化粧品產品資訊檔案(範例)

<亮澤護唇蜜>

＜PIF **無特定之格式**，本範例**僅提供參考用**＞

中華民國 112 年 10 月

目　　錄

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I. 產品敘述

(1) 產品基本資料

項目	內容描述
產品名稱	亮澤護唇蜜
產品類別	唇蜜、唇油
產品劑型	油膏
用途	彩妝
製造作業場所資訊	製造廠名稱：XX 化粧品股份有限公司 廠址：〇〇市〇〇區〇〇路〇〇號 國別：台灣
包裝作業場所資訊	包裝廠名稱：YY 股份有限公司 廠址：〇〇市〇〇區〇〇路〇〇號 國別：台灣
產品製造業者資訊	製造業者：AJP 化粧品股份有限公司 地址：〇〇市〇〇路〇〇段 XX 號 公司負責人：李〇基 聯絡電話：02-2xxx-xxxx 統一編號：0123XXXX

(2) 完成產品登錄之證明文件

登錄號碼：0123XXXX TEST 1000000011

(3) 全成分名稱及其各別含量

INCI Name	Cas No.	w/w%	功能
Mineral Oil	8042-47-5	69.1	溶劑
Diisostearyl Malate	81230-05-9	12.0	潤膚劑
Simmondsia Chinensis (Jojoba) Seed Oil	61789-91-1	5.0	潤膚劑
Quaternium-18 Bentonite	68953-58-2	4.0	黏度控制劑
Butylene/Ethylene/Styrene Copolymer	68648-89-5	3.9	黏度控制劑
Ethylene/Propylene/Styrene Copolymer	66070-58-4	3.8	黏度控制劑
Fragrance	-	1.0	香精
Calcium Titanium Borosilicate	65997-17-3	0.53	填充劑
Tocopheryl Acetate	7695-91-2	0.5	抗氧化劑
Titanium Dioxide	13463-67-7	0.076	色素
Tin Oxide	18282-10-5	0.004	填充劑
Iron Oxides	1309-37-1	0.09	色素
Total		100.0	

(4) 產品標籤、仿單、外包裝或容器

項目	資料
內包裝/容器 (正反面)	 
標籤/仿單	品名：亮澤護唇蜜 用途：滋潤唇部，增加唇部光澤感。 用法：沾取適量，均勻塗抹於唇部。 全成分：Mineral Oil、Diisostearyl Malate、Simmondsia Chinensis (Jojoba) Seed Oil、Quaternium-18 Bentonite、Butylene/Ethylene/Styrene Copolymer、Ethylene/Propylene/Styrene Copolymer、Fragrance、Calcium Titanium Borosilicate、Tocopheryl Acetate、Titanium Dioxide、Tin Oxide、Iron Oxides。 保存方法：請置於陰涼處請勿直接陽光照射以免變質。 製造業者/地址/電話：AJP化粧品股份有限公司/ oo市oo路oo段XX號 / 02-2xxx-xxxx 製造日期：2022.08.06　　有效期間： 3年 批號：IT22080F　　容量： 10 mL 使用注意事項：僅限使用於唇部，使用後若有不適請立即停止使用，若症狀持續未緩解，需立即就醫。

(5) 製造場所符合化粧品優良製造準則之證明文件或聲明書

衛 生 福 利 部

化 粧 品 優 良 製 造 證 明 書

證號：(C)GMPOOOO-OOO

製造廠（場所）名稱：

製造廠（場所）地址：

核定劑型及作業項目：

本證明書依據化粧品衛生安全管理法第 29 條規定發給。
本部係依據「化粧品優良製造準則」之規定進行查核，該優良製造準則之要求符合國際標準化組織(ISO)發布之 ISO 22716：2007。

衛生福利部

發 證 日 期：　　年　　月　　日
有 效 日 期：　　年　　月　　日

XXXX(流水號)

符合化粧品優良製造準則聲明書

Declaration of Conformity

本業者/本廠生產之化粧品符合中華民國之化粧品優良製造準則，產品資料如下：

I hereby declare that the products described below manufactured in conformity with Cosmetic Good Manufacturing Practice

一、製造廠名稱：

Manufacturer's Name

二、製造廠地址：

Manufacturer's Address

三、製造劑型：

Product forms

四、作業項目：

The process of operations

以上聲明書所保證之內容，如有造假不實或違背相關法規等情事，本業者/本人願自行負擔法律上一切責任。

Where violations of this declaration occur, I agree to take the legal responsibilities.

立聲明書人：　　(Signature)

Applicant

申請廠商蓋公司章

負責人/代表人：　　(Signature)

Person in charge

負責人或代表人章

統一編號或身分證字號：

Company Tax ID No. / ID Number

地址：

Address:

中 華 民 國　　年　　月　　日

Date　　year　　month　　day

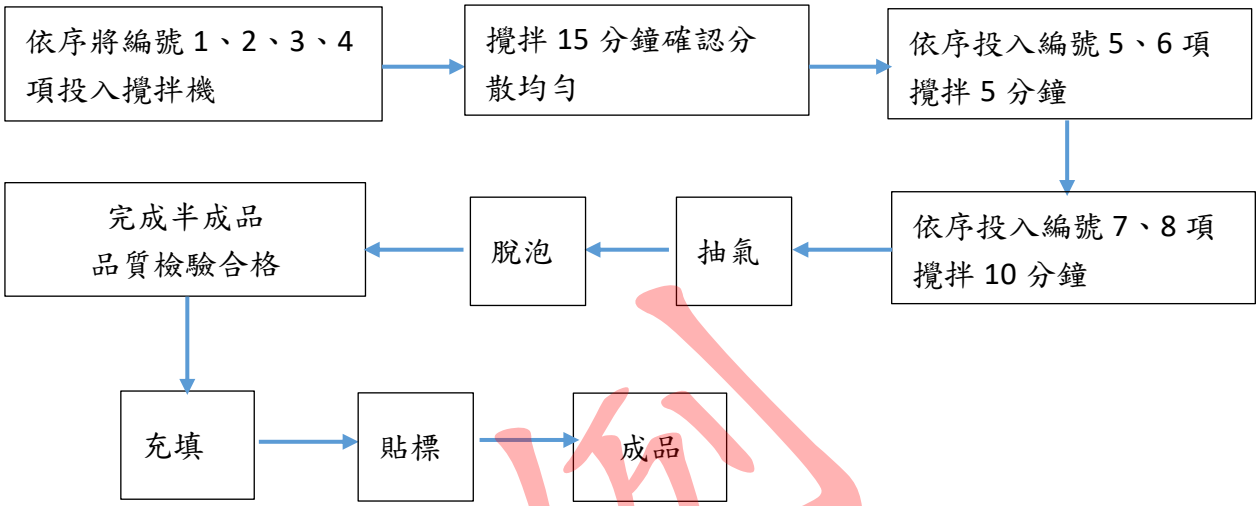
(6) 製造方法、流程

編號	Trade name (Product Name)	INCI name	Cas No.	w/w%
1	Versagel® M 750	Mineral Oil (90%)	8042-47-5	76.8
		Butylene/Ethylene/Styrene Copolymer (~5%)	68648-89-5	
		Ethylene/Propylene/Styrene Copolymer (~5%)	66070-58-4	
2	COSMOL 222	Diisostearyl Malate	81230-05-9	12
3	JOJOBA OIL GOLDEN	*Simmondsia Chinensis* (Jojoba) Seed Oil	90045-98-0	5
4	BENTONE® 38	Quaternium-18 Bentonite	68953-58-2	4
5	-	Fragrance	-	1
6	DL-alpha-Tocopheryl Acetate	Tocopheryl Acetate	7695-91-2	0.5
7	Glare® Glitter RedGL-7401E	Calcium Titanium Borosilicate(80%)	65997-17-3	0.4
		Titanium Dioxide(19%)	13463-67-7	
		Tin Oxide(1%)	18282-10-5	
8	Glare® Brilliant Russet GL-6600K	Calcium Titanium Borosilicate(70%)	65997-17-3	0.3
		Iron Oxides(30%)	1309-37-1	

製程簡述：

1. 依序將編號 1、2、3、4 項投入攪拌機下攪拌 15 分鐘確認分散均勻。
2. 依序投入編號 5、6 項攪拌 5 分鐘。
3. 依序投入編號 7、8 項攪拌 10 分鐘。
4. 抽氣。
5. 脫泡。
6. 完成半成品，品質檢驗合格後進行後續充填及貼標。

製程流程圖：


依序將編號 1、2、3、4 項投入攪拌機
攪拌 15 分鐘確認分散均勻
依序投入編號 5、6 項攪拌 5 分鐘
依序投入編號 7、8 項攪拌 10 分鐘
抽氣
脫泡
完成半成品品質檢驗合格
充填
貼標
成品

(7) 使用方法、部位、用量、頻率及族群

使用方法、部位及用量：沾取適量，均勻塗抹於唇部。

使用族群：青少年、成年人。

使用頻率：每日兩次。

(8) 產品使用不良反應資料

目前本產品尚未有任何不良反應事件報告。如有不良反應和嚴重不良反應的資料時會及時提供給安全資料簽署人員進行確認與評估，並更新於本產品資訊檔案中。

II. 品質資料

(9) 產品及各別成分之物理及化學特性

成品規格檢驗報告

亮澤護唇蜜 CoA			
檢測項目	**規格**	**實際檢驗結果**	**檢驗方法**
外觀	不流動油膏狀	不流動油膏狀	目視
顏色	紅棕色帶珠光粉	紅棕色帶珠光粉	目視
氣味	薄荷香	薄荷香	嗅覺
黏度(at 25 °C)	30,000~60,000 mPa·s	38,630 mPa·s	使用已校正之黏度計依黏度計檢測方法測定
密度(at 25 °C)	1.0±0.05 g/cm^3	0.95 g/cm^3	定量瓶
微生物規格	生菌數 < 100 cfu/g 不得檢出： 大腸桿菌 綠膿桿菌 金黃色葡萄球菌 白色念珠菌	生菌數 未檢出 (<10 cfu/g)； 大腸桿菌 陰性； 綠膿桿菌 陰性； 金黃色葡萄球菌 陰性； 白色念珠菌 陰性	參考衛生福利部食品藥物管理署 109.07.28 及 111.04.21 公告建議檢驗方法-化粧品中微生物檢驗方法及化粧品中白色念珠菌之檢驗方法。
檢測人員/日期		(請簽名並加上日期)	
複核人員/日期		(請簽名並加上日期)	

各成分物理化學特性

- 由 AJP 化粧品股份有限公司及安全資料簽署人員彙整各成分之安全資料表、檢驗成績書或技術資料表，另存放於成分物理化學特性檔案夾(附錄 1)。
- 安全資料簽署人員依據上述資料內容摘錄各成分物理化學特性如下：

1. Trade name (Product name)：VERSAGEL® M 750

INCI name：Mineral Oil (and) Ethylene/Propylene/Styrene Copolymer (and) Butylene/Ethylene/Styrene Copolymer

Mineral oil

Modify Date: 2022-01-11 13:13:40

	Common Name	Mineral oil		
	CAS Number	8042-47-5 (/en/baike/1198662.html)	**Molecular Weight**	23.9979
	Density	0.877	**Boiling Point**	N/A
	Molecular Formula	N/A	**Melting Point**	N/A
	MSDS	Chinese USA	**Flash Point**	220 °C

Chemical & Physical Properties

Density	0.877
Molecular Weight	23.9979
Flash Point	220 °C
Appearance of Characters	light oil | white
Index of Refraction	1.476-1.483
Storage condition	−20°C

BUTYLENE/ETHYLENE/STYRENE COPOLYMER

Modify Date: 2022-01-11 18:13:21

	Common Name	BUTYLENE/ETHYLENE/STYRENE COPOLYMER		
	CAS Number	68648-89-5 (/en/baike/1552980.html)	**Molecular Weight**	1129.848
	Density	N/A	**Boiling Point**	N/A
	Molecular Formula	$C_{34}H_{18}Cu_2N_6Na_4O_{17}S_4$	**Melting Point**	N/A
	MSDS	N/A	**Flash Point**	N/A

Chemical & Physical Properties

Molecular Formula	$C_{34}H_{18}Cu_2N_6Na_4O_{17}S_4$
Molecular Weight	1129.848
Exact Mass	1127.779419

polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene

Modify Date: 2022-01-13 11:56:41

	Common Name	polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene		
	CAS Number	66070-58-4 (/en/baike/83051.html)	**Molecular Weight**	158.24000
	Density	0.91 g/mL at 25°C(lit.)	**Boiling Point**	145.2°C at 760 mmHg
	Molecular Formula	$(C_8H_8.C_4H_6)x$	**Melting Point**	N/A
	MSDS	Chinese USA	**Flash Point**	31.1°C

Chemical & Physical Properties

Density	0.91 g/mL at 25°C(lit.)
Boiling Point	145.2°C at 760 mmHg

Molecular Formula	$(C_8H_8.C_4H_6)x$
Molecular Weight	158.24000
Flash Point	31.1°C
Exact Mass	158.11000
LogP	3.68800
Appearance of Characters	powder

Certificate of Analysis

PO Number:		Order Number:	824519
Product:	VERSAGEL® M 750	Shipping Date:	Jun 29, 2021
Cust. Mat. No:		Date of Mfg:	May 18, 2021
Product Code:	300537125002	Qty:	16 DR
Lot Number:	2105180028		
Carrier:	0060001343-A CUSTOMER TRUCK		

Billing: Shipping To: Shipping From:

Test Description	Units	Method	Min.	Max.	Results
SPECIFIC GRAVITY @ 25/25°C		D4052			0.8395
COLOR, SAYBOLT		D156	27		30
APPEARANCE, CLEAR	P/F	VISUAL	PASS		PASS
VISCOSITY @ 25°C	cPs	D2983	67000.00	83000.00	77600.00

Suggested retest date is 3 years from the Date of Manufacture.

It is hereby guaranteed that the product is produced from substances that are not carcinogens according to the EU Dangerous Substances Directive and of which the full refining history is known.

Caution: For manufacturing, processing, or repacking.

Analysis Certified By : _____ Printed Name _____ Laboratory Manager Title _____ Lab Signature/Loader

All sales of Products by and its subsidiaries and affiliates are subject to the applicable terms and conditions of sale at http://www.calumetspecialty.com/customer-service/terms-of-sale, which are incorporated herein by reference.

Printed: 07/02/2021 06:29:13 AM

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2. Trade name (Product name)：COSMOL 222

INCI name：Diisostearyl Malate

Diisostearyl malate

Modify Date: 2022-01-24 12:58:25

	Common Name	Diisostearyl malate		
	CAS Number	81230-05-9 (/en/baike/1506170.html)	**Molecular Weight**	639.044
	Density	0.9±0.1 g/cm3	**Boiling Point**	676.3±35.0 °C at 760 mmHg
	Molecular Formula	$C_{40}H_{78}O_5$	**Melting Point**	N/A
	MSDS	N/A	**Flash Point**	185.0±19.4 °C

Chemical & Physical Properties

Density	0.9±0.1 g/cm3
Boiling Point	676.3±35.0 °C at 760 mmHg
Molecular Formula	$C_{40}H_{78}O_5$
Molecular Weight	639.044
Flash Point	185.0±19.4 °C
Exact Mass	638.584900

LogP	16.87
Vapour Pressure	0.0±4.7 mmHg at 25°C
Index of Refraction	1.466

Certificate of Analysis

PRODUCT NAME: COSMOL 222
LOT NUMBER: X-X-G
DATE OF MANUFACTURE: 24th April 2020
EXPIRE DATE (BEFORE OPEN): 24th April 2023
DATE OF ISSUE: 3rd July 2020

PACKING: 16 Kg / 90 C/N
QUANTITY: 1440 Kg

Test Name	Result	Specification	Analytical Method
Appearance	Pass	Colorless or light yellow liquid, odorless or a faint characteristic odor	JSQI*
Identification	Pass	Absorbance at 2950cm-1, 1740cm-1, 1460~1465cm-1, 1365cm-1 and 1175cm-1	JSQI, IR-Liquid film method
Specific Gravity	0.914	0.910~0.915	JSQI, Method II, 20°C/20°C
Refractive Index	1.461	1.445~1.465	JSQI, 20°C
Viscosity (mPa·s)	1674	1800~2200	JSQI, Method II, BM type, rotor No.3, 30rpm, 60sec, 30°C
Freezing Point (°C)	-60	≦-30	JSQI
Acid Value (mgKOH/g)	0.3	≦1.0	JSQI, Method I, 5g
Saponification Value (mgKOH/g)	173.4	165.0~180.0	JSQI
Hydroxyl Value (mgKOH/g)	84.9	75.0~90.0	JSQI, 2.5g, 0.5mol/L KOH-Ethanol
Iodine Value (g)2/100g)	0.0	≦2.0	JSQI, 10g
Purity (1) Heavy Metals (ppm)	≦10	≦10	JSQI
Purity (2) Arsenic (ppm)	≦1	≦1	JSQI
Loss on Drying (%)	0.2	≦2.0	JSQI, 2g, 105°C, 2hr
Residue on Ignition (%)	≦0.10	≦0.10	JSQI, Method II, 5g
Color	20	≦70	The JOCS** Standard Methods for the Analysis of Fats, Oils and Related Materials, APHA Method

REMARKS: *JSQI-The Japanese Standards of Quasi-drug Ingredients 2006 by Ministry of Health, Labour and Welfare
**JOCS-Japan Oil Chemists' Society

3. Trade name (Product name)：JOJOBA OIL GOLDEN

INCI name：*Simmondsia Chinensis* (Jojoba) Seed Oil

Jojoba oil

Modify Date: 2022-01-15 15:58:59

	Common Name	Jojoba oil		
	CAS Number	61789-91-1 (/en/baike/1465582.html)	**Molecular Weight**	N/A
	Density	0.87 g/mL at 20 °C	**Boiling Point**	N/A
	Molecular Formula	N/A	**Melting Point**	N/A
	MSDS	USA	**Flash Point**	N/A

Chemical & Physical Properties

Density	0.87 g/mL at 20 °C
Index of Refraction	n20/D1.466
Storage condition	2-8°C

JOJOBA OIL (GOLDEN)

INCI: SIMMONDSIA CHINENSIS (JOJOBA) SEED OIL

Appearance: Amber Colored Liquid, Clear & Transparent, no sediment or turbidity at 25 degrees Centigrade.

