete set of  
50 supporting data was provided on one (or a few) rather than several different TiO<sub>2</sub>  
51 nanomaterials in a single submission.

- 1  
2 Physicochemical properties:
- 3 - The studies provided in the submission relate to a range of TiO<sub>2</sub> materials that comprise  
4 micronized, ultrafine, or nano-sized particles. The physicochemical characterisation data  
5 include coated and non-coated materials, composed of rutile and/or anatase forms of  
6 TiO<sub>2</sub>. On the basis of the physicochemical data provided, the SCCS has considered the  
7 materials in three broad groups on the basis of crystalline form and photocatalytic  
8 activity.
  - 9 - The SCCS agrees that TiO<sub>2</sub> nanoparticles, due to agglomerative behaviour, are likely to  
10 be present in the final sunscreen products mainly in the form of agglomerates, which  
11 can also be in the nanoscale. It can therefore be assumed that the consumer is likely to  
12 be exposed mainly to TiO<sub>2</sub> agglomerates. However, it is also possible for the  
13 agglomerates to de-agglomerate under certain conditions of formulation/use. Therefore,  
14 the SCCS has considered the size of the primary particles more important than the size  
15 of agglomerates for the purposes of risk assessment.
  - 16 - As nanoparticles may have different properties and biokinetic behaviour than their  
17 soluble equivalents, it is important to know the exact purity/impurity profile of a  
18 nanomaterial intended for use in a cosmetic product (SCCS Guidance, SCCS/1484/12).  
19 This opinion therefore does not cover TiO<sub>2</sub> nanomaterials that have TiO<sub>2</sub> purity less than  
20 99%, and for which an acceptable impurity profile has not been provided. The opinion  
21 may, however, be also applicable to other TiO<sub>2</sub> nanomaterials that are similar to the  
22 nanomaterials in this opinion in terms of the physicochemical parameters listed in Tables  
23 1-3, and other specific provisions laid out in Section 2.
  - 24 - None of the materials evaluated in the submission is comprised of completely spherical  
25 particles because their reported aspect ratios are >1.0. However, the SCCS has  
26 accepted an aspect ratio range between 1.0 and 4.5 on the basis that a lower aspect  
27 ratio particle is less likely to be of a concern compared to higher aspect ratio ones.
  - 28 - Zeta potential measurements have been provided for some materials, and not for others  
29 due to difficulties in measuring zeta potential for hydrophobic nanomaterials.
  - 30 - Among the nanomaterials assessed, the SCCS has noted a potential concern in relation  
31 to photocatalytic activity, and stability of the coating, of some of the materials. It is  
32 stated by the Applicant that all coatings on the materials included in the submission are  
33 stable. Three (3) studies have been provided, which show that coatings are stable.  
34 However, from the other physicochemical data provided, it is less clear how stable the  
35 coatings are in final formulations. The photocatalytic activity data, which is measured in  
36 formulations, clearly indicate that either some of the materials were not completely  
37 coated, or some of the coatings (e.g. organic, organosilanes) were not so stable in the  
38 formulations. This is an important aspect to ascertain because application of a  
39 formulation containing a nanomaterial that has a significant photocatalytic activity may  
40 lead to local effects on sun-exposed skin. Such effects may or may not manifest during  
41 the immediate use, and it is important to investigate the possibility of latent effects  
42 following the use of a skin product that contained photocatalytic nanoparticles. This is  
43 because, whilst most studies on dermal absorption indicate that TiO<sub>2</sub> nanoparticles are  
44 not able to penetrate the skin deep enough to reach live cells of the epidermis/dermis,  
45 they do show that nanoparticles can penetrate into stratum corneum, and can also enter  
46 hair follicles and sweat glands. It is therefore possible that a trace amount of  
47 nanoparticles may remain embedded in stratum corneum, in hair follicles, and/or sweat  
48 glands, potentially over several days after skin application of a product and washing off.  
49 If the nanoparticles have a significant photocatalytic activity, there is a possibility that  
50 they may cause generation of reactive radical species on exposure to sunlight, long after  
51 the skin formulation had been applied and washed off. This, in a close proximity of living  
52 cells, raises a concern over the possibility of harmful effects. Generally metal(oxide)  
53 nanomaterials which exhibit a high photocatalytic activity are those that are either  
54 uncoated, partially coated, or have not been quenched by other means (e.g. doping) to  
55 adequately reduce photoreactivity. The TiO<sub>2</sub> nanomaterials in the current submission