Typical Properties	Method	Units	Range	
			Min.	Max.
Iodine Value	AOCS Cd 1-25	g/100g	79	86
Melting Point	AOCS Cc 18-80	°C	12	15
Refractive Index@40°C	AOCS Cc 7-25	n	1.458	1.460
Saponification Value	AOCS Cd 3-25	mgKOH/g	90	95
Specific Gravity @ 25°C	AOCS Cc 10a-25	- - -	0.8400	0.8650
Triglyceride Content	AOCS Ci 2-91	wt%	Less than 0.5	
Specification Values				
Acid Value	AOCS Ci 4-91	mg KOH/g	Less than 2	
Color	AOCS Cc13b-45	Gardner	10 Maximum	
Odor	AOCS Cg2-83		Characteristic/Slight	
Peroxide Value	AOCS Cd 8b-90	meq/kg	Less than 5	

Additives: None Shelf Life: 3 Years

Pesticide Residue Analysis Results (ppm) (N.D. = none detected)

Alpha BHC:	N.D. <0.25
Gamma BHC:	N.D. <0.25
Beta BHC:	N.D. <0.25
Delta BHC:	N.D. <0.25
Heptachlor:	N.D. <0.25
Heptachlor Epoxide:	N.D. <0.25
Aldrin:	N.D. <0.25
Endosulfan I:	N.D. <0.25
Endosulfan II:	N.D. <0.25
Endosulfan Sulfate:	N.D. <0.25
P,p DDE:	N.D. <0.25
P,p DDD:	N.D. <0.25
P,p DDT:	N.D. <0.25
Dieldrin:	N.D. <0.25
Endrin:	N.D. <0.25
Endrin Aldehyde:	N.D. <0.25

4. Trade name (Product name)：BENTONE® 38

INCI name：Quaternium-18 Bentonite

Bentone 38

Modify Date: 2022-01-15 16:39:01

	Common Name	Bentone 38		
	CAS Number	12001-31-9 (/en/baike/46313 2.html)	**Molecular Weight**	343.57900
	Density	N/A	**Boiling Point**	N/A
	Molecular Formula	$HLiMgNaO_{11}Si_4$	**Melting Point**	N/A
	MSDS	N/A	**Flash Point**	N/A

Chemical & Physical Properties

Molecular Formula	$HLiMgNaO_{11}Si_4$
Molecular Weight	343.57900
Exact Mass	342.85000
PSA	170.68000

Certificate of Analysis

Customer:

Material:	10796AIE0025KGBG
Customer Part:	
Description	BENTONE® 38

Customer Order:	Customer Specification:
Our Order:	Ship From:

Lot Number: 1007X25601 Ship Date: 27 OCT 2021 Quantity Shipped: 80.000 EA

Date Mfg: 13 SEP 2021 Date Exp: 12 SEP 2025

Test	Result	UOM	Min	Max	Property	Method
% Drying Loss	1.59		0.10	3.00		X-101
% Loss of Ignition	38.78		36.50	39.50		X-922, X-922A
% Thru 200 Mosh Sieve	96.82		95.00	100.00		X-9041
Viscosity 2% in Toluene	352	mPa.s	220	9999		X-9155

Date: 27 OCT 2021 Time: 11:57:01 Page: 1 of 1

5. Fragrance

IFRA CONFORMITY CERTIFICATE
Essential Oil

Product Name: PEPPERMINT OIL

We certify that the above product is in compliance with the Standards of the INTERNATIONAL FRAGRANCE ASSOCIATION (IFRA - *50th* Amendment / published *June 2021*), provided it is used in the following category(ies) at a maximum concentration level of:

IFRA class(es)	Maximum Level of use (%)	*IFRA class(es)*	Maximum Level of use (%)
Category 1	*20.000*	*Category 7A*	*3.900*
Category 2	*6.000*	*Category 7B*	*3.900*
Category 3	*2.000*	*Category 8*	*1.300*
Category 4	*59.000*	*Category 9*	*18.000*
Category 5A	*20.000*	*Category 10A*	*18.000*
Category 5B	*3.900*	*Category 10B*	*43.000*
Category 5C	*5.900*	*Category 11A*	*1.300*
Category 5D	*1.300*	*Category 11B*	*1.300*
Category 6	*66.000*	*Category 12*	*100.000*

Regulated Substance	Max Concentration (%)	Limiting Substance
Carvone	1.0	Yes
Limonene	4.0	No
Linalool	1.0	No

The essential oil of Peppermint on its own, is not impacted by the IFRA code for its use in perfuming compositions, however, the components listed above are impacted.

The IFRA Standards regarding use restrictions are based on safety assessments by the Panel of Experts of the RESEARCH INSTITUTE FOR FRAGRANCE MATERIALS (RIFM) and are enforced by the IFRA Scientific Committee. Evaluation of individual Fragrance ingredients is made according to the safety standards contained in the relevant section of the IFRA Code of Practice.

This certificate is based on statistical analytical data and is not carried out on each batch. The stated use levels relate to this specific material alone and may not be valid when used in combination with other restricted materials. It is the ultimate responsibility of our customer to ensure the safety of the final product (containing this fragrance) by further testing if need be.

Issue date 13th October 2021 Page 1/5

List of IFRA categories and subcategories with corresponding product

IFRA Category Product Type	IFRA Sub-category	Flavour use	Phototoxicity	IFRA category for Certificate of Conformity of fragrance mixtures with IFRA Standards
Category 1				
Lip Products of all types (solid and liquid lipsticks, balms, clear or colored, etc.)		YES	Applicable (leave-on products)	Category 1
Children's toys		YES	Applicable (leave-on products)	Category 1
Category 2				
Deodorant and antiperspirant products of all types including any product with intended or reasonably foreseeable use on the axillae or labelled as such (spray, stick, roll-on, under-arm, deo- cologne, etc.)		NO	Applicable (leave-on products)	Category 2
Body sprays (including body mist)		NO	Applicable (leave-on products)	Category 2
Category 3				
Eye products of all types (eye shadow, mascara, eyeliner, eye make-up, eye masks, eye pillows, etc.) including eye care and moisturizer		NO	Applicable (leave-on products)	Category 3
Facial make up and foundation		NO	Applicable (leave-on products)	Category 3
Make-up remover for face and eyes		NO	Applicable (leave-on products)	Category 3
Nose pore strips		NO	Applicable (leave-on products)	Category 3
Wipes or refreshing tissues for face, neck, hands, body		NO	Applicable (leave-on products)	Category 3
Body and face paint (for children and adults)		NO	Applicable (leave-on products)	Category 3
Facial masks for face and around the eyes		NO	Applicable (leave-on products)	Category 3
Category 4				
Hydroalcoholic and non-hydroalcoholic fine fragrance of all types (Eau de Toilette, Parfum, Cologne, solid perfume, fragrancing cream, aftershaves of all types, etc.)		NO	Applicable (leave-on products)	Category 4
Fragranced bracelets		NO	Applicable (leave-on products)	Category 4
Ingredients of perfume kits and fragrance mixtures for cosmetic kits		NO	Applicable (leave-on products)	Category 4
Scent pads, foil packs		NO	Applicable (leave-on products)	Category 4
Scent strips for hydroalcoholic products		NO	Applicable (leave-on products)	Category 4
Category 5				
Body creams, oils, lotions of all types	A	NO	Applicable (leave-on products)	Category 5A
Foot care products (creams and powders)	A	NO	Applicable (leave-on products)	Category 5A
Insect repellent (intended to be applied to the skin)	A	NO	Applicable (leave-on products)	Category 5A
All powders and talc (excluding baby powders and talc)	A	NO	Applicable (leave-on products)	Category 5A

6. INCI name：Tocopheryl Acetate

Tocopheryl acetate

Modify Date: 2022-01-12 09:42:09

	Common Name	Tocopheryl acetate		
	CAS Number	7695-91-2 (/en/baike/1042041.html)	**Molecular Weight**	472.743
	Density	0.9±0.1 g/cm3	**Boiling Point**	485.3±0.0 °C at 760 mmHg
	Molecular Formula	$C_{31}H_{52}O_3$	**Melting Point**	-28℃
	MSDS	Chinese USA	**Flash Point**	235.6±24.7 °C

Chemical & Physical Properties

Density	0.9±0.1 g/cm3
Boiling Point	485.3±0.0 °C at 760 mmHg
Melting Point	-28℃
Molecular Formula	$C_{31}H_{52}O_3$
Molecular Weight	472.743
Flash Point	235.6±24.7 °C
Exact Mass	472.391632
PSA	35.53000
LogP	12.07
Vapour Pressure	0.0±1.2 mmHg at 25°C

DL-A-TOCOPHERYL ACETATE

CERTIFICATE OF ANALYSIS

Productcode :
Lot No. :
Analysis No. :

Test	Result	Limits / Specifications	Dimension / Units
Appearance visual	clear viscous oil	clear viscous oil	
Colour visual	almost colourless	colourless almost colourless slightly yellow slightly greenish yellow	
Identity GC	corresponds	corresponds	
Optical rotation Ph.Eur.	-0.00	-0.01 to +0.01	°
Heavymetals ICP-MS	corresponds*	max. 10	ppm
Lead ICP-MS	corresponds*	max. 2	ppm
Arsenic ICP-MS	corresponds*	max. 1	ppm
Mercury ICP-MS	corresponds*	max. 0.1	ppm
Cadmium ICP-MS	corresponds*	max. 0.5	ppm
Residual Solvents			
- Pyridine GC	corresponds*	max. 200	ppm
- Toluene GC	corresponds*	max. 890	ppm
Acidity Titration / USP	0.0	max. 1.0	ml 0.10N NaOH
Related Subst. (Ph.Eur.)			
- Impurity A GC	0.1	max. 0.5	%
- Impurity B GC	0.6	max. 0.6	%
- Impurity C (free tocopherol) GC	0.2	max. 0.5	%
- Impurity D and E GC	0.3	max. 1.0	%
- Any other impurity, each GC	0.12	max. 0.25	%
- Total GC	1.7	max. 2.5	%

Date of issue :

DL-A-TOCOPHERYL ACETATE

CERTIFICATE OF ANALYSIS

Productcode :
Lot No. :
Analysis No. :

Test	Result	Limits / Specifications	Dimension / Units
Assay Tocopherylacetate (Ph.Eur.) GC	99.1	96.5 to 102.0	%
Assay Tocopherylacetate (USP/FCC) GC	98.2	96.0 to 102.0	%

*) checked at regular intervals
This lot was analysed and released by our authorized Quality Control Department and was found to meet the specifications as given above.
The product meets all requirements of the following valid compendia when tested accordingly:
USP, FCC, Ph.Eur.

The Quality Assurance Manager

Date of issue :

7. Trade name (Product name)：Glare® Glitter Red GL-7401E

INCI name：Calcium Titanium Borosilicate (and) Tin Oxide (and) Titanium Dioxide

Fiber Glass Wool

Modify Date: 2022-10-27 08:10:30

(INCI：Calcium Titanium Borosilicate)

	Common Name	Fiber Glass Wool		
	CAS Number	65997-17-3 (/en/baike/1423821.html)	**Molecular Weight**	158.039
	Density	1.1 g/mL at 25 °C(lit.)	**Boiling Point**	1000 °C
	Molecular Formula	$CaNaO_4P$	**Melting Point**	680 °C
	MSDS	Chinese USA	**Flash Point**	N/A

Chemical & Physical Properties

Density	1.1 g/mL at 25 °C(lit.)
Boiling Point	1000 °C
Melting Point	680 °C
Molecular Formula	$CaNaO_4P$
Molecular Weight	158.039
Exact Mass	157.905777
Storage condition	Storage temperature: no restrictions.

titanium dioxide

Modify Date: 2022-09-27 11:40:49

	Common Name	titanium dioxide		
	CAS Number	13463-67-7 (/en/baike/804872.html)	**Molecular Weight**	79.866
	Density	4.26 g/mL at 25 °C(lit.)	**Boiling Point**	2900 °C
	Molecular Formula	O_2Ti	**Melting Point**	1840 °C
	MSDS	Chinese USA	**Flash Point**	2500-3000°C
	Symbol	 GHS05, GHS07, GHS08 (/GHS.jsp#_pict)	**Signal Word**	Danger

Chemical & Physical Properties

Density	4.26 g/mL at 25 °C(lit.)
Boiling Point	2900 °C
Melting Point	1840 °C

Molecular Formula	O_2Ti
Molecular Weight	79.866
Flash Point	2500-3000°C
Exact Mass	79.937775
PSA	34.14000
Appearance of Characters	powder
Index of Refraction	2.61
Storage condition	-20°C
Water Solubility	insoluble

Stannic oxide

Modify Date: 2022-10-27 18:38:25

	Common Name	Stannic oxide		
	CAS Number	18282-10-5 (/en/baike/895871.html)	**Molecular Weight**	150.69
	Density	6.95 g/mL at 25 °C(lit.)	**Boiling Point**	1800-900°C
	Molecular Formula	SnO_2	**Melting Point**	1127 °C
	MSDS	Chinese USA	**Flash Point**	1800-1900°C

Chemical & Physical Properties

Density	6.95 g/mL at 25 °C(lit.)
Boiling Point	1800-900°C
Melting Point	1127 °C
Molecular Formula	SnO_2
Molecular Weight	150.69
Flash Point	1800-1900°C
PSA	34.14000
Index of Refraction	1.9968
Water Solubility	INSOLUBLE

PRODUCT SPECIFICATIONS

Product Name:	
Trade Name:	**Glare® Glitter Red GL-7401E**
Manufacturer:	
Date Prepared:	

1. Appearance:	Sparkle Silver White, Free-flowing Powder				
2. Ingredients and Composition					
INCI Name	Composition	By Weight (%)		CAS No.	EINECS
Calcium Titanium Borosilicate	-	74 - 85		65997-17-3	266-046-0
Tin Oxide	SnO_2	0 - 1		18282-10-5	242-159-0
Titanium Dioxide	TiO_2	15 - 25		13463-67-7	236-675-5
3. Particle Size (㎛):	Mean Diameters	D10 - D90		(By Malvern Mastersizer 2000S)	
		15 - 105			
4. pH:	7.0 - 11.0			(10% Aqueous Suspension)	
5. Loss on Drying:	0.5 % max.				
6. Loss on Ignition:	2.0 % max.				
7. Water Soluble Substances:	0.3 % max.				
8. Acid Soluble Substances:	2.0 % max				
9. Trace Elements:	Mercury (Hg)			1 ppm max.	Internal Method
	Arsenic (As)			1 ppm max.	
	Lead (Pb)			3 ppm max.	
	Cadmium (Cd)			1 ppm max.	
	Barium (Ba)			30 ppm max.	
	Antimony (Sb)			1 ppm max.	
	Copper (Cu)			30 ppm max.	
	Chromium (Cr)			30 ppm max.	
	Nickel (Ni)			10 ppm max.	
10. Microorganisms:	100 CFU/g max.				No Pathogens

* Note : The suitability of Glare® product for any special cosmetic application has not been established, and the responsibility belongs to the end user.

8. Trade name (Product name)：Glare® Brilliant Russet GL-6600K

INCI name：Calcium Titanium Borosilicate (and) Iron Oxides

Ferric oxide

Modify Date: 2022-10-13 18:24:56

	Common Name	Ferric oxide		
	CAS Number	1309-37-1 (/en/baike/965634.html)	**Molecular Weight**	159.688
	Density	5.24	**Boiling Point**	N/A
	Molecular Formula	Fe_2O_3	**Melting Point**	1538°C
	MSDS	Chinese USA	**Flash Point**	>230°F
	Symbol	 GHS02, GHS05 (/GHS.jsp#_pict)	**Signal Word**	Danger

Chemical & Physical Properties

Density	5.24
Melting Point	1538°C
Molecular Formula	Fe_2O_3

Molecular Weight	159.688
Flash Point	>230°F
Exact Mass	159.854630
PSA	43.37000
Stability	Stable.
Water Solubility	INSOLUBLE

PRODUCT SPECIFICATIONS

Product Name:	
Trade Name:	Glare® Brilliant Russet GL-6600K
Manufacturer:	
Date Prepared:	

1. Appearance:	Lustrous Reddish, Free-flowing Powder				
2. Ingredients and Composition					
INCI Name	Composition	By Weight (%)		CAS No.	EINECS
Calcium Titanium Borosilicate	-	67 - 77		65997-17-3	266-046-0
Iron Oxides	Fe_2O_3	23 - 33		1309-37-1	215-168-2
3. Particle Size (㎛):	Mean Diameters	D10 - D90		(By Malvern Mastersizer 2000S)	
		6 - 41			
4. pH:	7.0 - 11.0			(10% Aqueous Suspension)	
5. Loss on Drying:	0.5 % max.				
6. Loss on Ignition:	2.0 % max.				
7. Water Soluble Substances:	0.3 % max.				
8. Acid Soluble Substances:	2.0 % max				
9. Trace Elements:	Mercury (Hg)			1 ppm max.	Internal Method
	Arsenic (As)			1 ppm max.	
	Lead (Pb)			3 ppm max.	
	Cadmium (Cd)			1 ppm max.	
	Barium (Ba)			30 ppm max.	
	Antimony (Sb)			1 ppm max.	
	Copper (Cu)			30 ppm max.	
	Chromium (Cr)			30 ppm max.	
	Nickel (Ni)			60 ppm max.	
10. Microorganisms:	100 CFU/g max.				No Pathogens

* Note : The suitability of Glare® product for any special cosmetic application has not been established, and the responsibility belongs to the end user.

(10) 成分之毒理資料

- 由 AJP 化粧品股份有限公司及安全資料簽署人員查詢蒐集各成分之毒理資料，另存放於成分毒理資料檔案夾(附錄 2)。
- 安全資料簽署人員依據上述資料內容摘錄各成分相關毒理資料如下：

1. INCI name：Mineral Oil

- 急性毒性：在口服(類似 OECD 401)、皮膚(類似 OECD 402)和吸入(類似 OECD 403)進行的動物研究中，對於高度精煉的礦物油，雄性和雌性大鼠的口服 LD_{50} > 5000 mg/kg bw；雄性和雌性大鼠吸入其氣溶膠之 LD_{50} 大於 5 mg/L (5000 mg/m^3)；雄性和雌性兔子的皮膚 LD_{50} > 2000 mg/kg/bw。[1]
- 重複劑量毒性：關於慢性經口暴露，一項 2 年大鼠飼料慢性毒性/致癌性合併研究(Trimmer 等人，2004)透過飲食給予 0、60、120、240 或 1200 mg/kg/day 劑量的高度精煉礦物油 P70H 和 P100H，研究符合 GLP 且按照 OECD 453 測試指引進行。P70H 和 P100H 兩種化合物的結果相似，在任何劑量下存活率不受影響，並且未觀察到給予受測物質相關的臨床效果，NOAEL 為≥ 1200 mg/kg bw/day，研究顯示通過飲食攝入的高度精煉礦物油 24 個月沒有致癌潛力或慢性毒性。關於吸入重複毒性，為期 4 週的大鼠吸入研究(Dalbey 等人，1991)以 OECD 412 測試兩種精煉礦物油和一種白礦油，氣溶膠濃度為 0、50、220 和 1000 mg/m^3，由於肺重量增加，測試結果亞急性吸入暴露的 NOEL 為 50 mg/m^3 而 LOEL 為 210 mg/ m^3，這種效應是由於組織中的油累積造成的而不一定是毒理學終點。關於皮膚暴露途徑，在一項 80SUS 白礦油的亞慢性研究(Mobil 1988)為生殖研究的一部分，大鼠以 0、125、500 或 2000 mg/kg 的劑量給予 13 週 (5 天/週)。結果顯示施用於皮膚在所有劑量組大鼠皆產生紅斑、皮膚剝落和結痂等皮膚刺激現象。與對照組相比，給予 500 和 2000 mg/kg bw/day 的雄性和雌性大鼠的體重下降，尿液分析和血液學可能具有毒理學意義，但死亡率、臨床症狀、食物消耗、器官重量或臨床化學皆未發現其他與測試物質相關的影響，在最高測試劑量下沒有引起關注的顯著毒理學發現。此亞慢性皮膚暴露試驗顯示，暴露於白礦油後的 NOAEL ≥ 2000 mg/kg bw/day (OECD 411)。交互參照另一項潤滑礦物油在紐西蘭白兔的 28 天重複給藥皮膚毒性

研究，透過酵素測量和組織病理學評估肝毒性(OECD 410)，其保守 NOAEL 為 1000 mg/kg bw/day。[1]