- 1 that have a high photocatalytic activity include anatase materials in uncoated (S75-G)  
2 and coated forms (S75-F, S75-O). Three (3) other rutile coated nanomaterials also have  
3 comparatively lower but still significant levels of photocatalytic activity (S75-C, S75-D,  
4 S75-E).
- 5 - The SCCS considers up to 10% photocatalytic activity compared to corresponding non-  
6 coated or non-doped reference as acceptable.
  - 7 - In view of this, the SCCS does not recommend the use of nanomaterials that have a  
8 high photocatalytic activity (S75-F, S75-G, S75-O) in dermal formulations. These  
9 materials can only be recommended after appropriate coating/doping has been applied  
10 to quench their photocatalytic activity down to acceptable levels.
  - 11 - Three rutile materials (S75-C, S75-D, S75-E) with relatively lower but still significant  
12 levels of photocatalytic activity may be used in dermal formulations, but further  
13 investigations over longer post-application periods may be necessary to ascertain that  
14 they do not pose a risk due to photocatalytic activity.

#### 15 Acute toxicity:

- 16 - The studies provided on acute oral toxicity in the submission mainly relate to TiO<sub>2</sub>  
17 nanomaterials that are anatase/rutile mixtures, coated with trimethoxy-n-octyl-silane.  
18 From the limited relevant information provided, and considering that oral intake is not  
19 likely to be the major route of exposure to TiO<sub>2</sub> nanomaterials from dermal application  
20 of formulations, the acute oral toxicity of TiO<sub>2</sub> is unlikely to be of a concern.
- 21 - The studies provided on acute dermal toxicity relate to an ultrafine TiO<sub>2</sub> material and a  
22 material described as 'natural colour', and are therefore of no relevance to the  
23 assessment of nanomaterials.
- 24 - No study has been provided on acute inhalation toxicity. Sub-chronic (inhalation) and  
25 chronic (instillation) studies have indicated substantial inflammatory responses and  
26 overload associated with diminishing particle clearance in a dose dependent manner,  
27 and histological indications of epithelial hypertrophy and hyperplasia.
- 28 - The limited relevant information provided in the submission, and other information in  
29 the open literature, indicates that TiO<sub>2</sub> nanomaterials are likely to be non-toxic via oral  
30 or dermal application routes. However, inhalation exposure to TiO<sub>2</sub> nanoparticles is  
31 likely to cause substantial inflammatory effects in the lung.

32

#### 33 Skin irritation:

- 34 - Only two of the studies provided are relevant to the TiO<sub>2</sub> nanomaterials. They relate to  
35 anatase/rutile mixtures, coated with trimethoxy-n-octyl-silane. The results showed  
36 primary irritation index between zero and 0.3. Two studies using ultrafine grade  
37 materials showed the mean irritation scores of 0.3 and 1.58-1.92 during 5 day repeat  
38 applications on rabbit skin. Other studies also showed the tested materials to be either  
39 mild- or non- irritant to rabbit and guinea pig skin, but it is not clear whether the tested  
40 materials were nanomaterials.
- 41 - From the limited relevant information, it can be considered that TiO<sub>2</sub> nanomaterials are  
42 likely to mild- or non- irritant to skin.

43

#### 44 Eye irritation:

- 45 - Two studies tested TiO<sub>2</sub> anatase/rutile mixtures, coated with trimethoxy-n-octyl-silane.  
46 From the studies, the derived primary irritation index was between zero and 0.3. A  
47 different study used ultrafine rutile material coated with alumina/silica and regarded the  
48 tested material as slightly irritant to rabbit eye. Another study found the tested TiO<sub>2</sub>  
49 materials to be moderately irritant to rabbit eye, but it is not clear whether the  
50 material was a nanomaterial.
- 51 - From the limited relevant data provided, eye irritation potential of nano-TiO<sub>2</sub> appears to  
52 be low.

- 1  
2 Skin sensitisation:
- 3 - Two of the provided studies have regarded TiO<sub>2</sub> nanomaterials (anatase/ rutile mixture,  
4 coated with trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane) as non-sensitiser.  
5 Another ultrafine material (rutile, coated with alumina/silica) is classified as a weak  
6 sensitiser, but characterisation data (particle size distribution) has not been reported to  
7 indicate what proportion of the particles was in the nano-scale.
  - 8 - Due to the absence of skin penetration of TiO<sub>2</sub> as demonstrated by many studies  
9 included in this dossier, the usefulness of the Buehler test for assessing sensitisation  
10 potency of nanomaterials is doubtful as it is based on exposure to intact skin.
  - 11 - From the limited relevant data provided, TiO<sub>2</sub> nanomaterials appear to be non- or weak  
12 skin sensitisers.

- 13  
14 Dermal absorption:
- 15 - A number of *in vitro* and *in vivo* dermal penetration studies have been provided with the  
16 submission. In addition, there is a body of open literature on this subject. The evidence  
17 from these studies supports the conclusion that TiO<sub>2</sub> nanoparticles are unlikely to  
18 penetrate across the skin to reach viable cells of the epidermis. In these studies, TiO<sub>2</sub>  
19 nanoparticles have been shown to penetrate only to the outer layers of the stratum  
20 corneum, and there is as yet no conclusive evidence to show that they do reach living  
21 cells of the epidermis/dermis. Studies have also shown that TiO<sub>2</sub> nanoparticles do not  
22 penetrate the (simulated) sunburnt skin.
  - 23 - Despite the extensive database showing a general lack of TiO<sub>2</sub> nanoparticle absorption  
24 via the dermal route, there are a few gaps in the knowledge. For example, it is not clear  
25 whether TiO<sub>2</sub> nanoparticles will be able to penetrate through cuts and bruises, or over  
26 repeated or long term applications of a sunscreen formulation.
  - 27 - A number of studies have indicated that TiO<sub>2</sub> nanoparticle can enter the hair follicles  
28 and sweat glands, and that they may remain there for a number of days. This is a  
29 scenario in which TiO<sub>2</sub> nanoparticles are likely to get and remain in a close proximity to  
30 the living cells for a length of time. A photocatalytic nanoparticle in such a situation may  
31 cause generation of reactive oxyradical species (ROS) and potential harmful effects  
32 when exposed to sunlight. As mentioned before, more data would be needed to justify  
33 the use of those TiO<sub>2</sub> nanoparticles in skin applications that have a considerable level of  
34 photocatalytic activity.

- 35  
36 Repeated dose toxicity:
- 37 - Only two of the four provided subchronic studies on repeated dose toxicity are relevant  
38 to the TiO<sub>2</sub> nanomaterials under evaluation. However, these studies relate to oral  
39 exposure only, from which a LOAEL of 5 mg/kg bw/d has been derived.
  - 40 - No chronic toxicity study (>12 months) is provided, although a chronic inhalation study  
41 has been provided (Section 3.3.1.3).

- 42  
43 Inhalation toxicity:
- 44 - Studies in open literature indicate that subacute repeated dose respiratory toxicity  
45 studies with nano size TiO<sub>2</sub> induce an acute inflammation in the lungs that may be  
46 reversible depending on the dose and the time evaluated after exposure. In view of this,  
47 acute inflammation (spray) applications, which may result in inhalation exposure is not  
48 recommended by the SCCS.

- 49  
50 Mutagenicity/ Genotoxicity:
- 51 - Although an extensive range of studies on mutagenicity has been provided in the  
52 submission, most of them have not been conducted in any special consideration of the  
53 nano-related properties of the test materials.