- 皮膚腐蝕性/刺激性：一項皮膚刺激研究在雄性白兔身上測試高度精煉礦物油，動物在兩個磨損和兩個未磨損的測試點暴露於 0.5 mL 未稀釋的白礦油 24 小時。暴露後，在施用後 24 小時和 72 小時對皮膚刺激進行評分(Draize)的紅斑和水腫反應。所有部位的平均紅斑和水腫評分均為 0.0。基於上述高度精煉的礦物油被認為是一種無皮膚刺激性物質。[1]
- 眼刺激性：一項眼睛刺激研究分析高度精煉礦物油對雄性紐西蘭白兔的影響(OECD 405)，當暴露於 0.1 mL 未稀釋的礦物油時，發現高度精煉的礦物油對兔子眼睛無刺激性。平均未清洗結膜評分(24-72 小時)為 0.22，而平均角膜和虹膜評分為 0 (24-72 小時)。基於上述高度精煉的礦物油被認為對眼睛無刺激性。[1]
- 皮膚致敏性：一項皮膚致敏研究中，雄性天竺鼠在刺激、誘導和激發試驗中進行測試以確定高度精煉的礦物油的皮膚致敏性。研究條件下與誘導處理相比結果顯示，白礦油不是皮膚致敏原。[1]
- 致癌性：2 年大鼠飼料慢性毒性/致癌性合併研究(Trimmer 等人，2004)顯示高度精煉的礦物油不會通過口腔接觸致癌(OECD 453)；一項皮膚致癌性研究，將白油(通常 75 µL) 每週兩次或三次塗抹在 C3H 小鼠的皮膚上持續長達 104 週，藥用白油通常用作陰性對照和測試物質的載體 (Chasey and McKee, 1993)。在使用高度精煉的礦物油進行的所有生物測定中，皮膚腫瘤的發生率接近於零。[1]
- 致突變性/遺傳毒性：在體外改良 Ames 測試中，發現高度精煉的礦物油對誘變性呈陰性(類似 OECD 471)。在體外哺乳動物細胞染色體畸變試驗中，充分精煉的潤滑油礦物油 55/60 Pale Oil 在任何劑量下均未顯示明顯的畸變(OECD 473)。在體外哺乳動物細胞基因突變測定結果顯示高度精煉的礦物油不具有致突變性(OECD 476)。[1]
- 生殖毒性：1987 年關於高度精煉礦物油(白礦油)的研究，設計類似於 OECD 415，使用的劑量為 0、125、500 和 2000 mg/kg/day，在任何受測組中均未觀察到死亡，雌性親代未觀察到與受測物質相關的體重增加或食物消耗變化，在接受試驗物質的動物中，著床數、產仔數或妊娠期均未發現不良影響。雌性親代的 NOAEL 大於或等於 2000 mg/kg bw/day。[1]
- 光毒性：以 52 名受試者使用含有 53.25%石油餾的睫毛膏進行的

皮膚致敏/光敏化組合測試，在施用 1、4、7 天時另於貼片測試位點處暴露紫外線 1 分鐘。52 名受試者均未發現皮膚致敏現象或光敏反應。[2]

- 經皮吸收：13 項體內(人類、動物)和體外研究調查礦物油和蠟的皮膚滲透性，大多數物質被吸附於皮膚角質層，只有一小部分到達更深層皮膚。各種研究都沒有證據表明礦物油和蠟可被經皮吸收並全身作用，因此，由於不存在皮膚吸收，化粧品中使用的礦物油和蠟不會對消費者的健康構成風險。[3]
- 毒理代謝動力學：在人體中進行的毒代動力學研究顯示，白色礦物碳氫化合物的血液濃度低於檢測極限 (0.16 µg/mg)，表示在 1 mg/kg 的膳食暴露量下吸收可忽略不計，數據亦顯示總全身暴露的固有應變差異(Fischer 344 與 Sprague-Dawley 大鼠的全身劑量相比高約 4 倍)、代謝率以及高度精煉的礦物油碳氫化合物的肝和淋巴結滯留，這可能與不同物種的敏感性與形成肝肉芽腫和 MLN 組織細胞增多症有關。[1]
- 人體數據：毒代動力學以人體試驗進行。
- 其他安全資料：液體石蠟(CAS No. 8012-95-1 or 8042-47-5)為高度精煉的石油礦物油，為烴類的複雜組合組成，透過用硫酸和發煙硫酸對石油餾分進行深度處理或通過加氫獲得，或通過氫化和酸處理的組合。物質特性和性質為碳數於 C15 至 C50 間的飽和烴組成，平均分子量(g/Mol) 230–700，Log Kow 於 7.7–24.2。[3]
- 參考資料：

1. White mineral oil (petroleum) Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15514/7/1
2. Final Report on the Safety Assessment of Petroleum Distillate. JACT 5(3):225-248, 1986.
3. Review of data on the dermal penetration of mineral oils and waxes used in cosmetic applications. Toxicol Lett. 2017;280:70-78.

2. INCI name：Diisostearyl Malate

- 急性毒性：以等同於 OECD TG 401 的小鼠急性經口毒性研究中通過口服管飼法給予單次劑量 5000 mg/kg bw，結果無臨床毒性跡象，觀察期間未發生死亡，急性經口 LD_{50} 大於 5000 mg/kg bw。參照 Di C12-13 Alkyl Malate (CAS 149144-85-4)的大鼠急性皮膚毒性試驗，皮膚施用劑量為 2000 mg/kg bw，在研究期間沒有發生死亡且未觀察到臨床症狀，急性皮膚 LD_{50} 大於 2000 mg/kg bw。[1]
- 重複劑量毒性：無 Diisostearyl Malate 的數據，交叉參照在 Fischer 344 大鼠中以 1600、3100、6300、12500 和 25000 ppm 的劑量對 Bis(2-ethylhexyl) adipate (CAS 103-23-1) 進行與 OECD 408 等效的 90 天亞慢性經口毒性研究，在研究期間未觀察到任何動物的毒性作用和死亡且所有動物的組織病理學檢查均未發現不良反應。結果得出雄性大鼠的 NOAEL 為 6300 ppm，相當於 630 mg/kg bw/day，雌性大鼠的 NOAEL 為 25000 ppm，相當於 2187 mg/kg bw/day。[1]
- 皮膚腐蝕性/刺激性：一項與 OECD TG 404 等效的體內皮膚刺激研究中，將 0.5 mL 測試物質以半封閉方式施於紐西蘭白兔的剃毛皮膚上 4 小時後去除，於施用後 1、24、48 和 72 小時和 4 天觀察評分，貼片去除 1 小時後 3 隻動物皆有輕微紅斑(1 級)，但 4 天後復原，所有動物均無水腫發生，測試物質在測試條件下被認為是無皮膚刺激性。[1]
- 眼刺激性：一項與 OECD TG 405 等效的眼刺激研究中，0.1 mL 測試物質滴入三隻雄性紐西蘭白兔的眼睛中，每隻動物的未經處理的眼睛用作對照。檢查眼睛並根據 Draize 評分系統在施用後 1、24、48 和 72 小時對變化進行分級，在研究期間，在所有動物中使用測試物質後，均未發現角膜或虹膜效應。在施用 1 小時後的發紅效應於 24 小時內完全可逆，測試物質被認為對眼睛無刺激性。[1]
- 皮膚致敏性：一項與 OECD TG 429 等效並符合 GLP 的局部淋巴結檢測 (LLNA)試驗以 CBA/J 小鼠進行皮膚致敏測試，於丙酮/橄欖油(4:1 v/v)添加測試物質濃度為 1、2.5、5、10 和 25%，每天在耳朵的背側表面局部施用 25 µL 連續三天。結果所有在測試條件下，測試物質無出現皮膚致敏現象。在濃度為 1、2.5、5、10 和 25% 時計算的刺激指數(SI)分別為 1.21、1.14、1.32、0.87 和 1.39 (皆小於 3)。結論在此測試條件下測試物質無出現皮膚致敏現象。[1]

- 致癌性：無相關研究數據。
- 致突變性/遺傳毒性：在鼠傷寒沙門氏菌菌株 TA98、TA100、TA1535 和 TA1537 中，使用回復突變試驗研究 Diisostearyl Malate 誘導基因突變的潛力，濃度 312.5、625、1250、2500 和 5000 mg/平板下並測試有或無代謝活化的情況，測試結果物質沒有致突變性。[2]
- 生殖毒性：無相關研究數據。
- 光毒性：無相關研究數據。
- 經皮吸收：無 Diisostearyl Malate 的相關研究數據。由於 Diisostearyl Malate 分子量約為 639.06 g/mol、log Pow >10 和水溶性 <1 mg/L 等特性，因此較無法被皮膚吸收。[1]
- 毒理代謝動力學：無 Diisostearyl Malate 的相關研究數據。根據相關可用資訊評估 Diisostearyl Malate 在人體中的全身生物利用度被認為是低的；預計母體脂肪組織中不會有顯著生物蓄積；因為只有 Diisostearyl Malate 的水解產物才可能分佈在體內，且吸收非常有限預計不會分佈體內；預計不會進行廣泛的代謝，而是直接排除。[1]
- 人體數據：一項以 51 位受測者進行 Diisostearyl Malate (100%；0.2 mL，0.2 g)的 HRIPT 試驗，結果於誘導或激發期間均未觀察到皮膚致敏反應。[2]
- 參考資料：

1. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/17702/7/1
2. Safety Assessment of Dialkyl Malates as Used in Cosmetics. IJT 34(Suppl. 1):5-17, 2015.

3. INCI name：*Simmondsia Chinensis* (Jojoba) Seed Oil

- 急性毒性：對於小鼠或大鼠不具有急性口服毒性(LD_{50} 大於 5.0 g/kg)。[1]
- 重複劑量毒性：精製荷荷巴蠟的亞慢性皮膚毒性試驗中，使用 32 隻 DH 天竺鼠分為 4 組(4 隻雄性，4 隻雌性/組)進行。前 2 組分別施用荷荷巴油蠟 0.25 和 0.5 g/kg，每週 6 天共 20 週，施用部位無覆蓋。另 2 個對照組分別接受 0.5 g/kg 橄欖油或生理鹽水。結束後進行肉眼和顯微鏡檢查，體重或器官重量沒有差異，在組織中未觀察到器官及皮膚施用部位的損傷。Verschuren (1989) 餵食 Simmondsia Chinensis (Jojoba) 種子油(2.2、4.5 和 9%於飼料中)雄性和雌性 SPF Wistar 大鼠（馴化時 6 週大）4 週，所有大鼠沒有出現死亡和臨床症狀。[1] 使用 4 組 10 隻雄性 Sprague-Dawley 大鼠評估精製荷荷巴油的口服毒性，其中兩組每天一次餵食含有 0.5 或 1.0 g 荷荷巴油的基礎日糧(5 g/飼料)持續 7 天，其餘 2 組每天飼餵一次含有 2.0 或 3.0 g 荷荷巴油的基礎日糧持續 4 天。在餵食 1.0 g 的 5 隻大鼠和餵食 2.0 和 3.0 g 的所有大鼠中觀察到毒性跡象，這 3 組的死亡率均為 10%，餵食 0.5 克荷荷巴油的大鼠沒有死亡。(Hamm 1984)。[2]
- 皮膚腐蝕性/刺激性：精製荷荷巴油(100%)的皮膚刺激性試驗中，使用 10 隻雄性白化天竺鼠，以橄欖油和輕質液體石蠟作為對照，一半動物以 0.5 ml 樣品施用於剃毛皮膚上持續 15 天，剩下動物以相同程序持續 30 天。根據 Draize 量表評分結果未觀察到荷荷巴油、種子油或橄欖油有明顯反應。[1]
- 眼刺激性：在兔子的眼部刺激試驗中，經過除臭或脫臭和變色精製的荷荷巴油在滴注後 1 小時引起輕微可逆的結膜刺激，24 小時後反應已清除。[3]
- 皮膚致敏性：用未稀釋的荷荷巴油測試的白化天竺鼠貼劑中未觀察到明顯的皮膚刺激反應。在最大化測試中，在施用 10.0%荷荷巴酒精溶液測試的 20 隻雄性和雌性白化土撥鼠中未觀察到致敏反應。[3]
- 致癌性：無致癌毒性數據。[1]
- 致突變性/遺傳毒性：Ames 試驗中，荷荷巴油和氫化荷荷巴蠟的混合物在代謝活化和不活化的情況下都沒有致突變性。[3]

- 生殖毒性：無生殖或發育毒性相關研究數據。
- 光毒性：一項含有 20.0%荷荷巴油護唇膏產品的光毒性試驗中以 10 名受試者評估評估種子油。半數將約 0.2 g 的測試物質施於到右前臂內側 24 小時，剩下一半則施於左前臂內側。未照射對照部位於右前臂或左前臂的內側。貼布去除後按量表評分，然後對測試部位照射 15 分鐘 UVA 光(劑量= 4,400 μW/cm^2)，距離約為 10 公分，未照射對照部位於照射期間以鋁箔屏蔽。於照射結束後 24 及 48 小時對反應進行評分結果無受試者有反應，產品是歸類為無光毒性。[1]
- 經皮吸收：Yaron (1987)發表的裸鼠皮膚研究以溶液含有約 4 meq 荷荷巴油/面積測試，22 小時後其滲透為 1 小時的 6.7 倍，經根據皮膚組織學檢查，主要滲透途徑是毛囊。[1]
- 毒理代謝動力學：以 0.5 至 1.69 mg/10 g 餵食該成分的小鼠的糞便中檢測到荷荷巴油。荷荷巴油可滲透裸鼠皮膚，滲透的主要途徑是毛囊，荷荷巴油於含 Brij 96 及 Capmul 在 40%水的乳液比凝膠基質更高的速率通過新生小鼠皮膚遞送 Fluconazole。只有少量放射性標記的荷荷巴蠟被皮下注射到白化小鼠體內會被吸收至體內及大腦和肝臟的脂質部分中。在小鼠體內注射放射性標記的荷荷巴蠟後，最大的放射性計數出現在肝臟、大腦、肺和身體脂質中。[1]
- 人體數據：含有 0.5%荷荷巴油的外用油產品和含有 20.0%荷荷巴油的潤唇膏產品被歸類為對人類無刺激性和非致敏劑。在一組 28 名沒有已知敏感性的患者中，未觀察到對未稀釋的荷荷巴油的致敏反應。[3]
- 其他安全資料：CIR 的安全性評估報告就該安全評估中討論的使用和濃度，結論目前 Simmondsia Chinensis (Jojoba) Seed Oil, Simmondsia Chinensis (Jojoba) Seed Wax, Hydrogenated Jojoba Oil, Hydrolyzed Jojoba Esters, Isomerized Jojoba Oil, Jojoba Esters, Simmondsia Chinensis (Jojoba) Butter, Jojoba Alcohol 及合成的 Jojoba Oil 用於化粧品成分是安全的。[1]
- 參考資料：

1. Final Report on the Safety Assessment of Simmondsia Chinensis (Jojoba) Seed Oil, Simmondsia Chinensis (Jojoba) Seed Wax, Hydrogenated Jojoba Oil, Hydrolyzed Jojoba Esters, Isomerized Jojoba Oil,Jojoba Esters, Simmondsia Chinensis (Jojoba) Butter, Jojoba Alcohol, and Synthetic Jojoba Oil. CIR, 09/23/2008.

2. Preparation and evaluation of trialcoxytricarballylate, trialkoxycitrate, trialkoxyglycerylether, jojoba oil, and sucrose polyester as low calorie replacements of edible fats and oils. J. Food Sci. 49:419-428, 1984.
3. Final Report on the Safety Assessment of Jojoba Oil and Jojoba Wax. JACT 11(1):57-74, 1992.

4. INCI name：Quaternium-18 Bentonite

- 急性毒性：以 8 g/kg 的劑量將季銨鹽- 18 膨潤土懸浮至棉籽油懸浮液中口服給予 20 隻大鼠，給藥後兩週內未發生死亡。因懸浮液難以操作故未給予更高的劑量。現有數據顯示 LD_{50} 大於 8 g/kg。[1]
- 重複劑量毒性：每組 12 隻斷奶大鼠餵食含有 1%、5%或 25%季銨鹽-18 膨潤土的飲食 12 週，兩個相似組被餵食基本飲食並作為對照。12 週結束後所有組的血液學、器官重量、大體病理學和顯微病理學檢查結果基本相同，且無跡象顯示該成分會產生任何亞慢性口服毒性。[1]

  十隻兔子的背部剃毛(15 x 18 cm)並在封閉下暴露於 0.5 g 季銨鹽-18 膨潤土中每天 6 小時持續 90 天(預估約 333 mg mg/kg/day)，同時使用十隻對照動物。暴露結束時和下一次暴露開始時根據 Draize 標準對暴露部位的刺激進行評分。兩組的血液學和大體病理結果均正常，顯微病理學顯示實驗組和對照組都有輕微的肝臟和腎臟異常且有慢性原蟲感染，但未發現季銨鹽-18 膨潤土的局部或全身毒性證據。[1]
- 皮膚腐蝕性/刺激性：將 0.5 g 未稀釋的季銨鹽- 18 膨潤土施用於完整和磨損的兔皮膚，每天暴露 6 小時連續 5 天，之後休息 10 天再接觸 5 天，結果沒有發現反應，認為測試物質是惰性的。[1]
- 皮膚致敏性：以皮內注射研究對天竺鼠皮膚產生致敏反應，給予 12 隻天竺鼠初始注射 0.05 ml 測試樣品(0.1%於生理食鹽水) ，後續三週內每週再注射 3 次 0.1 ml，之後休息兩週。結束後注射 0.05 ml 的激發劑量。結果激發劑量的反應比誘導劑量的反應小，顯示無皮膚致敏作用。[1]
- 眼刺激性：將 0.1ml 10%的生理鹽水懸浮液滴入 10 隻兔子眼睛中，滴注 24 小時後評估結果為陰性。[1]
- 致癌性：無相關研究數據。
- 致突變性/遺傳毒性：無相關研究數據。
- 生殖毒性：無相關研究數據。
- 光毒性：無相關研究數據。
- 經皮吸收：季銨鹽- 18 膨潤土是季銨鹽-18 與粘土的反應產物，此化合物很難通過皮膚吸收。[1]
- 毒理代謝動力學：無相關研究數據。

◆ 人體數據：50 名人類受試者的兩種眉毛顏色製劑(4.1 或 4.0% 季銨鹽-18 膨潤土)的重複斑貼測試。兩種產品均未發現皮膚刺激或致敏的證據。臨床試驗濃度大於 4.1%是可行的，由於化粧品配方中含有 > 5-10%的受測成分。[1]

◆ 參考資料：

1. Final Report on the Safety Assessment of Quaternium-18, Quaternium-18 Hectorite, and Quaternium-18 Bentonite Quaternium. JACT 1(2):71-83, 1982.