- 1 - Several studies have been performed mainly to investigate mechanistic effects relating  
2 to DNA damage and genotoxic properties. These studies are usually not performed  
3 according to specific genotoxicity guidelines (e.g. OECD). Many of the studies have not  
4 evaluated the effects in a dose- and/or time- dependent manner. Those that have  
5 addressed this, often reveal no clear dose- or time- dependent effects.
- 6 - From the provided studies, and open literature, TiO<sub>2</sub> particles have also been  
7 reported, or suggested, to interfere with the assays, because:
- 8 - Micronucleus scoring is difficult in the presence of TiO<sub>2</sub> particles. This effect  
9 was suggested to explain for the occasionally observed decreases in MN counts  
10 after TiO<sub>2</sub> treatment (Falck et al., 2009).
- 11 - It has been suggested (although not shown) that artefacts may be caused in  
12 relation to the use of cytochalasin B for micronucleus testing. On one hand, it is  
13 suggested that nanoparticles may interfere with cytochalasin B (binding), and  
14 on the other, that the cytochalasin B may act as an inhibitor of the uptake of  
15 nanoparticles in cells potentially leading to false negatives (Landsiedel et al.,  
16 2010).
- 17 - Due to the current lack of information on the possible cellular uptake and  
18 subsequent translocation of TiO<sub>2</sub> nanoparticles to nucleus, it is not possible to  
19 draw a conclusion on whether or not exposure to TiO<sub>2</sub> nanomaterials can lead  
20 to mutagenic effects.
- 21 - Overall in a number of assays, TiO<sub>2</sub> nano particles were observed to induce DNA  
22 damage, so TiO<sub>2</sub> nano particles have to be considered genotoxic.
- 23 - It is also of note that appropriate coating of nanomaterial to quench surface  
24 photocatalytic activity will also reduce the likelihood of generation of reactive oxygen  
25 species (ROS), which may in turn reduce the chances of genotoxicity.

26

#### 27 Carcinogenicity:

- 28 - Pigmentary and ultrafine TiO<sub>2</sub> materials have been tested for carcinogenicity by oral  
29 administration in mice and rats, by inhalation exposure in rats and female mice, by  
30 intratracheal administration in hamsters and female rats and mice, and by subcutaneous  
31 injection in rats and by intraperitoneal administration in male mice and female rats.
- 32 - According to the evaluation of TiO<sub>2</sub> by IARC (2010), induction of lung tumours was  
33 observed in two inhalation studies with rats. Two other inhalation studies in rats, and  
34 one in female mice gave negative results. Intratracheally instilled female rats showed  
35 an increased incidence of lung tumours following treatment with two types of titanium  
36 dioxide. Tumour incidence was not increased in intratracheally instilled hamsters and  
37 female mice. Oral, subcutaneous and intraperitoneal administration did not produce a  
38 significant increase in the frequency of any type of tumour in mice or rats. IARC  
39 concluded that there is inadequate evidence in humans for the carcinogenicity of  
40 titanium dioxide but sufficient evidence in experimental animals for the carcinogenicity  
41 of titanium dioxide. Both nano and non nano size Titanium dioxide was classified as a  
42 Group 2B carcinogen (Possibly carcinogenic to humans).
- 43 - In their recent evaluation of TiO<sub>2</sub> NIOSH has determined that ultrafine TiO<sub>2</sub> which  
44 contains nano-sized TiO<sub>2</sub> is a potential occupational carcinogen and, that there is  
45 insufficient data to classify fine TiO<sub>2</sub> as potential occupational carcinogen after inhalation  
46 (NIOSH 2011).
- 47 - Nano titanium dioxide has been studied in 2 two-stage skin carcinogenicity studies with  
48 mice, 2 two-stage skin carcinogenicity studies with rats, and one two-stage lung study  
49 with rats. Both noncoated (ncTiO<sub>2</sub>) and coated titanium dioxide have been studied in the  
50 two-stage mouse skin carcinogenicity studies with CD1 mice and a transgenic mouse  
51 strain (rasH2). In one well performed study with non-coated and alumina and stearic  
52 acid coated TiO<sub>2</sub>, no promoter activity was found (Furukawa et al., 2011). Promoter

1 activity was also not found for ncTiO<sub>2</sub> in the other study (Sagawa et al., 2012).  
2 However, it is difficult to draw a firm conclusion from this study with silica coated  
3 titanium dioxide due to lack of positive controls and very high tumour incidence in the  
4 'initiated' mice.