5. INCI name：Butylene/Ethylene/Styrene Copolymer

- 急性毒性：一項急性口服毒性研究評估含有乙烯/丙烯/苯乙烯共聚物(Ethylene/Propylene/Styrene Copolymer) (4~15%)和丁烯/乙烯/苯乙烯共聚物(Butylene/Ethylene/Styrene Copolymer) (0.1~ 2%)的市售混合物。該混合物以大劑量餵養雄性和雌性大鼠，估算之急性口服 LD_{50} > 5,050 mg/kg。[1]
- 重複劑量毒性：無 Butylene/Ethylene/Styrene Copolymer 的數據。參考苯乙烯(Styrene)以米格魯犬進行的 560 天重複劑量毒性試驗，分別以含苯乙烯劑量 0、200、400 或 600 mg/kg bw/day 的花生油灌食。200 mg/kg/day 劑量未觀察到不良反應，但較高劑量組中觀察到紅血球中亨氏小體數量增加、細胞堆積體積減少、血紅蛋白和紅血球計數零星減少，且在肝臟中發現鐵沉積增加和亨氏小體數量增加。本研究中的 NOAEL 為 200 mg/kg/day，LOAEL 為 400 mg/kg/day。基於上述對狗的紅血球和肝臟的影響，美國 EPA 已經確定出苯乙烯的慢性經口暴露參考劑量(RfD)為 1 mg/kg/day。[1]
- 皮膚腐蝕性/刺激性：以白化兔評估含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer(0.1~ 2%)之市售混合物的皮膚刺激性。研究結果該混合物非主要的皮膚刺激物，而根據 EPA 指南第 81-5 號，該混合物為“輕微刺激性”且是可逆的因此被歸為 IV 類，為 EPA 皮膚刺激的最低毒性類別。[1]
- 眼刺激性：以白化兔評估含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer (0.1~ 2%)之市售混合物的眼部刺激性。研究結果該混合物非主要的眼部刺激物，而根據 EPA 指南第 81-4 號，該混合物對沖洗過和未沖洗過的眼睛“刺激性極小”，觀察到的輕微刺激是可逆的而被歸為 IV 類，為 EPA 眼部刺激的最低毒性類別。[1]
- 皮膚致敏性：無 Butylene/Ethylene/Styrene Copolymer 的數據。參考 Styrene/Acrylates Copolymer 依 OECD 406 進行的天竺鼠致敏試結果為非致敏劑。另參考 Styrene 的天竺鼠最大化試驗，測試包括皮內注射 10% (w/v) Styrene、局部施用 20% (w/v) Styrene 和含 2% (w/v) Styrene 的丙酮溶液激發，在任何受試動物中均未觀察到皮膚過敏現象，結果均為無皮膚致敏性。[1]
- 致癌性：無 Butylene/Ethylene/Styrene Copolymer 的數據。參考國家

毒理學計劃(NTP)於 Fischer 344 大鼠和 B6C3F1 小鼠進行關於 Styrene 的口服致癌性生物測定，未有充分證據表示 Styrene 在大鼠或小鼠中具有致癌性，但由暴露於 Styrene 的工人研究發現淋巴造血系統癌症的死亡率或發病率增加，以及 DNA 加合物和遺傳基因水平增加，因此根據 NTP，Styrene 單體被預期為人類致癌物[1]；但 ECHA 根據取得最新數據並未表明接觸苯乙烯會增加癌症風險，包括淋巴造血癌和肺腫瘤，認為尚無令人信服的證據表明 Styrene 對人類具有顯著的致癌潛力。[2]

- 致突變性/遺傳毒性：無 Butylene/Ethylene/Styrene Copolymer 的數據。參考 Styrene 可用的體外測定結果在某些測試系統中(包括 Ames 測試和哺乳動物細胞中的體外染色體畸變和姐妹染色單體交換研究)，Styrene 在體外確實具有一定的遺傳毒性潛力而該活性需要代謝活化(推測為 styrene oxide)，但在大鼠、倉鼠和小鼠中透過吸入、口服和腹腔內途徑單次或重複暴露於高達引起全身毒性的濃度或劑量的 Styrene 後，體內染色體畸變和微核的結果為陰性，因此尚無令人信服的證據表明 Styrene 在人類具有致突變性。[2]
- 生殖毒性：無 Butylene/Ethylene/Styrene Copolymer 的數據。參考 Styrene 在大鼠和兔的發育毒性研究，吸入 500 ppm 苯乙烯不會引起發育神經毒性，另一項為期 2 年 COBS (SD) BR 大鼠的慢性毒性研究，在飲用水中添加 250 ppm Styrene，結果表明對母親的食物消耗或體重增加沒有與受測物質相關影響且沒有對幼崽的顯著發育影響，因此 NTP CERHR 專家小組得出結論，Styrene 不會對實驗動物造成發育或生殖毒性。[1]
- 光毒性：無相關研究數據。
- 經皮吸收：無 Butylene/Ethylene/Styrene Copolymer 的數據。一項測定皮膚吸收率的人體試驗，9 名男性志願者將一隻手浸入液態 Styrene 中暴露 10 至 30 分鐘，數據顯示 Styrene 的吸收低(平均吸收率為 60 μg / cm^2 / h)。[2]
- 毒理代謝動力學：無 Butylene/Ethylene/Styrene Copolymer 的數據。
- 人體數據：含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer (0.1~ 2%)的市售混合物以 117 名受試者進行人體重複損傷斑貼試驗，結果該混合物未在任何受試者中誘發過敏性接觸性皮膚炎。[1]
- 其他安全資料：Butylene/Ethylene/Styrene Copolymer(丁烯/乙烯/苯

乙烯共聚物)為丁烯、乙烯和苯乙烯單體通過氫化終止的聚合物，主要用於非水性增粘劑。CIR 的安全評估報告提供化粧品中使用的苯乙烯(Styrene)和乙烯基型苯乙烯(Vinyl-type Styrene)共聚物的安全性相關資訊與評估，這些成分最常見的功能為增黏劑、遮光劑和成膜劑，而苯乙烯是此安全評估中審查的所有共聚物的組成部分。在考慮這些成分的大分子量，專家小組同意預計不會經皮吸收，不存在經皮吸收的可能性以及陰性毒性試驗結果為專家組評估這些聚合物用於化粧品的安全性提供充分的依據，專家小組得出結論該安全評估所述的 35 種苯乙烯和乙烯基型苯乙烯共聚物(其中包含 Butylene/Ethylene/Styrene Copolymer)在目前化粧品中的使用和濃度是安全的。[1]

◆ 參考資料：

1. Safety Assessment of Styrene and Vinyl-type Styrene Copolymers as Used in Cosmetics. CIR Final Report 09/09/2014.
2. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15565/7/8

6. INCI name：Ethylene/Propylene/Styrene Copolymer

- 急性毒性：一項急性口服毒性研究評估含有乙烯/丙烯/苯乙烯共聚物(Ethylene/Propylene/Styrene Copolymer) (4~15%)和丁烯/乙烯/苯乙烯共聚物(Butylene/Ethylene/Styrene Copolymer) (0.1~ 2%)的市售混合物。該混合物以大劑量餵養雄性和雌性大鼠，估算之急性口服 LD50 > 5,050 mg/kg。[1]
- 重複劑量毒性：無 Ethylene/Propylene/Styrene Copolymer 的數據。參考苯乙烯(Styrene)以米格魯犬進行的 560 天重複劑量毒性試驗，分別以含苯乙烯劑量 0、200、400 或 600 mg/kg bw/day 的花生油灌食。200 mg/kg/day 劑量未觀察到不良反應，但較高劑量組中觀察到紅血球中亨氏小體數量增加、細胞堆積體積減少、血紅蛋白和紅血球計數零星減少，且在肝臟中發現鐵沉積增加和亨氏小體數量增加。本研究中的 NOAEL 為 200 mg/kg/day，LOAEL 為 400 mg/kg/day。基於上述對狗的紅血球和肝臟的影響，美國 EPA 已經確定出苯乙烯的慢性經口暴露參考劑量(RfD)為 1 mg/kg/day。[1]
- 皮膚腐蝕性/刺激性：以白化兔評估含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer(0.1~ 2%)之市售混合物的皮膚刺激性。研究結果該混合物非主要的皮膚刺激物，而根據 EPA 指南第 81-5 號，該混合物為“輕微刺激性”且是可逆的因此被歸為 IV 類，為 EPA 皮膚刺激的最低毒性類別。[1]
- 眼刺激性：以白化兔評估含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer (0.1~ 2%)之市售混合物的眼部刺激性。研究結果該混合物非主要的眼部刺激物，而根據 EPA 指南第 81-4 號，該混合物對沖洗過和未沖洗過的眼睛“刺激性極小”，觀察到的輕微刺激是可逆的而被歸為 IV 類，為 EPA 眼部刺激的最低毒性類別。[1]
- 皮膚致敏性：無 Ethylene/Propylene/Styrene Copolymer 的數據。參考 Styrene/Acrylates Copolymer 依 OECD 406 進行的天竺鼠致敏試結果為非致敏劑。另參考 Styrene 的天竺鼠最大化試驗，測試包括皮內注射 10% (w/v) Styrene、局部施用 20% (w/v) Styrene 和含 2% (w/v) Styrene 的丙酮溶液激發，在任何受試動物中均未觀察到皮膚過敏，結果均為無皮膚致敏性。[1]
- 致癌性：無 Ethylene/Propylene/Styrene Copolymer 的數據。參考國

家毒理學計劃(NTP)於 Fischer 344 大鼠和 B6C3F1 小鼠進行關於 Styrene 的口服致癌性生物測定，未有充分證據表示 Styrene 在大鼠或小鼠中具有致癌性，但由暴露於 Styrene 的工人研究發現淋巴造血系統癌症的死亡率或發病率增加，以及 DNA 加合物和遺傳基因水平增加，因此根據 NTP，Styrene 單體被預期為人類致癌物 1；但 ECHA 根據取得最新數據並未表明接觸苯乙烯會增加癌症風險，包括淋巴造血癌和肺腫瘤，認為尚無令人信服的證據表明 Styrene 對人類具有顯著的致癌潛力。[2]

- 致突變性/遺傳毒性：無 Ethylene/Propylene/Styrene Copolymer 的數據。參考 Styrene 可用的體外測定結果在某些測試系統中(包括 Ames 測試和哺乳動物細胞中的體外染色體畸變和姐妹染色單體交換研究)，Styrene 在體外確實具有一定的遺傳毒性潛力而該活性需要代謝活化(推測為 styrene oxide)，但在大鼠、倉鼠和小鼠中透過吸入、口服和腹腔內途徑單次或重複暴露於高達引起全身毒性的濃度或劑量的 Styrene 後，體內染色體畸變和微核的結果為陰性，因此尚無令人信服的證據表明 Styrene 在人類具有致突變性。[2]
- 生殖毒性：無 Ethylene/Propylene/Styrene Copolymer 的數據。參考 Styrene 在大鼠和兔的發育毒性研究，吸入 500 ppm 苯乙烯不會引起發育神經毒性，另一項為期 2 年 COBS (SD) BR 大鼠的慢性毒性研究，在飲用水中添加 250 ppm Styrene，結果表明對母親的食物消耗或體重增加沒有與受測物質相關影響且沒有對幼崽的顯著發育影響，因此 NTP CERHR 專家小組得出結論，Styrene 不會對實驗動物造成發育或生殖毒性。[1]
- 光毒性：無相關研究數據。
- 經皮吸收：無 Ethylene/Propylene/Styrene Copolymer 的數據。一項測定皮膚吸收率的人體試驗，9 名男性志願者將一隻手浸入液態 Styrene 中暴露 10 至 30 分鐘，數據顯示 Styrene 的吸收低(平均吸收率為 60 µg / cm2 / h)。[2]
- 毒理代謝動力學：無 Ethylene/Propylene/Styrene Copolymer 的數據。
- 人體數據：含有 Ethylene/Propylene/Styrene Copolymer (4~15%)和 Butylene/Ethylene/Styrene Copolymer (0.1~ 2%)的市售混合物以 117 名受試者進行人體重複損傷斑貼試驗，結果該混合物未在任何受試者中誘發過敏性接觸性皮膚炎。[1]
- 其他安全資料：Ethylene/Propylene/Styrene Copolymer (乙烯/丙烯/

苯乙烯共聚物)為乙烯、丙烯和苯乙烯單體經加氫終止的聚合物，主要用於非水性增粘劑。CIR 的安全評估報告提供化粧品中使用的苯乙烯(Styrene)和乙烯基型苯乙烯(Vinyl-type Styrene)共聚物的安全性相關資訊與評估，這些成分最常見的功能為增黏劑、遮光劑和成膜劑，而苯乙烯是此安全評估中審查的所有共聚物的組成部分。在考慮這些成分的大分子量，專家小組同意預計不會經皮吸收，不存在經皮吸收的可能性以及陰性毒性試驗結果為專家組評估這些聚合物用於化粧品的安全性提供充分的依據，專家小組得出結論該安全評估所述的 35 種苯乙烯和乙烯基型苯乙烯共聚物(其中包含 Ethylene/Propylene/Styrene Copolymer)在目前化粧品中的使用和濃度是安全的。[1]

◆ 參考資料：

1. Safety Assessment of Styrene and Vinyl-type Styrene Copolymers as Used in Cosmetics. CIR Final Report 09/09/2014.
2. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15565/7/8

7. INCI name：Calcium Titanium Borosilicate

- 急性毒性：交互參照硼矽酸鈣的皮膚毒性試驗，約 20%用量以蒸餾水潤濕並在封閉狀態下塗抹於紐西蘭白兔的皮膚，皮膚 LD_{50} 死劑量>2000 mg/kg(最高測試量)。[1] 硼矽酸鈣於 Sprague Dawley CD 大鼠的口服毒性試驗(30%於 1%羧甲基纖維素溶液)結果，大鼠的口服 LD_{50} > 5000 mg/kg。[1]
- 重複劑量毒性：無鹼玻璃微纖維(E-glass microfibre)在反覆經口或經皮接觸後沒有毒性。兩項關於雄性大鼠亞慢性反復吸入無鹼玻璃微纖維(104E)的研究(1000 WHO 纖維/ml 空氣，7 小時/天，持續 1、3、8 或 14 天； 15、50 或 150 纖維/ml 空氣，每天 6 小時，每週 5 天，持續 3 個月)，結果證明吸入無鹼玻璃微纖維會在肺部引起炎症反應。由於無鹼玻璃微纖維不具有化學反應性，反覆暴露 3 個月的大鼠肺部誘導炎症反應的 LOAEC 為 15 WHO 纖維/ml 空氣。[2]
- 皮膚腐蝕性/刺激性：將硼矽酸鈣(0.5 g 在 0.5 mL 食鹽水)施用於紐西蘭大白兔的磨損和未磨損皮膚，在閉塞下持續 24 小時。在去除後 30 分鐘和 48 小時，完整皮膚和磨損皮膚的反應沒有差異。移除時，所有受測部位均出現從非常輕微到中度到嚴重的紅斑。2 隻動物的磨損和未磨損皮膚部位出現輕微水腫，在 72 小時刺激性大大降低。結果顯示硼矽酸鈣對兔子皮膚有輕微刺激性。[1]
- 眼刺激性：在使用測試(n = 31)中，每天使用含有 53%硼矽酸鈣鈉的眼影持續 4 週。在測試期前後進行了 24 次眼科檢查。在受試者中有 21 人自認為有敏感的眼睛，無不良反應報告，所有眼科檢查均正常。含有 31%硼矽酸鈣鋁的粉末眼影進行的 Epiocular 人體細胞測定，測試結果顯示無眼刺激性。[1]
- 皮膚致敏性：在半封閉下對含有硼矽酸鈣鈉 (53%; 0.2 g) 的粉末眼影進行的人體重複損傷斑貼試驗 (HRIPT; n = 98)中，沒有觀察到刺激或致敏的跡象。在半封閉下含有硼矽酸鈣鈉（60%-97%；施用 0.2 g）的化妝品的 HRIPT (n = 104)試驗中，沒有觀察到刺激或致敏的跡象。含有硼矽酸鈣鋁（31%；0.2 g）的粉末眼影的 HRIPT(n = 105)試驗中，沒有觀察到不良反應也沒有致敏的證據。[1]
- 致癌性：將雄性大鼠長期全身暴露於吸入無鹼玻璃微纖維氣溶膠(1000 WHO 纖維/ml)每天 7 小時，每週 5 天，最長 12 個月，之後是最長 12 個月的恢復期，研究顯示無鹼玻璃微纖維可誘導大鼠肺

部纖維化、癌、腺瘤和間皮瘤。[2]

- 致突變性/遺傳毒性：交互參照硼矽酸鈣的 Ames 試驗，將硼矽酸鈣(1 g/mL) 浸泡在 DMSO 中過夜並將上清液以鼠傷寒沙門氏菌(TA100、TA98、TA1535 和 TA1537) 進行試驗。結果無論有無代謝活性存在，上清液都無致突變性。[1]
- 生殖毒性：無相關研究數據。
- 光毒性：無相關研究數據。
- 經皮吸收：硼矽酸鹽成分是不溶和惰性，不會顯著滲透皮膚。[1]
- 毒理代謝動力學：無相關研究數據。
- 人體數據：在半封閉下對含有硼矽酸鈣鈉(53%; 0.2 g)的粉末眼影進行的人體重複斑貼試驗 (HRIPT; n = 98)中，沒有觀察到刺激或致敏的跡象。在半封閉下含有硼矽酸鈣鈉(60%-97%；施用 0.2 g)的化粧品的 HRIPT (n = 104)試驗中，沒有觀察到皮膚刺激或致敏的跡象。在半封閉下含有硼矽酸鈣鈉(53.5%；0.2 g)的化粧品的 HRIPT(n = 103)中，沒有觀察到刺激或致敏的跡象。含有硼矽酸鈣鋁(31%；0.2 g)的粉末眼影的 HRIPT(n = 105)試驗中，沒有觀察到不良反應也沒有皮膚致敏的證據。[1]
- 其他安全資料：化粧品中使用的硼矽酸鈣鈉、硼矽酸鋁鈣、硼矽酸鈦鈣、硼矽酸銀和硼矽酸鋅等硼矽酸鹽玻璃成分，硼矽酸鹽玻璃主要用作填充劑，這些成分的相似結構、特性、功能和用途使它們能夠分組並使用可用的毒理學數據來評估整組成分的安全性。[1] 無鹼玻璃微纖維的熔點為 700°C 以上，物理化學特性表明其穿過生物膜的可能性較低，因此通過胃腸道和皮膚吸收的可能性較低。[2]
- 參考資料：

1. Safety Assessment of Borosilicate Glasses as Used in Cosmetics. IJT 32(Suppl. 3):65-72, 2013.
2. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15936/7/1

8. INCI name：Tocopheryl Acetate

- 急性毒性：生育酚乙酸酯對囓齒動物和非囓齒動物的急性毒性很低。現有的大鼠急性經口毒性研究結果口服 LD_{50} > 10000 mg/kg bw，小鼠和兔子的口服 LD_{50} 分別為>4000 和>2000 mg/kg bw (類似 OECD 401)，狗的口服 LD_{50} > 14.4 g/kg bw。在急性經皮毒性測試(類似 OECD 402)中測試觀察到皮膚 LD_{50} >3000 mg/kg，無死亡。測試物質僅在施用後 24-48 小時出現引起輕微的局部紅斑，只有少數動物表現出局部磨損的跡象。[1]
- 重複劑量毒性：一項大鼠 90 天重複給藥(口服管飼法)研究，給藥劑量為 125、500 和 2000 mg/kg，高劑量雌鼠的相對肝臟重量顯著增加；2000 mg/kg bw/day 在雄鼠及雌鼠皆引起血液學變化。所有受試劑量的受試動物均觀察到肺部病變，主因為吸入測試物質，這在正常情況下不會發生，且在長期飼料研究中沒有發現這些影響。由於在 500 mg/kg 時只有 APTT 值增加，而 PT 和纖維蛋白原值沒有增加，所以 NOAEL 設定為 500 mg/kg bw/day。[1]
- 皮膚腐蝕性/刺激性：未觀察到不良反應(無皮膚刺激性)。依 OECD 404 進行的皮膚刺激研究中，3 隻兔子(2 隻雄性，1 隻雌性)暴露於生育酚乙酸酯。於 24、48 和 72 小時對紅斑和水腫進行評分，所有時間點的平均評分為 0.0。[1]
- 眼刺激性：未觀察到不良反應(無眼刺激性)。依 OECD 405 進行的眼部刺激研究中，3 隻兔子(1 隻雄性，2 隻雌性)暴露於於生育酚乙酸酯。於 24、48 和 72 小時對不透明度、虹膜、發紅和腫脹進行評分，平均評分分別為 0.0、0.0、0.2 和 0.0。[1]
- 皮膚致敏性：依 CTFA 安全測試指南以 30 隻雌性(20 隻測試和 10 隻對照)喜馬拉雅斑點天竺鼠進行生育酚乙酸酯的光致敏試驗。為了誘導致敏，將未稀釋的生育酚乙酸酯經皮施用到 8 cm^2 的皮膚區域 (之前用 4 次皮內注射弗氏完全佐劑進行標記)，然後將測試部位暴露於 1.8 J/cm^2 UVB 和 10 J/cm^2 UVA 照射，在誘導期的 2 週內重複 4 次，對照動物僅用弗氏完全佐劑。誘導開始後三週以濃度為 100%（未稀釋）、75%、50%和 25%(稀釋液在乙醇中)，然後將處理部位暴露於 10 J/cm^2 UVA 照射(左側)或未照射(右側)。於激發暴露後 24、48 和 72 小時評估紅斑和水腫的皮膚反應。觀察到 20 隻測試動物中有兩隻出現輕微的紅斑皮膚反應，在動物的照射和未照射