5 - Non-coated titanium dioxide was studied in 2 two-stage rat skin carcinogenicity studies.  
6 Although, no tumour promoter activity was observed, it is difficult to draw any  
7 conclusion since little experience with the model used is available and no positive  
8 controls have been used in the studies.

9 - One, two-stage rat lung carcinogenicity study has been carried out with non coated  
10 titanium dioxide. The rats were 'initiated' by DHPN in the drinking water prior to intra-  
11 pulmonary spraying with ncTiO<sub>2</sub>. The experiment demonstrated promoter activity of  
12 ncTiO<sub>2</sub> (Xu et al., 2011).

13 - Since TiO<sub>2</sub> particles have shown carcinogenic activity (after inhalation) and since nano  
14 ncTiO<sub>2</sub> showed promoter activity after intra-pulmonary spraying, the use of nano TiO<sub>2</sub>  
15 in sprayable applications is not recommended by the SCCS.

16  
17 Reproductive toxicity  
18 - No study has been provided on reproductive toxicity that is relevant to the  
19 nanomaterials under assessment. A review article covering exploratory studies in mice  
20 has been provided, which relates to the use of a TiO<sub>2</sub> material which is <10µm (with no  
21 further information), and a TiO<sub>2</sub> nanomaterial with primary particle size 25-70 nm (no  
22 further information).

23 - Other studies in open literature have indicated the possibility of placental transport in  
24 pregnant animals into the foetus, or found effects in the offspring for various  
25 manufactured nanomaterials including nano-TiO<sub>2</sub>. However, the information relating to  
26 this endpoint is patchy and therefore inconclusive.

27  
28 Photo-induced toxicity  
29 - Only a few studies have been provided that are relevant to the nanomaterials under  
30 assessment.  
31 - These indicate that TiO<sub>2</sub> materials may not be photo-sensitisers. However, concerns  
32 regarding the use of photocatalytic nanomaterials in dermal formulations discussed  
33 above need to be taken into consideration.

34 - Several studies have specifically addressed photo-sensitization effects TiO<sub>2</sub>. However,  
35 the outcomes of these studies need to differentiate between photo-sensitization and  
36 other local effects on skin (taking into account the aspect of penetration), versus  
37 potential effects at other target sites.

38  
39 Toxicokinetics:  
40 - Two studies have been provided in the submission on toxicokinetics of TiO<sub>2</sub> following  
41 intravenous injection in rats and mice. In addition, there are few other relevant studies  
42 in the open literature relating to inhalation and intravenous, as well as limited  
43 (questionable) information on oral administration routes.

44 - The available evidence suggests that, if TiO<sub>2</sub> particles become systemically available by  
45 the oral and inhalation uptake pathway, they are likely to accumulate mainly in the liver,  
46 followed by a very slow rate of clearance.

47  
48 Special investigations:  
49 No relevant specific studies have been provided apart from those already discussed above  
50 under relevant endpoints.  
51



1  
2

## 2. CONCLUSIONS

1  
2 This opinion is based on the risk assessment of nano-sized titanium dioxide (TiO<sub>2</sub>) for use  
3 as a UV filter in sunscreen formulations. It is important to note that risk assessment of  
4 nanomaterials in general still has certain gaps in the knowledge - for instance in relation to  
5 the behaviour of nanoparticles in a test medium, or in the animals. This has led to  
6 uncertainties over whether the nanoparticles are able to reach and interact with various  
7 moieties and biological target sites, and whether, on dermal application, they may penetrate  
8 through damaged skin, or during repeated or long term applications. There are also  
9 uncertainties over the validity of the currently available tests used for nanomaterials.  
10 However, a positive toxic response in these tests is still considered valid for risk assessment  
11 as it would indicate a hazard potential.

12 As discussed above, the safety data provided in support of the fifteen (15) nanomaterials is  
13 quite patchy, and is only partially useful for any of the given nanomaterials. However, the  
14 SCCS took the view that this submission could be considered for evaluation as an exception.  
15 This is because some additional information on TiO<sub>2</sub> nanomaterials is available in open  
16 literature which is relevant for this evaluation. Also, for example, although the safety data  
17 provided in the submission on rutile nanomaterials is insufficient, the studies on anatase  
18 form (or rutile/anatase mixtures) could be considered as a surrogate because published  
19 studies in open literature have regarded anatase a greater safety concern than the rutile  
20 form. However, as the evaluation is still based on limited information which could be related  
21 to specific nanomaterial types in the submission, this opinion is limited to the nanomaterials  
22 indicated below:

- 23
- 24 - On the basis of physicochemical considerations discussed above, this opinion applies to  
25 the TiO<sub>2</sub> nanomaterials presented in this submission. In addition, the opinion may also  
26 be applicable to other TiO<sub>2</sub> nanomaterials that are similar to the nanomaterials covered  
27 in this opinion in terms of physicochemical parameters listed in Tables 1-3, and the  
28 specific provisions laid out in the overall conclusions below.
  - 29 - It needs to be stressed that the main consideration in the current assessment is the  
30 apparent lack of penetration of TiO<sub>2</sub> nanoparticles through skin, which is supported by a  
31 body of evidence both in the form of studies provided by the Applicant and other studies  
32 reported in open literature. In the absence of a systemic exposure, a margin of safety  
33 (MoS) could not be calculated for TiO<sub>2</sub> nanomaterials in this assessment. From the  
34 limited relevant information provided in the submission, and the information from open  
35 literature, the SCCS considers that TiO<sub>2</sub> nanomaterials in a sunscreen formulation are  
36 unlikely to lead to:
    - 37 ○ systemic exposure to nanoparticles through human skin to reach viable cells of  
38 the epidermis, dermis, or other organs;
    - 39 ○ acute toxicity via dermal application or incidental oral ingestion. This, however,  
40 does not apply to sprayable applications that may lead to inhalation exposure of  
41 TiO<sub>2</sub> nanomaterials, which may result in lung inflammation;
    - 42 ○ skin irritation, eye irritation, or skin sensitisation when (repeatedly) applied on  
43 healthy skin (except possible phototoxicity of insufficiently coated nanomaterials);
    - 44 ○ reproductive effects when applied on healthy skin.
  - 45 - Some TiO<sub>2</sub> nanoparticles have been shown to be able to damage DNA and should be  
46 considered genotoxic. However as negative results have also been reported, the current  
47 evidence in relation to potential genotoxicity of TiO<sub>2</sub> nanomaterials is not conclusive.  
48 TiO<sub>2</sub> particles have also shown to lead to carcinogenic effects after inhalation. These  
49 manifestations are a major hazard concern. However, no penetration was found through  
50 the stratum corneum of reconstructed human full thickness skin models and no DNA  
51 damage was detected by the Comet assay in these cells in contrast to epidermal cell  
52 line. Considering the absence of a systemic exposure, the SCCS considers that the use

- 1 of nano TiO<sub>2</sub> in dermally applied cosmetic products should not pose any significant risk  
2 to the consumer.
- 3 - Evidence on acute and sub-chronic inhalation toxicity does not support the overall safety  
4 of use of TiO<sub>2</sub> nanomaterial formulations for spray applications. In addition, tumour  
5 promoter activity of nano (non-coated) TiO<sub>2</sub> has been shown after intra-pulmonary  
6 spraying. Therefore the SCCS does not recommend the use of nano TiO<sub>2</sub> in sprayable  
7 applications. This may be reconsidered if further evidence is provided to rule out the  
8 possibility that the nanoparticles can reach the lower respiratory tract during spray  
9 applications.
- 10 - Although there is no conclusive evidence at present to indicate penetration of TiO<sub>2</sub>  
11 nanoparticles through the skin to viable cells of the epidermis, a number of studies have  
12 shown that they can penetrate into the outer layers of the stratum corneum, and can  
13 also enter hair follicles and sweat glands. It is therefore recommended not to use TiO<sub>2</sub>  
14 with substantially high photocatalytic activity (e.g. S75-F, S75-G, S75-O) in sunscreen  
15 formulations. Other TiO<sub>2</sub> nanomaterials that have a relatively lower but still significant  
16 level of photocatalytic activity (e.g. S75-C, S75-D, S75-E) may be used, but further  
17 investigations over longer post-application periods taking into account the potential  
18 photocatalytic activity post-application, whilst allowing for appropriate lag-time and  
19 using realistic application scenarios may be necessary to ascertain that they do not pose  
20 a risk due to photocatalytic activity.