測試部位之間沒有檢測到一致或顯著的差異。[1]

- 致癌性：在一項飼料研究中給予大鼠 500、1000 和 2000 mg/kg bw 的生育酚乙酸酯共 104 週，研究得出的結論受試物質沒有致癌作用。[1]
- 致突變性/遺傳毒性：Ames 試驗(OECD 471，GLP)以五種鼠傷寒沙門氏菌測試菌株(TA1535、TA97、TA98、TA100 和 TA102)進行在有或無代謝活化的情況下生育酚乙酸酯的誘變活性，濃度範圍為 50 至 5000 µg/平板。結果回復菌落的數量沒有明顯增加。結論-生育酚乙酸酯在所描述的實驗條件下不致突變。[1]
- 生殖毒性：在一代生殖大鼠毒性研究中，受測組的生殖指數不受影響，後代正常發育。NOAEL 為 800 mg/kg bw/day (相當於 2%)。在大鼠和兔子的致畸性研究中(類似 OECD 414)，對照組和受測組之間的畸形沒有顯著差異，兩種物種的 NOAEL 為>1600 mg/kg bw/day。[1]
- 光毒性：以喜馬拉雅斑點天竺鼠進行生育酚乙酸酯的光致敏試驗結果無光致敏性。[1]
- 經皮吸收：在體外皮膚吸收試驗(類似 OECD 428，非 GLP)中，測試 3 種 α-羥基酸乳膏中的生育酚乙酸酯滲透到並穿過完整和剝離的豬皮膚的情形。生育酚乙酸酯的總皮膚滲透率取決於時間、配方類型和皮膚狀況，儘管沒有顯著差異。在 1 小時、6 小時和 18 小時暴露時測試 3 種不同的製劑(劑量 5%)觀察到的經皮吸收在 1.1 ~4.2 %。[1]
- 毒理代謝動力學：給予 10 隻大鼠單次口服 α-生育酚菸酸酯和 α-生育酚乙酸酯後 3、6、12、24 或 48 小時，觀察大鼠組織脂質中總放射性的分佈及代謝，分析結果 α-生育酚菸酸酯和 α-生育酚乙酸酯均被大鼠組織廣泛代謝，腎上腺、卵巢、脂肪組織和心臟似乎在吸收後長達 48 小時內從血液中提取維生素 E，出現最多的代謝物是 α-生育酚醌(α-tocopheryl quinone)。而在腎上腺中，標記最高的化合物是未酯化的生育酚。作者得出結論腎上腺組織在維生素 E 的代謝中發揮了一定的作用。[1]
- 人體數據：對 203 名人類志願者進行 Draize 測試，以確定生育酚乙酸酯對人體的皮膚致敏潛力。在誘導階段，受試者在 2 週內十次暴露於 100% 維生素 E，兩週休息期後再重新施用 3 天，每天一次。誘導期後的平均刺激指數為：0.076/受試者(n=203)，沒有受試者有高於 1 的刺激等級，在挑戰階段(n = 203)後均無陽性反應。[1]

- 其他安全資料：生育酚和生育酚乙酸酯通常被認為是安全的食品成分(GRAS)，而化粧品使用導致的全身暴露預計不會超過食品使用的安全量。FAO/WHO 的食品添加劑聯合專家委員會確定 DL-α-生育酚和 D-α-生育酚濃縮物、單獨或組合的每日允許攝入量為 0.15 至 2 mg/kg。[2]
- 參考資料：

1. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/13377/7/1
2. Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics. IJT 37(Suppl. 2):61-94, 2018.

9. INCI name：Titanium Dioxide

- 急性毒性：現有的急性經口毒性研究，在大鼠中單次經口給予顏料級或超細二氧化鈦至 25,000 mg/kg bw 後，沒有死亡或其他毒性跡象。以證據權重的方法得出結論二氧化鈦的 LD_{50} 為>5,000 mg/kg bw；在二氧化鈦粉塵(顏料級和超細)的急性吸入毒性研究中，大鼠的頭部和鼻子僅在高達 6.82 mg/L 的空氣中暴露 4 小時。進行研究期間沒有發生死亡。得出大鼠吸入 LC_{50} > 6.82 mg/L 空氣；在公共領域沒有任何關於急性皮膚毒性的可靠報告，認為不需要進行急性經皮毒性試驗，由於吸入被認為是人類接觸最相關的途徑，並且物理化學性質和毒理學特性表明沒有顯著通過皮膚吸收的潛力。[1]
- 重複劑量毒性：JECFA 於 1970 年評估 Lehmann 和 Herget 等人對對二氧化鈦的研究，給兩隻天竺鼠、兩隻兔子、兩隻貓和一隻狗餵食工業級二氧化鈦(含量≥99%)達 390 天。從飲食中狗每天攝入 9 克(相當於 900 mg/kg bw/day)、兔子攝入為 1170 克(相當於 1.5 g/kg bw/day)、貓攝入 3 克/天(相當於 1.5 g/kg bw/day)、天竺鼠攝入 0.6 克(相當於 800 mg/kg bw/day)，另外兩隻貓接受每天 3 g 二氧化鈦分別 175 天和 300 天。結果未見不良反應，組織病理學檢查未發現異常，在膽汁、心臟、脾臟和骨骼肌中檢測到少於 5 mg 的鈦。[2]
- 皮膚腐蝕性/刺激性：根據 OECD 404 對紐西蘭白兔進行半封閉斑貼試驗，未觀察到紅斑，物質被歸類為無皮膚刺激性。[1]
- 眼刺激性：根據 OECD 405 對紐西蘭白兔進行急性眼刺激測試，物質被歸類為無眼刺激性。[1]
- 皮膚致敏性：超細二氧化鈦以 OECD 429 相當的小鼠局部淋巴結檢測 (LLNA)測試濃度 5、25、50 及 100%，觀察到所有的刺激指數 (SI) 小於 3.0，無法計算在研究條件下測試物質的 EC3 值。另一以 OECD 406 進行的天竺鼠 Buehler 方法，結果無論是在誘導期，還是在測試物質的激發後 24 和 48 小時，任何動物都沒有觀察到發紅現象。因此二氧化鈦非皮膚致敏劑。[1]
- 致癌性：1979 年美國 NCI 以 Fischer 344 大鼠和 B6C3F1 小鼠(50 隻動物/性別)進行致癌性研究。在 B6C3F1 小鼠的飲食中添加二氧化鈦(銳鈦礦，未指定粒度，純度 98%)，劑量為 0、25,000 和 50,000 mg/kg 飼料 (相當於雄性 0、3250、6500 mg TiO2/kg bw/day 和雌性 0、4175、8350 mg TiO2/kg bw/day)，該研究進行 103 週並於 104 週

犧牲動物，研究結論口服二氧化鈦是在 B6C3F1 小鼠中不致癌。在 Fischer 344 大鼠以同樣方式進行(相當於雄性 0、1125、2250 mg /kg bw/day，和雌性 0、1450、2900 mg/kg bw/day)，結果測試物質沒有影響雄性和雌性大鼠的存活率且給藥組的腫瘤發生率沒有顯著高於對照組研究，作者結論口服二氧化鈦對 Fischer 344 大鼠沒有致癌作用。根據這項研究 EFSA Panel 小組確定 NOAEL 為 50000 mg/kg bw 飼料，相當於雄性 2250 和雌性 2900 mg/kg bw/day，分別是測試的最高劑量。[2] 大鼠暴露於 10、50 和 250 mg/ m^3 的二氧化鈦粉塵 24 個月，在最高劑量的動物中出現了鱗狀細胞癌。[3]

- 致突變性/遺傳毒性：二氧化鈦在細菌回復突變試驗、體外基因突變和染色體斷裂試驗以及體內試驗中進行多項均顯示陰性反應。[1]
- 生殖毒性：根據現有嚙齒動物長期毒性/致癌性研究的證據權重和大鼠毒代動力學行為的相關信息，得出的結論是二氧化鈦不存在生殖毒性危害。[1]
- 光毒性：依 OECD 432 進行奈米二氧化鈦 T805 (有塗層, A/R, PSMA 1 type)、T817 (有塗層, A/R, PSMA 1 type)及 P25 (無塗層)的光毒性試驗，T805 和 T817 在高達 100mg/L 的濃度下沒有細胞毒性及光毒性。P25(無塗層)在最高濃度下沒有細胞毒性但照射後觀察細胞存活率於 50 mg/L 和 100mg/L 分別降低 82%和 44%，P25 對 Balb/c 3T3 細胞有光毒性，表示塗層對奈米二氧化鈦的光毒性之重要性。[4]
- 經皮吸收：依 OECD 428 進行微細氧化鋅和二氧化鈦通過豬皮膚的體外吸收試驗中，測試配方以 4 mg/cm^2 的標稱劑量應用於 1cm^2 暴露的皮膚 24 小時，結果完全質量平衡(回收率 86-100%)、受體吸收劑量為 0%而皮膚潛在可吸收劑量為 0.1-0.5% (不包括表面膠帶剝離)，表示受試物質不能穿透豬角質層。[1]
- 毒理代謝動力學：口服二氧化鈦後，在組織中沒有觀察到鈦的大量累積，已證實二氧化鈦不會以任何顯著程度滲透人體皮膚，因此二氧化鈦通過人體皮膚的真皮吸收被認為極低。[1]
- 人體數據： SCCS 安全評估意見報告中評估多項人體經皮吸收試驗結果，這些研究結果表示二氧化鈦米粒子在防曬配方中施用於皮膚時，可能大部分留在皮膚上而小部分粒子可能會滲透到角質層的外層，一些報告表示二氧化鈦奈米粒子可能會更深地滲透以到達顆粒層。然而，一致且大量的證據表示奈米粒子的滲透深度不足以到達健康皮膚的活表皮或真皮細胞。在乾癬患者皮膚防曬配方中的奈米

二氧化鈦比健康皮膚更能滲透到角質層的更深區域，但在乾癬患者皮膚或健康皮膚中都無法到達活細胞層。[4]

- 其他安全資料：SCCS 安全評估指出在頭髮造型氣溶膠噴霧產品中使用濃度為 25%的色素二氧化鈦 (TiO_2) 對普通消費者或美髮師來不安全，而在臉部彩妝應用於鬆散粉末中使用最高濃度 25%的顏料二氧化鈦對普通消費者來說是安全的，此結論是基於在可能分類為 2 類致癌物(通過吸入)的情況下對二氧化鈦的安全評估，表示該意見書的結論適用於在化粧品中可能通過吸入途徑(即氣霧劑、噴霧劑和粉末狀產)品引起消費者暴露的顏料二氧化鈦，而該意見不適用於任何珠光顏料，因為此類材料具有複合性質，其中二氧化鈦只是微量成分。[5] 二氧化鈦 TiO_2 (CI 77891)為美國食品和藥物管理局免於認證的顏色添加劑，可安全用於著色產品，包括塗抹在嘴唇和眼睛區域的化粧品和個人護理產品，歐盟以化粧品法規附件 IV (E171)管理。我國以化粧品色素成分使用限制表管理，所有化粧品均可使用[6]。
- 參考資料：

1. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15560/7/1
2. Re-evaluation of titanium dioxide (E 171) as a food additive. EFSA ANS Panel, 2016.
3. SCCNFP/0005/98- SCCNFP Opinion on Titanium Dioxide (S75), 2000.
4. SCCS/1516/13-SCCS OPINION ON Titanium Dioxide (nano form), 2014.
5. SCCS/1617/20-OPINION on Titanium dioxide (TiO2) used in cosmetic products that lead to exposure by inhalation, 2020.
6. 化粧品色素成分使用限制表(衛授食字第 1091605373 號)

10.INCI name：Tin Oxide

- 急性毒性：小鼠和大鼠(數量和菌株未說明)對 Tin Oxide 的急性口服 LD50 為>20 g/kg，腹腔給藥的急性 LD_{50}>6.6 g/kg。[1]
- 重複劑量毒性：大鼠餵食含有 0%、0.03%、0.10%、0.30%或 1.0% Tin Oxide 的飼料 28 天，未觀察到相關的不良反應。[2]
- 皮膚腐蝕性/刺激性：無相關研究數據。
- 眼刺激性：絨毛尿囊膜 (CAM)血管測定法(CAMVA-14 天)和牛角膜混濁度和通透性 (BCOP)測試評估含有 1.11% Tin Oxide 的眼影的眼刺激性，於稀釋至 0.6%和 0.2%濃度時兩試驗均為陰性。31 名受試者每日使用含有 0.3% Tin Oxide 的眼影，4 週後無眼部刺激反應。[1]
- 皮膚致敏性：人體重複損傷斑貼試驗結果，評估口紅(含 0.5%，103 名受試者)、唇彩(0.35%，108 名受試者)、眼影粉(0.3%，98 名受試者)，眼影(1.3%，209 名受試者)均未引起皮膚刺激或過敏性接觸致敏。[1]
- 致癌性：無相關研究數據。
- 致突變性/遺傳毒性：無相關研究數據。
- 生殖毒性：無相關研究數據。
- 光毒性：無相關研究數據。
- 經皮吸收：無相關研究數據。
- 毒理代謝動力學：無相關研究數據。
- 人體數據：31 名受試者每日使用含有 0.3% Tin Oxide 的眼影，4 週後不會引起眼部刺激。人體重複損傷斑貼試驗結果，評估口紅(含 0.5%，103 名受試者)、唇彩(0.35%，108 名受試者)、眼影粉(0.3%，98 名受試者)，眼影(1.3%，209 名受試者)均未引起皮膚刺激或過敏性接觸致敏。[1]
- 其他安全資料：氧化錫 SnO_2 在化粧品中用作研磨劑、填充劑和遮光劑，在沖洗型產品使用濃度高達 0.4%而在駐留型產品中高達 1.3%。CIR 專家小組指出 SnO_2 為不溶於水的無機金屬化合物，不應經皮吸收而全身暴露，應確認的是施用部位的毒性，而專家小組得出結論目前的使用和濃度是安全的。[1]
- 參考資料：

1. Safety Assessment of Tin(IV) Oxide as Used in Cosmetics. IJT 33(Suppl 4):40-46, 2014.

11.INCI name：Iron Oxides

- 急性毒性：一項未發表的研究(Bayer, 1977 年)報告大鼠口服氧化鐵紅(Fe_2O_3)的 LD_{50} 高於 10 g/kg bw，另一項使用氧化鐵紅的大鼠研究(Ramm，1986 年)，LD_{50} 高於 5 g/kg bw。[1]
- 重複劑量毒性：奈米氧化鐵紅 (Fe_2O_3-30 nm) 和微米氧化鐵紅(Fe_2O_3-Bulk)的大鼠 28 天亞急性口服毒性進行 0、30、300 或 1 000 mg/kg bw/day 的比較。在暴露於微米級紅色氧化鐵或 30 或 300 mg/kg bw/day 的奈米氧化鐵紅的大鼠中，沒有觀察到體重下降、攝食量沒有變化，也沒有觀察到任何不良症狀或死亡率，而服用高劑量奈米氧化鐵紅的大鼠表現出體重和攝食量下降、嚴重的中毒症狀以及生化參數的一些紊亂以及肝臟、腎臟和肝臟的不良組織病理學變化。確定微米氧化鐵紅的 NOAEL 為 1 000 mg/kg bw/day，為測試的最高劑量。另一根據 OECD TG 408 以 Sprague-Dawley 大鼠管飼奈米氧化鐵紅(Fe_2O_3，60-118 nm)劑量 250、500 或 1000 mg/kg bw/day，持續 13 週，結果顯示亞慢性口服 Fe_2O_3 奈米顆粒對大鼠沒有全身毒性，奈米氧化鐵的 NOAEL 為 1000 mg/kg bw/day。[1]
- 皮膚腐蝕性/刺激性：根據 OECD 404 對白化兔進行斑貼試驗，未觀察到紅斑，物質被歸類為無皮膚刺激性。[2]
- 眼刺激性：根據 OECD 405 對紐西蘭白兔進行急性眼刺激測試，物質被歸類為無眼刺激性。[2]
- 皮膚致敏性：IUCLID 數據集文件報告稱，在 Maurer (1979)使用天竺鼠的優化致敏試驗方法中，紅色氧化鐵(Fe_2O_3)和黑色氧化鐵($FeO·Fe_2O_3$)均為陰性反應。[1]
- 致癌性：關於長期毒性和致癌性，10 隻狗在 1 至 9 歲期間以約 570 mg/磅(相當於 1.25 g/kg 飼料，0.312 mg /kg bw /19 天)餵食含有氧化鐵著色劑(未指定化合物)的飼料，每日消耗量估計為 428 mg/狗，未報告有不良反應。國際癌症研究機構 (IARC)專著指出，證據表示赤鐵礦(紅色氧化鐵)和三氧化二鐵(未指明的化合物)對動物沒有致癌性，而對人類的致癌性證據不足。[1]
- 致突變性/遺傳毒性：紅色(Fe_2O_3)和黑色 ($FeO·Fe_2O_3$)氧化鐵，無論是奈米形式還是微米形式(分別為 7-30 nm 和>100 nm)，在哺乳動物細胞的體外遺傳毒性試驗中均呈陽性反應，其中誘導 DNA 鏈斷裂並觀察到微核。但奈米和微米尺寸的紅色氧化鐵的體內口服給藥

在大鼠造血系統中未引起遺傳毒性作用，沒有關於胃腸道接觸部位的數據。由於數據庫的限制，並考慮到無法在不同氧化還原狀態的氧化鐵之間進行交叉讀取，無法根據現有數據評估氧化鐵的遺傳毒性。[1]

- 生殖毒性：在一項未發表的研究(JECFA，1983 年)，10 隻雄性和 3 隻雌性水貂在其飼料中添加了 0.75%的氧化鐵(未指定的化合物)觀察到繁殖、產仔和泌乳與對照組相似。六隻雄性幼崽和四隻雌性幼崽繼續使用氧化鐵飼料，直到蛻皮(165 天)時觀察到急性腎病和肝病。皮毛質量和生長正常，未觀察到毒性跡象。[1]
- 光毒性：無相關研究數據。
- 經皮吸收：無相關研究數據。
- 毒理代謝動力學：以 OECD417 於 Crl:CD(SD)大鼠口服 Sicovit Red 30 E172 的試驗，以 1000 mg/kg bw 劑量單次口服 Sicovit Red 30 E172 後，任何雄性或雌性動物均未發現與測試項目相關的行為變化或外觀異常跡象，雄性和雌性大鼠 0 至 72 小時血漿中的 Cmax 分別為 3.17 µg Fe/g 和 4.39 µg Fe/g。與靜脈給藥相比，口服給藥後鐵的絕對生物利用度為 0.22%/0.23%(雄性/雌性)，評估是在未考慮載體的情況下對特定物質數據進行的，而給藥組的血漿鐵含量實際上落在載體對照組的範圍內，因此，由毒代動力學分析得出的絕對生物利用度因此可以被視為保守的高估，從而得出結論即來自測試氧化物的鐵的生物利用度同樣最小到可以忽略不計。
- 人體數據：一項流行病學研究法國碳鋼生產廠僱用的工人中氧化鐵暴露與肺癌風險之間可能存在的關聯性，受試者共 16,742 名男性和 959 名女性的評估發現與強度、暴露持續時間和累積指數沒有劑量反應關係，結論未發現接觸氧化鐵與肺癌死亡率之間存在任何關係。[2]
- 其他安全資料：1980 年 JECFA 確定 ADI 為 0.5 mg/kg bw/day。[1] 紅色鐵氧化物 Fe_2O_3 (CI 77491)為美國食品藥品監督管理局免於認證的顏色添加劑，可安全用於著色產品，包括塗抹在嘴唇和眼睛區域的化粧品和個人護理產品，歐盟以化粧品法規附件 IV (E172)管理。我國以化粧品色素成分使用限制表管理，所有化粧品均可使用[3]。
- 參考資料：

1. Scientific Opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives. EFSA Journal 2015;13(12):4317.

2. Registration Dossier. ECHA 網站: https://echa.europa.eu/registration-dossier/-/registered-dossier/15552/7/1
3. 化粧品色素成分使用限制表(衛授食字第 1091605373 號)

(11) 產品安定性試驗報告

試驗結果評估：針對外觀、顏色、氣味、pH、黏度、密度、微生物、包材外觀項目進行 6 個月產品安定性試驗，結果判定均合格，將持續執行達宣稱效期之長期安定性試驗。

產品名稱	亮澤護唇蜜			
包裝材質	ABS、PET、PETG、PE			
試驗時間 / 試驗項目	第 0 個月 40 ℃ 75 %RH	第 1 個月 40 ℃ 75 %RH	第 3 個月 40 ℃ 75 %RH	第 6 個月 40 ℃ 75 %RH
外觀	不流動油膏	不流動油膏	不流動油膏	不流動油膏
顏色	紅色帶珠光粉	紅色帶珠光粉	紅色帶珠光粉	紅色帶珠光粉
氣味	薄荷香	薄荷香	薄荷香	薄荷香
黏度(at 25 °C)	38,630 mPa·s	37,520 mPa·s	40,240 mPa·s	39,550 mPa·s
密度(at 25 °C)	0.95 g/cm^3	0.94 g/cm^3	0.96 g/cm^3	0.98 g/cm^3
微生物檢測結果	未檢出	未檢出	未檢出	未檢出
包材外觀	無膨脹、變色、腐蝕及脆裂之現象	無膨脹、變色、腐蝕及脆裂之現象	無膨脹、變色、腐蝕及脆裂之現象	無膨脹、變色、腐蝕及脆裂之現象
結果判定	■合格 □不合格	■合格 □不合格	■合格 □不合格	■合格 □不合格
參考試驗方法	ISO/TR 18811 Cosmetics-Guidelines on the stability testing of cosmetics products,2018. 參考 5.3.2 建議之溫度及濕度進行加速安定性試驗			
檢測人員/日期	(請簽名並加上日期)	(請簽名並加上日期)	(請簽名並加上日期)	(請簽名並加上日期)
複核人員/日期	(請簽名並加上日期)	(請簽名並加上日期)	(請簽名並加上日期)	(請簽名並加上日期)

(12)微生物檢測報告

產品名稱	**亮澤護唇蜜**		
產品批號	IT22080F		
產品製造日期	111.08.06		
包裝材質	ABS、PET、PETG、PE	**試驗日期**	111.08.12
檢測項目	**規 格**	**檢測結果**	**參考測試方法**
生菌數	<100 cfu/g	未檢出 (<10 cfu/g)	參考衛生福利部食品藥物管理署 109.07.28 及 111.04.21 公布建議檢驗方法-化粧品中微生物檢驗方法及化粧品中白色念珠菌之檢驗方法。
大腸桿菌	不得檢出	未檢出	
綠膿桿菌	不得檢出	未檢出	
金黃色葡萄球菌	不得檢出	未檢出	
白色念珠菌	不得檢出	未檢出	
結果判定	■合格 □不合格		
檢測人員/日期	(請簽名並加上日期)		
複核人員/日期	(請簽名並加上日期)		

(13) 防腐效能試驗報告

樣品名稱 (Sample Name)		亮澤護唇蜜			
測試日期(Date Tested): 111.05.03~06.15					
試驗參考方法(Method Code): 衛福部食藥署 110.05.13 公告之化粧品防腐效能試驗指引					
測試菌種 (Microbial strains)					
分析時間點 (Assay Time)	大腸桿菌 *Escherichia coli* (ATCC 8739) (CFU/g or ml)	金黃色葡萄球菌 *Staphylococcus aureus* (ATCC 6538) (CFU/g or ml)	綠膿桿菌 *Pseudomonas aeruginosa* (ATCC 9027) (CFU/g or ml)	白色念珠菌 *Candida albicans* (ATCC 10231) (CFU/g or ml)	黑麴菌 *Aspergillus brasiliensis* (ATCC 16404) (CFU/g or ml)
第 0 天	9.2×10^5	9.6×10^5	8.5×10^5	8.4×10^4	9.3×10^4
第 7 天	<10	<10	<10	4.3×10^2	4.8×10^3
第 14 天	<10	<10	<10	<10	1.2×10^2
第 28 天	<10	<10	<10	<10	<10
檢測人員/日期		(請簽名並加上日期)			
複核人員/日期		(請簽名並加上日期)			

(14) 功能評估佐證資料

相關功能性測定，依產品宣稱之功能提供相關佐證資料。

(15) 與產品接觸之包裝材質資料

產品容量 ：10 ml

包裝材料	包裝材質
瓶蓋	ABS (Acrylonitrile Butadiene Styrene) 
刷柄+刷毛	PET (polyethylene terephthalate) +絨毛 
內塞	PE (polyethylene) 
瓶身	PE (polyethylene) 

III. 安全評估資料

(16) 產品安全資料

亮澤護唇蜜每日皮膚暴露量計算

參考 2023 年 5 月發布之歐盟消費者安全科學委員會(Scientific Committee on Consumer Safety, SCCS)化粧品成分測試及其安全性評估指引第 12 版(SCCS/1647/22)，並依用途、部位、頻率進行皮膚暴露量計算。

基本數據	
平均體重	60 kg
接觸部位	唇部
接觸種類	駐留產品
每日使用頻率	2/day
使用表面積(cm^2)	4.8
駐留因子	1.00

每日皮膚暴露量($E_{product}$)

對於此亮澤護唇蜜，參考 2023 年 5 月發布之 SCCS 化粧品成分測試及其安全性評估指引第 12 版(SCCS/1647/22)表 3A，查表得知每日皮膚暴露量：

Product type	*Estimated daily amount applied* q_x *(g/d)*	*Relative daily amount applied*[1] q_x/bw *(mg/kg bw/d)*	*Retention factor*[2] f_{ret}	*Calculated daily exposure* $E_{product}$ *(g/d)*	*Calculated relative daily exposure*[1] $E_{product}/bw$ *(mg/kg bw/d)*
Make-up					
Liquid foundation	0.51	7.90	1.00	**0.51**	**7.90**
Lipstick, lip salve	0.057	0.90	1.00	**0.057**	**0.90**

在 MoS 計算中使用的每日皮膚暴露量為 0.9 mg/kg bw/day。

亮澤護唇蜜各成分 MoS 值計算

計算各個成分之 Margin of Safety (MoS) 安全邊際值如下表：

SED= $E_{product}$ (每日皮膚暴露量)×C/100(配方百分比)×Dap/100(皮膚吸收率)

MoS = POD_{sys}/SED

SED (mg /kg bw/day)為全身暴露劑量；Eproduct (mg /kg bw/day)為每日皮膚暴露量；

C(%)為配方百分比；Dap(%)為皮膚吸收率；PODsys 一般常用 NOAEL 估算。

SCCS 化粧品成分測試及其安全性評估指引第 12 版 (SCCS/1647/22) 提及 90 天口服毒性試驗是化粧品成分最常用的重複劑量毒性試驗，當有科學合理的 90 天研究確認明確的每日使用的劑量反應點(Point of Departure, PoD)時 SCCS 會考慮以該研究計算 MoS，當對亞慢性毒性研究的品質存疑或缺乏支持 90 天研究的 PoD 時，則建議應用不確定性因子來推估，為了保守嚴謹評估，故亦將各成分之 NOAEL 在考慮各別的毒理試驗條件後將不確定因子進行校正。以校正後之 NOAEL 值計算結果如下:

INCI name	配方百分比 C(%)	皮膚吸收率 DA_P(%)	NOAEL (mg /kg bw/day)	SED (mg /kg bw/day)	MoS
Mineral Oil	69.1	10	1428.6	0.0622	22968
Diisostearyl Malate	12.0	10	315.0	0.0108	29167
Simmondsia Chinensis (Jojoba) Seed Oil	5.0	100	-	0.0450	-
Quaternium-18 Bentonite	4.0	10	333.0	0.0036	92500
Butylene/Ethylene/ Styrene Copolymer	3.9	10	200.0	0.0035	57143
Ethylene/Propylene/ Styrene Copolymer	3.8	10	200.0	0.0034	58824
Fragrance	1.0	100	-	0.0090	附 IFRA 符合性聲明
Calcium Titanium Borosilicate	0.53	10	-	0.0005	-
Tocopheryl Acetate	0.5	10	250.0	0.0005	500000
Titanium Dioxide	0.076	10	2250.0	0.0001	22500000
Tin Oxide	0.004	10	-	0.0000	-
Iron Oxides	0.09	10	155.6	0.0001	1556000

INCI name	NOAEL 校正說明
Mineral Oil	13 週每週 5 天的大鼠皮膚毒性得知 NOAEL 為 2000 mg/kg bw/day，考慮試驗天數之不確定因子，2000*5/7 =1428.6 mg/kg bw/day。
Diisostearyl malate	交叉參照 Bis(2-ethylhexyl) adipate 的 90 天大鼠亞慢性經口毒性研究得知 NOAEL 為 630 mg/kg bw/day,,考慮口服生物可用率 50%之不確定因子，630*50% =315 mg/kg bw/day。
Quaternium-18 Bentonite	兔子的 90 天重複皮膚毒性試驗得知 NOAEL 約為 333 mg mg/kg/day。
Butylene/Ethylene/Styrene Copolymer	交叉參照 Styrene 的 560 天狗重複劑量毒性試驗得知 NOAEL 為 200 mg/kg bw/day，此為更保守值故未以不確定因子進行校正。
Ethylene/Propylene/Styrene Copolymer	交叉參照 Styrene 的 560 天狗重複劑量毒性試驗得知 NOAEL 為 200 mg/kg bw/day，此為更保守值故未以不確定因子進行校正。
Tocopheryl Acetate	90 天大鼠口服毒性得知 NOAEL 為 500 mg/kg bw/day，考慮口服生物可用率 50%之不確定因子，500*50% =250 mg/kg bw/day。
Titanium Dioxide	大鼠 103 週大口服致癌試驗得出 NOAEL 為 2250 mg/kg bw/day，此為更保守值故未以不確定因子進行校正。
Iron Oxides	28 天大鼠口服毒性得知 NOAEL 為 1000 mg/kg bw/day,考慮口服生物可用率 50%及試驗天數等不確定因子，1000*50%*28/90 =155.6 mg/kg bw/day。

亮澤護唇蜜安全評估結論

安全評估結論簡述

經分析所有可取得之安全性資料，根據上述評估計算結果並根據當前科學知識，推定亮澤護唇蜜在預期正常合理使用條件下，本產品為可安全使用之產品，對人體健康造成傷害風險低。

標籤警語和使用說明

亮澤護唇蜜的包裝材料/標籤上提到了以下警告和使用說明：

使用方式：沾取適量，均勻塗抹於唇部。

使用注意事項：僅限使用於唇部，使用後若有強烈刺痛感，請立即停止使用。

安全評估理由

亮澤護唇蜜的安全性評估基於每種成分的毒理學特徵並評估所收集之產品數據。

1. 該產品在符合化粧品優良製造規範之場所和生產設施中生產，並進行微生物品質管理以及倉儲管理作業。
2. 本產品添加之色素，符合我國化粧品色素成分使用限制表之規定。
3. 根據本產品「亮澤護唇蜜」之化粧品的物理/化學特性、安定性試驗報告、微生物檢測報告及防腐效能試驗報告，結果由數據顯示產品符合規格特性，證實了「亮澤護唇蜜」產品配方具有足夠安定性及微生物安全性。由六個月之加速安定性試驗推測本產品於架儲期間品質穩定，上市後將同時進行長期安定性試驗確認之。
4. 微生物檢測報告結果符合我國化粧品微生物容許量基準之要求。防腐效能試驗報告顯示符合衛福部食藥署 110.05.13 公告之化粧品防腐效能試驗指引標準 A，表示產品微生物汙染風險受到管控，可保護產品避免受到潛在微生物汙染之風險。。
5. 本產品使用之包裝材質為 ABS、PET、PETG 及 PE，根據過去類似配方及此包材之使用經驗，評估此包裝材料合適且安全。
6. 根據 SCCS 化粧品成分測試及其安全性評估指引第 12 版，計算化粧品中產品和各別成分的暴露程度。對於產品使用暴露量，採用國際間常用 SCCS 用於口紅或潤唇膏產品之標準暴露值以計算安全邊際值 (MoS)。
7. 使用之香精符合國際香料協會標準(IFRA 50th Amendment)，應用於唇部

產品之最大濃度為 20 %，此亮澤護唇蜜添加 1%香精，推測不具皮膚致敏性。

8. 此亮澤護唇蜜中的所有原材料和成分均可使用於化粧品中，而針對可取得重複劑量毒性試驗數據之成分計算出的安全邊際值(MoS)皆高於 100，這支持此產品的安全性。目前未取得重複劑量毒性試驗數據之 Simmondsia Chinensis (Jojoba) Seed Oil、Calcium Titanium Borosilicate 及 Tin Oxide，根據其物理化學特性及取得之毒理數據資料可得知，導致全身毒性風險低且對皮膚不會引起刺激性及致敏性。
9. 目前此產品尚未出現不良反應和嚴重不良反應，如有不良反應和嚴重不良反應的相關資料時，會及時提供給安全資料簽署人員重新評估此產品之安全性，並更新於本產品資訊檔案。

（請簽名並加上日期）

_____

安全資料簽署人員簽名及日期

*請檢附安全資料簽署人員之符合之學歷及資格證明文件

附錄 1：產品及各別成分之物理及化學特性資料

註：本範例僅提供其中一成分之物理化學特性資料為示範，實際執行時應包含所有蒐集到之產品及內含各成分之品質規格或各成分之檢驗報告(Certificate of Analysis, COA)、安全資料表(Safety Data Sheet, SDS)、檢驗標準或試驗方法等分析規格書，且內容如有變更應隨時更新。

INCI name：Titanium Dioxide

SAFETY DATA SHEET

1. Identification of the substance or mixture and of the supplier

A. GHS product identifier Glare® Glitter Red GL-7401E
B. Recommended use of the chemical and restrictions on use
Recommended use Cosmetic
Restrictions on use Not available
C. Manufacturers

2. Hazards identification

A. GHS classification of the substance/mixture
Not classified
B. GHS label elements, including precautionary statements
Piotogram and symbol : Not applicable
Signal word : Not applicable
Hazard statements : Not applicable
Precautionary statements
Precaution : Not applicable
Treatment : Not applicable
Storage : Not applicable
Disposal : Not applicable
C. Other hazard information not included in hazard classification (NFPA)
Health 0
Flammability Not available
Reactivity Not available

3. Composition/information on ingredients

Chemical Name (INCI Name)	CAS number	EC number	Content (%)
Calcium Titanium Borosilicate	65997-17-3	266-046-0	74 – 85
Tin Oxide (CI 77861)	18282-10-5	242-159-0	0 – 1
Titanium Dioxide (CI 77891)	13463-67-7	236-675-5	15 – 25

4. First aid measures

A. Eye contact
– In case of contact with substance, immediately flush eyes with running water at least 20 minutes.
B. Skin contact
– In case of contact with substance, immediately flush skin with running water at least 20 minutes.

- Remove and isolate contaminated clothing and shoes.
- Wash contaminated clothing and shoes before reuse.
- Get immediate medical advice/attention.

C. Inhalation
- Specific medical treatment is urgent.
- Move victim to fresh air.
- Give artificial respiration if victim is not breathing.
- Administer oxygen if breathing is difficult.

D. Ingestion
- Do not let him/her eat anything, if unconscious.
- Get immediate medical advice/attention.

E. Indication of immediate medical attention and notes for physician
- Ensure that medical personnel are aware of the material(s) involved and take precautions to protect themselves.

5. Fire fighting measures

A. Suitable (and unsuitable) extinguishing media
- Suitable extinguishing media: Dry sand, dry chemical, alcohol-resistant foam, water spray, regular foam, CO2
- Unsuitable extinguishing media: High pressure water streams

B. Specific hazards arising from the chemical
- If inhaled, may be harmful.

C. Special protective equipment and precautions for fire-fighters
- Dike fire-control water for later disposal; do not scatter the material.
- Move containers from fire area if you can do it without risk.
- Fire involving Tanks: Cool containers with flooding quantities of water until well after fire is out.
- Fire involving Tanks: Withdraw immediately in case of rising sound from venting safety devices or discoloration of tank.
- Fire involving Tanks: Always stay away from tanks engulfed in fire.

6. Accidental release measures

A. Personal precautions, protective equipment and emergency procedures
- Eliminate all ignition sources.
- Stop leak if you can do it without risk.
- Please note that materials and conditions to avoid.
- Ventilate the area.
- Do not touch or walk through spilled material.
- Prevent dust cloud.

B. Environmental precautions and protective procedures
- Prevent entry into waterways, sewers, basements or confined areas.

C. The methods of purification and removal
- Small Spill: Flush area with flooding quantities of water. And take up with sand or other non-combustible absorbent material and place into containers for later disposal.
- Large Spill: Dike far ahead of liquid spill for later disposal.
- With clean shovel place material into clean, dry container and cover loosely; move containers from spill area.

7. Handling and storage

A. Precautions for safe handling
- Please note that materials and conditions to avoid.
- Wash thoroughly after handling.
- Please work with reference to engineering controls and personal protective equipment.
- Be careful to high temperature.

B. Conditions for safe storage
- Store in a closed container.
- Store in cool and dry place.

8. Exposure controls/personal protection

A. Occupational Exposure limits

Korea regulation

Titanium Dioxide TWA = 10 mg/m³

ACGIH regulation

Titanium Dioxide TWA 10 mg/m³

Biological exposure index : Not available

OSHA regulation

Calcium Titanium Borosilicate TWA = 15 mg/m³ (total dust) TWA = 5 mg/m³ (Respirable fraction)

Titanium Dioxide TWA = 15 mg/m³

NIOSH regulation

Calcium Titanium Borosilicate TWA = 3 fibers/cm³ (fibers ≤ 3.5 μm in diameter & ≥ 10 μm in length) TWA = 5 mg/m³ (total dust)

Tin Oxide TWA = 2 mg/m³ (as Sn)

EU regulation : Not available

Other

Tin Oxide Belgium: TWA = 2 mg/m³ (as Sn) Canada: TWA = 2 mg/m³ (as Sn) Finland: TWA = 2 mg/m³ (as Sn) Spain: TWA = 2 mg/m³ (as Sn)

Titanium Dioxide Austria: TWA = 10 mg/m³ France: TWA = 10 mg/m³ (as Ti) Italy: TWA = 10 mg/m³ United Kingdom: TWA = 10 mg/m³ Russia: TWA = 10 mg/m³

B. Appropriate engineering controls
- Provide local exhaust ventilation system or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value.