21

## 22 Overall conclusion

23 *1. Does SCCS consider that use of titanium dioxide in its nanoform as an UV-filter in*  
24 *cosmetic products in a concentration up to maximum 25.0 % is safe for the consumers*  
25 *taken into account the scientific data provided?*

26

27 On the basis of the available evidence, the SCCS has concluded that the use of TiO<sub>2</sub>  
28 nanomaterials with the characteristics as indicated below, at a concentration up to 25% as a  
29 UV-filter in sunscreens, can be considered to not pose any risk of adverse effects in humans  
30 after application on healthy, intact or sunburnt skin. This, however, does not apply to  
31 applications that might lead to inhalation exposure to TiO<sub>2</sub> nanoparticles (such as powders  
32 or sprayable products). Furthermore, this assessment applies to the TiO<sub>2</sub> nanoparticles  
33 presented in the submission, but may also be applicable to other TiO<sub>2</sub> nanomaterials that  
34 are similar to the parameters in Tables 1-3, i.e. TiO<sub>2</sub> nanomaterials that:

35 • have TiO<sub>2</sub> purity of  $\geq 99\%$ , or in case of a lesser purity, the impurities must be  
36 demonstrated to be safe for use in cosmetic formulations;

37 • are composed of mainly the rutile form, or rutile with up to 5% anatase, with  
38 crystalline structure and physical appearance as described in the current submission,  
39 i.e. clusters of spherical, needle, or lanceolate shapes;

40 • have a median particle size based on number size distribution of 30 to 100 nm  
41 (measured by different methods) as submitted in the dossier, or larger. Thus whilst  
42 primary particle size may be smaller (around 10 nm), the median particle size of TiO<sub>2</sub>  
43 nanomaterials in a cosmetic formulation must not be smaller than 30 nm in terms of  
44 number based size distribution;

45 • have an aspect ratio from 1.0 and up to 4.5, and volume specific surface area up to  
46 460 m<sup>2</sup>/cm<sup>3</sup>;

47 • are coated with one of the coating materials described in Table 1, and the coatings are  
48 stable in the final formulation and during use. Other cosmetic ingredients applied as  
49 stable coatings on TiO<sub>2</sub> nanomaterials can also be used, provided that they can be  
50 demonstrated to the SCCS to be safe and the coatings do not affect the particle  
51 properties related to behaviour and/or effects, compared to the nanomaterials covered  
52 in this opinion.

- 1       • are photostable in the final formulation;
- 2       • do not have photocatalytic activity. However, the SCCS considers up to 10%
- 3       photocatalytic activity compared to corresponding non-coated or non-doped reference
- 4       as acceptable.

5 It is also worth highlighting again that this opinion is based on the currently available  
6 scientific evidence which shows an overall lack of dermal absorption of TiO<sub>2</sub> nanoparticles.  
7 If any new evidence emerges in the future to show that the TiO<sub>2</sub> nanoparticles used in a  
8 sunscreen formulation can penetrate skin (healthy, compromised, or damaged skin) to  
9 reach viable cells, then the SCCS may consider revising this assessment.

10 It should also be noted that the risk assessment of nanomaterials is currently evolving. In  
11 particular, the toxicokinetics aspects have not yet been fully explored in the context of  
12 nanoparticles (e.g. the size dependency). Also, long term stability of the coatings remains  
13 unclear. At the moment, testing of nanomaterials and the present assessment, are both  
14 based on the methodologies developed for substances in non-nano form, and the currently  
15 available knowledge on properties, behaviour and effects of nanomaterials. This assessment  
16 is, therefore, not intended to provide a blue-print for future assessments of other  
17 nanomaterials, where depending on the developments in methodological risk assessment  
18 approaches and nano-specific testing requirements, additional/different data may be  
19 required and/or requested on a case-by-case basis.

20 It is also important to note that the potential ecotoxicological impacts of nano TiO<sub>2</sub> when  
21 released into the environment have not been considered in this opinion.