C. Personal protective equipment

Respiratory protection
- Wear NIOSH or European Standard EN 149 approved full or half face piece (with goggles) respiratory protective equipment when necessary.
- In case exposed to particulate material, the respiratory protective equipments as follow are recommended. :facepiece filtering respirator or air-purifying respirator, high-efficiency particulate air(HEPA) filter media or respirator equipped with powered fan, filter media of use(dust, mist, fume)
- In lack of oxygen(< 19.5%), wear the supplied-air respirator or self-contained breathing apparatus.oxygen

Eye protection
- Wear facepiece with goggles to protect.
- An eye wash unit and safety shower station should be available nearby work place.

- Wear breathable safety goggles to protect from particulate material causing eye irritation or other disorder.
- An eye wash unit and safety shower station should be available nearby work place.

Hand protection
- Wear chemical resistant gloves.
- Wear appropriate protective gloves by considering physical and chemical properties of chemicals.

Body protection
- Wear appropriate protective chemical resistant clothing.
- Wear appropriate protective clothing by considering physical and chemical properties of chemicals.

9. Physical and chemical properties

A. Appearance
Description Powder
Color White
B. Odor No odor
C. Odor threshold Not available
D. pH 7 – 11
E. Melting point/freezing point Not available
F. Initial boiling point and boiling range Not available
G. Flash point Not available
H. Evaporation rate Not available
I. Flammability (solid, gas) Not applicable
J. Upper/lower flammability or explosive limits Not available
K. Vapor pressure Not available
L. Solubility (ies) Not available
M. Vapor density Not available
N. Specific gravity 2.6 – 2.9 g/cm^3
O. Partition coefficient: n-octanol/water Not available
P. Auto ignition temperature Not available
Q. Decomposition temperature Not available
R. Viscosity Not available
S. Molecular weight Not available

10. Stability and reactivity

A. Chemical stability and Possibility of hazardous reactions:
- If inhaled, may be harmful.

B. Conditions to avoid:
- Heat, sparks or flames

C. Incompatible materials:
- Combustibles

D. Hazardous decomposition products:
- Not available

11. Toxicological information

A. Information of Health Hazardous

Acute toxicity

Oral : Not classified

- **Calcium Titanium Borosilicate** : Rat LD_{50} > 2,000 mg/kg (Read across: 1317-36-8)(OECD TG 423, GLP)
- **Tin Oxide** : Rat LD_{50} > 9,000 mg/kg
- **Titanium Dioxide** : Rat LD_{50} > 5,000 mg/kg (OECD Guideline 425, EPA OPPTS 870.1100)

Dermal : Not available

Inhalation : Not classified

- **Tin Oxide** : Rat LC_{50} > 5 mg/L/4hr (OECD TG 403, GLP)
- **Titanium Dioxide** : Rat LC_{50} > 6.82 mg/L/4hr

Skin corrosion/ irritation : Not classified

- **Calcium Titanium Borosilicate** : In test on skin irritation with rabbits, skin irritations were not observed. (Read across: 1317-36-8)(OECD TG 404, GLP)
- **Tin Oxide** : Skin irritation test using rabbit, not skin irritation. (OECD TG 404)
- **Titanium Dioxide** : In test on skin irritation with rabbits, skin irritations were not observed. (OECD Guideline 404)

Serious eye damage/ irritation : Not classified

- **Calcium Titanium Borosilicate** : In test on eyes irritation with rabbits, eyes irritations were not observed.(Read across: 1317-36-8)(OECD TG 405, GLP)
- **Tin Oxide** : The test substance was not irritating to the rabbit eyes. (OECD TG 405)
- **Titanium Dioxide** : In test on eye irritation with rabbits, eye irritations were not observed. (OECD Guideline 405, EU Method B.5, EPA OPPTS 870.2400)

Respiratory sensitization : Not classified

- **Titanium Dioxide** : Titanium oxide does not show respiratory sensitizing properties in animal studies or in exposure related observations in humans.

Skin sensitization : Not classified

- **Calcium Titanium Borosilicate** : In the test on guinea pigs, the test substance was not considered to be a dermal sensitizer in guinea pigs.(Read across: 1317-36-8)(OECD TG 406, GLP)
- **Tin Oxide** : No activation of the lymph nodes of mice were observed in the LLNA performed with the test material. (OECD TG 429)
- **Titanium Dioxide** : In test on skin sensitization with guinea pig, skin sensitizations were not observed. (OECD Guideline 406, EU Method B.6, EPA OPP 81-6, GLP)

Carcinogenicity : Not classified

Mutagenicity : Not classified

- **Calcium Titanium Borosilicate** : In the mammalian cell gene mutation assay, the result of the assay was positive. (OECD TG 476, GLP) But we can't classify as genetic toxicity because in vivo mutagenicity test is not available.
- **Tin Oxide** : Negative reactions were observed in these in vitro genotoxicity studies(bacterial reverse mutation assay(e.g. Ames test)(gene mutation)(OECD Guideline 471), mammalian cell gene mutation assay(OECD Guideline 476), mammalian cell micronucleus test(OECD Guideline 487)).
- **Titanium Dioxide** : Negative reactions were observed in in vitro (mammalian cell gene mutation test(OECD Guideline 476, GLP), mammalian chromosome aberration test(OECD Guideline 473, GLP), bacterial reverse mutation assay(OECD Guideline 471)) and in in vivo (micronucleus assay).

Reproductive toxicity : Not classified

- **Titanium Dioxide** : Based on the weight of evidence from the available long-term toxicity/carcinogenicity studies in rodents and the relevant information on the toxicokinetic behaviour in rats it is concluded that TiO2 does not present a reproductive toxicity hazard.

Specific target organ toxicity (single exposure) : Not available
Specific target organ toxicity (repeat exposure) : Not classified
- **Tin Oxide** : No toxicity related symptoms were observed in the 13-week repeat oral administration toxicity test using rats. (NOAEL ≥ 10000 mg / kg)
- **Titanium Dioxide** : Titanium dioxide did not show any adverse effects whatsoever in a chronic oral repeated dose toxicity study in rats, with a NOAEL of 3500 mg/kg bw/day. Titanium dioxide is not absorbed to any relevant extent through human skin, thus no toxic effects can be expected via the dermal route of exposure. Titanium dioxide showed fibrogenic effects in a chronic inhalation repeated dose toxicity study in rats with a NOAEC of 10 mg/m3.

Aspiration Hazard : Not available

12. Ecological information

A. Ecological toxicity
- Acute toxicity : Not classified
- Chronic toxicity : Not classified

Fish
- **Titanium dioxide** : 96hr-NOEC(Oncorhynchus mykiss) > 100 mg/L (OECD Guideline 203)

crustacean
- **Calcium Titanium Borosilicate** : 48hr-NOEC (Mytilus galloprovincialis) =0.232 mg/L (Read across; 10099-74-8)(GLP)

Algae
- **Calcium Titanium Borosilicate** : 96hr-NOEC (Skeletonema costatum) =0.0227 mg/L (Read across; 10099-74-8)(GLP)
- **Titanium dioxide** : 72hr-EC_{50} (other) = 61 mg/L , 72hr-NOEC(Pseudokirchnerella subcapitata) = 12.7 mg/L

B. Persistence and degradability

Persistence
- **Tin oxide** : Low persistency (log Kow is less than 4 estimated.) (Log Kow = 1.29) (estimated)
- **Titanium dioxide** : Low persistency (log Kow is less than 4 estimated.) (Log Kow = 2.23) (estimated)

Degradability : Not available

C. Bioaccumulative potential

Bioaccumulation
- **Tin oxide** : Bioaccumulation is expected to be low according to the BCF < 500 (BCF = 100) (estimated)
- **Titanium dioxide** : Bioaccumulation is expected to be low according to the BCF < 500 (BCF = 13.73) (estimated)

Biodegradation
- **Tin oxide** : not readily biodegradable (estimated)
- **Titanium dioxide** : not readily biodegradable (estimated)

D. Mobility in soil
- **Tin oxide** : Low potency of mobility to soil. (Koc = 13.16) (estimated)
- **Titanium dioxide** : Low potency of mobility to soil. (Koc = 86.1) (estimated)

E. Other hazardous effect : Not available

F. HAZARDOUS TO THE OZONE LAYER : Not classified

13. Disposal considerations

A. Disposal method

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

B. Disposal precaution

Consider the required attentions in accordance with waste treatment management regulation.

14. Transport information

A. UN Number Not applicable
B. UN Proper shipping name Not applicable
C. Transport Hazard class Not applicable
D. Packing group Not applicable
E. Marine pollutant Not applicable
F. IMDG/IATA/ICAO Not applicable
G. Special precautions
in case of fire Not applicable
in case of leakage Not applicable

15. Regulatory information

A. Occupational Safety and Health Regulation
Calcium Titanium Borosilicate : Occupational exposure limits listed
Calcium Titanium Borosilicate : Work environment monitoring listed (dust 6 months)
Tin Oxide : Work environment monitoring listed (6 months)
Tin Oxide : Administration subject listed
Titanium Dioxide : Administration subject listed
Titanium Dioxide : Occupational exposure limits listed
Titanium Dioxide : Work environment monitoring listed (6 months)
B. Chemical Control Act
Calcium Titanium Borosilicate : Existing Chemical Substance (KE-17630)
Tin oxide : Existing Chemical Substance (KE-33849)
Titanium dioxide : Existing Chemical Substance KE-33900
C. Dangerous Material Safety Management Regulation
Tin oxide : Dangerous Material Safety Management Regulation
Titanium dioxide : Dangerous Material Safety Management Regulation
D. Wastes Control Act
Calcium Titanium Borosilicate : Wastes Control Act Controlled Wastes
E. Other regulation (internal and external)
Internal information
Persistant Organic Pollutants Acts : Not regulated
② Foreign Regulatory Information
External information
EU classification(classification)
Calcium Titanium Borosilicate : Not classified
Tin oxide : Not classified
Titanium dioxide : Not classified
EU classification(risk phrases)
Calcium Titanium Borosilicate : Not applicable
Tin oxide : Not applicable

Titanium dioxide : Not applicable
EU classification(safety phrases)
Calcium Titanium Borosilicate : Not applicable
Tin oxide : Not applicable
Titanium dioxide : Not applicable
EU SVHC list : Not regulated
EU Authorisation List : Not regulated
EU Restriction list : Not regulated
U.S.A management information (OSHA Regulation) : Not regulated
U.S.A management information (CERCLA Regulation) : Not regulated
U.S.A management information (EPCRA 302 Regulation) : Not regulated
U.S.A management information (EPCRA 304 Regulation) : Not regulated
U.S.A management information (EPCRA 313 Regulation) : Not regulated
Substance of Roterdame Protocol : Not regulated
Substance of Stockholme Protocol : Not regulated
Substance of Montreal Protocol : Not regulated
Foreign Inventory Status
Calcium Titanium Borosilicate
U.S.A management information Section 8(b) Inventory (TSCA): Present
Japan management information Existing and New Chemical Substances (ENCS): (1)-189
China management information Inventory of Existing Chemical Substances (IECSC): Present[04789]
Canada management information Domestic Substances List (DSL): Present
Australia management information Inventory of Chemical Substances (AICS): Present
New Zealand management information Inventory of Chemicals (NZIoC): May be used as a single component chemical under an appropriate group standard.
Philippines management information Inventory of Chemicals and Chemical Substances (PICCS): Present
Tin oxide
U.S.A management information Section 8(b) Inventory (TSCA): Present
Japan management information Existing and New Chemical Substances (ENCS): (1)-551
Japan management information ISHL Harmful Substances Whose Names Are to be Indicated on the Label: ≥ 1% weight
Japan management information ISHL Notifiable Substances: ≥ 0.1% weight
China management information Inventory of Existing Chemical Substances (IECSC): Present 37645
Canada management information Domestic Substances List (DSL): Present
Australia management information Inventory of Chemical Substances (AICS): Present
New Zealand management information Inventory of Chemicals (NZIoC): HSNO Approval: HSR002805
Philippines management information Inventory of Chemicals and Chemical Substances (PICCS): Present
Titanium dioxide
U.S.A management information Section 8(b) Inventory (TSCA): Present
Japan management information Existing and New Chemical Substances (ENCS): (5)-5225, (1)-558
Japan management information ISHL Harmful Substances Whose Names Are to be Indicated on the Label: ≥ 1% weight

Japan management information ISHL Notifiable Substances: ≥ 0.1% weight
China management information Inventory of Existing Chemical Substances (IECSC): Present 11377
Canada management information Domestic Substances List (DSL): Present
Australia management information Inventory of Chemical Substances (AICS): Present
New Zealand management information Inventory of Chemicals (NZIoC): May be used as a single component chemical under an appropriate group standard.
Philippines management information Inventory of Chemicals and Chemical Substances (PICCS): Present

16. Other information

A. Information source and references
Emergency Response Guidebook 2008:
http://phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/erg2008_eng.pdf
U.S. National library of Medicine(NLM) ChemIDplus: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM
Korea Occupational Health & Safety Agency: http://www.kosha.net
EPISUITE v4.11: http://www.epa.gov/opt/exposure/pubs/episuitedl.html
Ministry of Public Safety and Security-Korea dangerous material inventory management system: http://hazmat.mpss.kfi.or.kr/index.do
IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:
http://monographs.iarc.fr
TOMES-LOLI®: http://www.rightanswerknowledge.com/loginRA.asp
National Chemicals Information System: http://ncis.nier.go.kr/ncis/
Waste Control Act enforcement regulation attached [1]
REACH information on registered substances: https://echa.europa.eu/information-on-chemicals/registered-substances
American Conference of Governmental Industrial Hygienists TLVs and BEIs.
NIOSH Pocket Guide: http://www.cdc.gov/niosh/npg/npgdcas.html
National Toxicology Program: http://ntp.niehs.nih.gov/results/dbsearch/
International Uniform Chemical Information Database(IUCLID)
Korea Maritime Dangerous Goods Inspection Center: http://www.komdi.or.kr/index.html
EU CLP: https://echa.europa.eu/information-on-chemicals/cl-inventory-database

B. Issuing date 13-03-2006

C. Revision number and date
revision number 8
date of the latest revision 01-03-2022

D. Others
• Since the user's working conditions are not known by us, the information supplied on this safety data sheet is based on our current level of knowledge and on national and community regulations.
• The product must not be used for any purposes other than those specified under heading 1 without first obtaining written handling instructions.
• It is at all times the responsibility of the user to take all necessary measures to comply with legal requirements and local regulations.
• The information given on this safety data sheet must be regarded as a description of the safety requirements relating to our product and not a guarantee of its properties.

附錄 2：各成分之毒理相關資料

註：本範例僅提供其中一成分之毒理資料為示範，實際執行時應包含所有蒐集之各個成分之毒理資料，且內容如有變更應隨時更新。

INCI name：Titanium Dioxide

Re-evaluation of titanium dioxide (E 171) as a food additive. EFSA ANS Panel, 2016.

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 28 June 2016

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Re-evaluation of titanium dioxide (E 171) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

Abstract

The present Opinion deals with the re-evaluation of the safety of titanium dioxide (TiO_2, E 171) when used as a food additive. From the available data on absorption, distribution and excretion, the EFSA Panel on Food Additives and Nutrient Sources added to Food concluded that the absorption of orally administered TiO_2 is extremely low and the low bioavailability of TiO_2 appears to be independent of particle size. The Panel concluded that the use of TiO_2 as a food additive does not raise a genotoxic concern. From a carcinogenicity study with TiO_2 in mice and in rats, the Panel chose the lowest no observed adverse effects levels (NOAEL) which was 2,250 mg TiO_2/kg body weight (bw) per day for males from the rat study, the highest dose tested in this species and sex. The Panel noted that possible adverse effects in the reproductive system were identified in some studies conducted with material which was either non-food-grade or inadequately characterised nanomaterial (i.e. not E 171). There were no such indications in the available, albeit limited, database on reproductive endpoints for the food additive (E 171). The Panel was unable to reach a definitive conclusion on this endpoint due to the lack of an extended 90-day study or a multigeneration or extended-one generation reproduction toxicity study with the food additive (E 171). Therefore, the Panel did not establish an acceptable daily intake (ADI). The Panel considered that, on the database currently available and the considerations on the absorption of TiO_2, the margins of safety (MoS) calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the toxicological data available and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) would not be of concern. The Panel concluded that once definitive and reliable data on the reproductive toxicity of E 171 were available, the full dataset would enable the Panel to establish a health-based guidance value (ADI).

Keywords: titanium dioxide, E 171, anatase, rutile, food colour

Requestor: European Commission

Question number: EFSA-Q-2011-00348

Correspondence: fip@efsa.europa.eu

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Summary

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of titanium dioxide (TiO_2, E 171) when used as a food additive.

TiO_2 is a food colour authorised as a food additive in the European Union (EU). It was previously evaluated by the Scientific Committee on Food (SCF) in 1975 and 1977, by the Joint FAO/WHO Expert Committee of Food Additives (JECFA) in 1969. In 1969, JECFA allocated an acceptable daily intake (ADI) 'not limited except for good manufacturing practice'. In 1975, the SCF did not establish an ADI for TiO_2, whereas in 1977, the SCF included TiO_2 in the category 'colours for which an ADI was not established but which could be used in food'. The Panel is aware that the European Chemical Agency (ECHA) is carrying out an evaluation for a proposal for harmonised classification and labelling (CLH) on TiO_2, for which the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) is the Rapporteur on behalf of the French Member State Competent Authority. ANSES prepared a report in which concluded that TiO_2 should be considered as being potentially carcinogenic to humans when inhaled and thus be classified Carc. Cat 1B – H350i. However, it also concluded that there was no carcinogenic concern after oral or dermal administration. A public consultation on this report is currently underway.

In nature, TiO_2 exists in different crystalline forms; anatase and rutile are the two most important natural forms. The food additive TiO_2 (E 171) is a white to slightly coloured powder and it is insoluble in water and in organic solvents (Commission Regulation (EU) No 231/2012).

The Panel noted that, according to the data provided by interested parties and from the literature, TiO_2 (E 171) as a food additive would not be considered as a nanomaterial according to the EU Recommendation on the definition of a nanomaterial (i.e. 'a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm').

The Panel was aware of the extensive database on TiO_2 nanomaterials, however, most of these data were not considered relevant to the evaluation of TiO_2 as the food additive (E 171) in this opinion. Therefore, the Panel considered these data could not be directly applied to the evaluation of the food additive.

From the available data on absorption, distribution and excretion, the Panel concluded that:

- the absorption of orally administered TiO_2 is extremely low;
- the bioavailability of TiO_2 (measured either as particles or as titanium) is low;
- the bioavailability measured as titanium appeared to be independent of particle size;
- the vast majority of an oral dose of TiO_2 is eliminated unchanged in the faeces;
- a small amount (maximum of 0.1%) of orally ingested TiO_2 was absorbed by the gut-associated lymphoid tissue (GALT) and subsequently distributed to various organs and elimination rates from these organs were variable.

The Panel further concluded that there were significant and highly variable background levels of titanium in animals and humans, which presented challenges in the analysis at the low levels of titanium uptake reported and could complicate interpretation of the reported findings.

The Panel concluded that, based on the available genotoxicity database and the Panel's evaluation of the data on absorption, distribution and excretion of micro- and nanosized TiO_2 particles, orally ingested TiO_2 particles (micro- and nanosized) are unlikely to represent a genotoxic hazard *in vivo*.