22  
23 *2. In order for the COM to differentiate in the regulation between materials in its nanoform*  
24 *and its non-nano form, can the SCCS give quantitative and qualitative guidance on how this*  
25 *differentiation should be given based on the particle size distribution or other parameters?*

26 A detailed SCCS guidance on risk assessment of nanomaterials in cosmetics has recently  
27 been published (SCCS/1484/12). The guidance provides a detailed account of the important  
28 nano-related parameters that should be considered in relation to physicochemical  
29 characterisation, hazard identification, exposure assessment and risk assessment of  
30 nanomaterials.

31

### 32                   **3. MINORITY OPINION**

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## 1 **ABBREVIATIONS AND GLOSSARY OF TERMS**

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2		
3	BET	Brunauer-Emmett-Teller method based on nitrogen gas absorption
4	CAS	A chemical registry system established by the Chemical Abstracts
5		Service (CAS)
6	ECVAM	European Centre for the Validation of Alternative Methods
7	EDX	Energy Dispersive X-ray
8	HPLC	High performance liquid chromatography
9	ICP-MS	Inductively coupled plasma mass spectrometry
10	In vitro test method	Biological method that uses organs, tissue sections and tissue
11		cultures, isolated cells and their cultures, cell lines and subcellular
12		fractions, or non-biological method that uses chemical interaction
13		studies, receptor binding studies, etc [Rogiers and Beken 2000]
14	ISO	International Organization for Standardization
15	IARC	International Agency for Research against Cancer
16	IUPAC	A system of chemical nomenclature established by the International
17		Union of Pure and Applied Chemistry (IUPAC)
18	Local effects	A Local effect refers to an adverse health effect that takes place at
19		the point or area of contact. The site may be skin, mucous
20		membranes, the respiratory tract, gastrointestinal system, eyes, etc.
21		Absorption does not necessarily occur.
22	Nanomaterial	An insoluble or biopersistent and intentionally manufactured material
23		with one or more external dimensions, or an internal structure, on
24		the scale from 1 to 100 nm [Regulation (EC) No 1223/2009]
25	Nanoparticle	A nano-object with all three external dimensions in the nanoscale
26		[ISO/TS 27687:2008, Nanotechnologies -- Terminology and
27		definitions for nano]. For the purpose of this assessment the term
28		'nanoparticle' is used to also include other forms of nano-object, such
29		as nano-rods, nano-tubes, etc.
30	NPs	Nanoparticles
31	Nanoscale	Size range from approximately 1 nm to 100 nm [ISO/TS 80004-
32		1:2010, Nanotechnologies -- Vocabulary]
33	OECD	Organisation for Economic Co-operation and Development
34	PBS	Phosphate buffered saline
35	ROS	Reactive Oxygen Species
36	SCCNFP	Scientific Committee on Cosmetic products and Non-Food Products
37		intended for consumers
38	SCCP	Scientific Committee on Consumer Products
39	SCCS	Scientific Committee on Consumer Safety
40	SED	Systemic Exposure Dosage
41	SEM	Scanning electron microscopy
42	Solubility	The terms 'solubility' and 'persistence' are often used to describe the
43		rate of "degradation". As such there are a number of definitions of
44		solubility (see SCENIHR Opinion 'Scientific Basis for the Definition of
45		the Term "Nanomaterial", 8 December 2010). In the context of this
46		assessment, solubility means disintegration of a nanomaterial in an



1		aqueous medium or biological environment into molecular
2		components with the loss of nano features.
3	Systemic effects	Systemic effect refers to an adverse health effect that takes place at
4		a location distant from the body's initial point of contact and
5		presupposes absorption has taken place.
6	TEM	Transmission electron microscopy
7	TiO <sub>2</sub> :	Titanium Dioxide
8	UV-Vis	Ultraviolet-visible spectrophotometry
9	Validated method	A standard method for which the relevance and reliability have been
10		established for a particular purpose, usually through an inter-lab
11		comparison, which found uncertainties in the measurements
12		acceptable..
13	VSSA	Volume specific surface area (see Kreyling et al., 2010)
14	XRD:	X-ray diffraction
15		