The Panel noted that possible adverse effects in the reproductive system were identified in some studies conducted with material which was either non-food-grade or inadequately characterised nanomaterial (i.e. not E 171). There were no such indications in the available, albeit limited, database on reproductive endpoints for the food additive (E 171). The Panel was unable to reach a definitive conclusion on this endpoint due to the lack of an extended 90-day study as in the Guidance for submission of food additives (EFSA ANS Panel, 2012) or a multigeneration or extended-one generation reproduction toxicity study with the food additive (E 171). Therefore, the Panel did not establish an ADI.

From a carcinogenicity study with TiO_2 in mice and in rats, the Panel chose the lowest no observable adverse effect level (NOAEL) reported which was 2,250 mg TiO_2/kg body weight (bw) per day for males from the rat study, the highest dose tested in this species and sex.

For the safety assessment of TiO_2 used as a food additive, based on information reported in the examined literature and information supplied following calls for data taking into account the following considerations:

- the food additive E 171 mainly consists of microsized TiO_2 particles, with a nanosized (< 100 nm) fraction less than 3.2% by mass;
- the absorption of orally administered TiO_2 particles (micro- and nanosized) in the gastrointestinal tract is negligible, estimated at most as 0.02–0.1% of the administered dose;
- no difference is observed in the absorption, distribution and excretion of orally administered micro- and nanosized TiO_2 particles;
- no adverse effect resulting from the eventual accumulation of the absorbed particles is expected based on the results of long-term studies which did not highlight any toxicity up to the highest administered dose;
- the uncertainties in the toxicological database arising from limitations in the available reproductive toxicity studies;

The Panel considered that an ADI should not be established, and that a margin of safety (MoS) approach would be appropriate (EFSA ANS Panel, 2012).

To assess the dietary exposure to TiO_2 (E 171) from its use as a food additive, the exposure was calculated based on: maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and reported use levels (defined as the *refined exposure assessment scenario*) as provided by industry and the Member States.

Based on the available dataset, the Panel calculated two refined exposure estimates based on different assumptions: a *brand-loyal consumer scenario*, in which it is assumed that the population is exposed over a long period of time to the food additive present at the maximum reported use/analytical levels for one food category and to a mean reported use/analytical level for the remaining food categories; and a *non-brand-loyal scenario*, in which it is assumed that the population is exposed over a long period of time to the food additive present at the mean reported use/analytical levels in all relevant food categories.

For the *maximum level exposure assessment scenario*, at the mean, the exposure estimates ranged from 0.4 mg/kg bw per day for infants and the elderly to 10.4 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 1.2 mg/kg bw per day for the elderly to 32.4 mg/kg bw per day for children.

For the *refined estimated exposure scenario*, in the *brand-loyal scenario*, the exposure estimates ranged, at the mean, from 0.4 mg/kg bw per day for infants and the elderly to 8.8 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 1.1 mg/kg bw per day for the elderly to 30.2 mg/kg bw per day for children. In the *non-brand-loyal scenario*, the exposure estimates ranged, at the mean, from 0.2 mg/kg bw per day for infants and the elderly to 5.5 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.5 mg/kg bw per day for the elderly to 14.8 mg/kg bw per day for children.

In the case of TiO_2, the Panel did not identify brand loyalty to a specific food category and therefore the Panel considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long term to food additives present at the mean reported use/analytical levels in processed food.

The Panel noted that the lowest MoS calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the available toxicological data and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) considered in this opinion is above 100. In the Guidance for submission of food additives (EFSA ANS Panel, 2012), the Panel considered that, for non-genotoxic and non-carcinogenic compounds 'a MoS of 100 or more between a NOAEL or BMDL and the anticipated exposure would be sufficient to account for uncertainty factors for extrapolating between individuals and species'. Consequently, the Panel considered that on the database currently available and the considerations on the absorption of TiO_2 the margins of safety calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the toxicological data available and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) considered in this opinion would not be of concern.

The Panel concluded that once definitive and reliable data on the reproductive toxicity of E 171 were available, the full dataset would enable the Panel to establish a health-based guidance value (ADI).

The Panel recommended that:

- In order to enable the Panel to establish a health-based guidance value (ADI) for the food additive TiO_2 (E 171), additional testing could be performed. An extended 90-day study or a multigeneration or extended-one generation reproduction toxicity study according to the current OECD guidelines could be considered. Such studies should be performed with TiO_2 (E 171) complying with the EU specifications and additionally including a characterisation of the particle size distribution of the test material. However, in deciding on actual testing, considerations of animal welfare need to be balanced against the improvement in the toxicological database within a tiered testing approach.
- The EU specifications for TiO_2 (E 171) should include a characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm), present in TiO_2 (E 171) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011).
- The maximum limits for the impurities of the toxic elements (arsenic, lead, mercury and cadmium) in the EU specification for TiO_2 (E 171) should be revised in order to ensure that TiO_2 (E 171) as a food additive will not be a significant source of exposure to those toxic elements in foods.

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Background as provided by the European Commission

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report 'Food Additives in Europe 2000'[4] submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010, the 2003 Terms of References are replaced by those below.

Terms of Reference as provided by the European Commission

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

Assessment

1. Introduction

The present Opinion deals with the re-evaluation of the safety of titanium dioxide (TiO_2, E 171) when used as a food additive.

TiO_2 (E 171) is authorised as a food additive in the EU in accordance to Annex with Annex II to Regulation (EC) No 1333/2008[1] in both anatase and rutile forms (Commission Regulation (EU) No 231/2012[5]).

TiO_2 (E 171) has been previously evaluated by the EU SCF in 1975 and 1977, by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1969 (JECFA, 1970) and by EFSA in 2004. It has also been reviewed by TemaNord in 2002.

The Panel noted the Scientific Committee on Consumer Safety (SCCS) Opinion on TiO_2 (nanoform) (SCCS, 2013a,b), and the recent commentary on this Opinion (SCCS and Chaudhry, 2015). However, the Panel noted that the aim of these reports was to provide an answer to the question of the

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[3] Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 1.10.2001, COM (2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.
[5] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes Annexes II and III to Regulation (EC) No 1331/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

European Commission on whether the use of TiO_2 in its nanoform as an ultraviolet (UV) filter in cosmetic products (e.g. sunscreens), at a concentration up to a maximum of 25.0% (250 g/kg product), was safe for consumers. Therefore, the Panel considered that the conclusions of the report cannot be extrapolated to the safety evaluation of TiO_2 (E 171) as a food additive.

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that had become available since then and information available following public calls for data.[6,7] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

2. Technical data

2.1. Identity of the substance

TiO_2 (E 171), Chemical Abstracts Service (CAS) Registry number 13463-67-7, European Inventory of Existing Commercial Chemical Substances (EINECS) number 236-675-5 and Colour Index (C.I.) number 77891, is an inorganic substance with the molecular formula TiO_2 and a molecular weight of 79.88 g/mol. The titanium atom is coordinated octahedrally with oxygen, but the position of the octahedral structure differs in the different crystalline forms (Diebold, 2003).

In nature, TiO_2 exists in different crystalline forms, anatase and rutile being the two most important natural forms: anatase (tetragonal, CAS Registry number 1317-70-0), rutile (tetragonal, CAS Registry number 1317-80-2) and brookite (orthorhombic, CAS Registry number 12188-41-9). Rutile is the thermodynamically stable form of TiO_2 (Kuznesof, 2006). TiO_2 also exists in an amorphous form (Mathews, 1976). Anatase rapidly transforms to rutile at a temperature > 700°C. Rutile melts at temperatures between 1,830 and 1,850°C (Kirk-Othmer, 1997, 2006).

Pure TiO_2 is a white powder that gives a white background colour. TiO_2 particles reflect light (pearlescent) over the majority of the visible spectrum and achieve opacity (i.e. making products impenetrable to light) by causing multiple reflections and refractions.

The food additive TiO_2 (E 171) is a white to slightly coloured powder (Commission Regulation (EU) No 231/2012). It is insoluble in water and organic solvents. It dissolves slowly in hydrofluoric acid and in hot concentrated sulfuric acid [JECFA, 2009; Commission Regulation (EU) No 231/2012].

Several synonyms exist for the different crystalline forms of TiO_2. Some of the more common synonyms for the pigment are: C.I. Pigment White 6, C.I. No 77891, Titania, INS No. 171, titanium white and titanium (IV) oxide [IARC (International Agency for Research on Cancer) (2010)].

2.1.1. Particle size and particle size distribution of TiO_2

Interested parties provided analytical data on the particle size characteristics of TiO_2 (E 171; anatase or rutile) used as a food/feed additive (Doc. provided to EFSA n. 6; Doc. provided to EFSA n. 15; Doc. provided to EFSA n. 9; Doc. provided to EFSA n. 12; Doc. provided to EFSA n. 19). The particle size distributions were determined using different analytical methods (dynamic light scattering (DLS), X-ray disc centrifugation (XSDC), transmission electron microscopy (TEM) and scanning electron microscopy (SEM)) and details of the analytical procedures were provided. The data are shown in Tables 1 and 2.

[6] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[7] Call for food additives usages level and/or concentration data in food and beverages intended for human consumption. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

Table 1: Data submitted by industries to EFSA on the particle size characteristics of TiO_2 as food/feed grade

Submitted by			Colorcon (2015; Doc. provided to EFSA n. 9)[a]	TDMA (2015; Doc. provided to EFSA n. 19)[b],[c]				TDMA (2015; Doc. provided to EFSA n. 19)[b],[d]		Interested party 1 (2012; Doc. provided to EFSA n. 15)[b]					
Crystal form			**Anatase**	**Anatase**			**Rutile**	**Anatase**		**Anatase (Sample 1)**			**Rutile (Sample 2)**		
Analytical method applied	DLS HD	Median particle size (d_{50}) (nm)	336	143	160	168	191								
		% particles by number < 100 nm	ND	12	ND	ND									
		% particles by mass < 100 nm		2	ND	ND	ND								
	XSDC HD	Median particle size (d_{50}) (nm)	176	151	166	179	202	168	202	230	250	250	330	340	340
		% particles by number < 100 nm	< 1	8	9	3	ND	ND	ND	14	5	4	< 1	< 1	< 1
		% particles by mass < 100 nm		1	< 1	< 1	ND	ND	< 1	0	0	0	0	0	0
	XSDC AECD	Median particle size (d_{50}) (nm)		121	135	148	169	137	122						
		% particles by number < 100 nm		32	29	20	3	17	26						
	TEM	Median particle size (d_{50}) (nm)	113	115	131	146	165	142	112						
		% particles by number < 100 nm	< 36	33	17	15	11	16	39						
	SEM	Median particle size (d_{50}) (nm)		123	134	147	172								
		% particles by number < 100 nm		23	19	12	10								

DLS HD: dynamic light scattering hydrodynamic diameter; ND: not detected; XSDC HD: X-ray scanning disc centrifugation hydrodynamic diameter; XSDC AECD: X-ray disc scanning centrifugation area equivalent circular diameter; TEM: transmission electron microscopy; SEM: scanning electron microscopy.
(a): Not clear if results are expressed based on particle number or by mass.
(b): Results expressed on a number basis.
(c): Data gathered with TDMA samples.
(d): Data generated with IACM samples, 2013.

Table 2: Data provided by CEFIC in 2011 (Doc. provided to EFSA n. 6)

E 171 TiO_2 products	Particles size of batches of TiO_2 E 171										
	A	A	B	C (lot 1)	C (lot 1)	C (lot 1)	C (lot 2)	C (lot 2)	D	E	F
Measured average particle size (nm)	390	680	556	577	394	225	441	190	169	420	550
Weight % < 100 nm	0.15	0.00	0.00	0.00	0.40	0.90	0.40	3.10	3.20	0.05	0.50
Measurement method	Spinning disc centrifuge	Laser diffraction	Laser diffraction	Laser diffraction	Spinning disc centrifuge	TEM	Spinning disc centrifuge	TEM	TEM	Spinning disc centrifuge	Laser diffraction
Method of dispersion	Ultra Turrax	Ultra Turrax	Ultrasonic probe	Ultrasonic probe	Ultrasonic probe	Ultrasonic probe	Ultrasonic probe	Ultrasonic probe	High-shear/ high-speed mixer	Ultrasonic probe	Ultrasonic probe
Dispersion paramcter	9,500 rpm/ 60 s	9,500 rpm/60 s	200 W/120 s	350 W/120 s	55 W/600 s	350 W/60 s	55 W/600 s	350 W/60 s	2,500 rpm/ 4 min speed mixer + manual rub out	9,500 rpm/60 s	50 W/180 s
Dispersion aid	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

TEM: transmission electron microscopy.

According to CEFIC (2011a; Doc. provided to EFSA n. 5):

'data on the particle size distribution of titanium dioxide will always vary depending on the measurement method. Optimum light scattering (i.e. whitening power) requires a primary particle size of approximately half the wavelength of the light to be scattered (i.e. half of 400–700 nm for visible light). Products with a mean primary particle size in the nano range (< 100 nm) would not be suitable and would not be supplied for this application'.

This statement from CEFIC is in line with Wang et al. (2007b) who reported that TiO_2 became transparent when its particle size was < 100 nm. In addition, CEFIC (Doc. provided to EFSA n. 5) reported that:

'There has been no significant change in the particle size of products supplied for the food market, however, as with other particulate materials, there will be a distribution of primary particle sizes around the average value and it is possible that a small fraction of the primary particles would be below 100 nm. It is indicated that in practice any products supplied would be aggregated so the actual particle size would be larger than the primary particle size'. (Doc. provided to EFSA n. 5)

CEFIC (2011b) provided information on the measured average particle size of 11 commercial samples of TiO_2 (E 171) in dispersions, using different methods of dispersion (ultra Turrax, ultrasonic probe, high-shear/high-speed mixer) and different measurement methods (laser diffraction, spinning disc centrifuge, TEM). The results showed an average particle size of 169–680 nm; the smaller particle sizes were reported from application of the TEM measurement technique. The weight percentage of particles with a size < 100 nm ranged between 0.0% and 3.2% (Doc. provided to EFSA n. 6; Table 2).

Limited information from anatase and rutile (E 171) samples was submitted by Interested party 1 (2012; Doc. provided to EFSA n. 15, Table 1).

Colorcon (2015; Doc. provided to EFSA n. 9) provided information on one sample of anatase (E 171) analysed by DLS, XSDC and TEM. The Panel noted that, when using XSDC, the median particle size (d_{50}) value was significantly lower than that obtained with DLS. However, using the former method and even after sonication of the suspension, < 1% of the particles had a size below 100 nm. As regards the data obtained with TEM, it was noted that dispersed TiO_2 showed an aggregated morphology with very few individual particles observed. At higher magnification, the diameters of the discrete particles within the aggregate were predominantly in the range of 80–180 nm. The d_{50} of these discrete (but aggregated) particles was found to be 113 nm and ~ 36% had a diameter of < 100 nm.

TDMA (2015; Doc. provided to EFSA n. 19) provided a report on the analysis of commercial E 171 and pigmentary TiO_2 (Table 1).

The Panel noted the difficulty of comparing the data available from different sources of information, resulting from the use of different analytical methodologies. Therefore, the results from the TDMA 2015 report (Doc. provided to EFSA n. 19) were considered to be most appropriate for assessing the possible presence of the nanoparticle fraction in titanium dioxide (E 171) for the following reasons: six samples of six 'anonymised' commercial products of the food additive E 171 were analysed; all samples had at least some methods of dispersion of the particles in common; four of the samples were analysed with DLS hydrodynamic diameter (HD), XSDC HD, X-ray disc scanning centrifugation area equivalent circular diameter (XSDC AECD), SEM and TEM, and two of them by XSDC HD, XSDC AECD and TEM. The Panel noted that the results on the percentage of nanoparticles by number for each sample were lower when DLS HD (from non-detected to 12%) and XSDC HD (from non-detected to 9%) were used, whereas the maximum percentage of nanoparticles by number were reported when TEM (from 11% to 39%) or XSDC AECD (from 3% to 32%) were used.

Additional information on the particle size characteristics of 'food-grade' TiO_2 gathered from the public literature is given in Table 3.

Table 3: Data on the particle size of food-grade TiO_2 from the literature

	Peters et al. (2014)	Theissmann et al. (2014)	Yang et al. (2014)	Weir et al. (2012)	Athinarayanan et al. (2015) – Periasamy et al. (2015)
Samples[a]	• Food-grade TiO_2 materials (E 171) 7 samples • 24 food products • 3 personal care products	• KRONOS K1171 a food-grade pigment with an anatase structure • KRONOS K2360 a pigment with a rutile structure for use in coatings and paints	• Food-grade TiO_2 (E 171) 5 samples • Synthetic TiO_2 (P 25)	• Food-grade TiO_2 (E 171) 1 sample • Synthetic TiO_2 (P 25) • Consumer products	• Food-grade TiO_2 (E 171) • Food products (confectionary)
Analytical method(s) used	• SEM • Flow field-flow fractionation (combined with inductively coupled mass spectrometry) • Single-particle inductively coupled mass spectrometry	• SEM	• TEM • PALS	• SEM • DLS	• TEM • DLS
Results (SEM/TEM)	• Size distribution in the range of 30–600 nm • 10% particles < 100 nm	• Equivalent circle diameter: 146 nm • Minimum Feret diameter: 133 nm	• Average diameters: 106–132 nm • 17–35% particles < 100 nm	• Mean particle size: 110 nm (range 30–400 nm) • 36% particles < 100 nm	• Spherical particles with a diameter of 30–250 nm
Results (DLS/PALS)			• Mean hydrodynamic size: 127–504 nm • 0% particles < 100 nm (four samples) and 29% particles < 100 nm (one sample)	• Mean diameter: 150 nm with a primary peak at 225 nm but a shoulder at 37 nm	• Average size of TiO_2 particles: 152 nm[b] • Average size of TiO_2 particles: 42 nm[c]
Comments	• Limitation: particles below 20 nm are excluded		• Primary particles aggregate in ultrapure water (DLS/PALS)		

SEM: scanning electron microscopy; TEM: transmission electron microscopy; DLS: dynamic light scattering; PALS: phase analysis light scattering.
(a): Results are reported only for the food-grade samples.
(b): Athinarayanan et al. (2015).
(c): Periasamy et al. (2015).

Weir et al. (2012) used TEM to analyse one single batch of food-grade TiO_2 and reported that at least 36% of the particles (it was not specified whether this refers to the weight or the number of particles) had a particle size < 100 nm.

Using SEM analysis of seven TiO_2 E 171 types, Peters et al. (2014) reported that ~ 10% of the particles had a size < 100 nm.

Theissmann et al. (2014) used a microscopic imaging methodology (similar to TEM) and determined that the d_{50} primary particle size of anatase TiO_2 food-grade was in the range of 133–146 nm.

Yang et al. (2014) analysed five different samples of food-grade TiO_2 using TEM and DLS. Four of the samples contained TiO_2 in the anatase form, whereas one sample contained both rutile and anatase. TEM was used to determine the number-based particle size distributions and the average diameters were shown to be in the range of 106–132 nm. The five samples contained 17–35% nanosized particles, based on the size distribution with a confidence level of 95%. However, when suspended in water, the mean hydrodynamic sizes of the five samples were in the range of 127–504 nm, as determined by DLS. The hydrodynamic diameter distributions of four samples showed that all particle sizes were > 100 nm, whereas in one sample, 29% of particles were < 100 nm.

Athinarayanan et al. (2015) and Periasamy et al. (2015) reported the results of the characterisation by TEM of TiO_2 from two different food products (confectionary) and a commercial TiO_2, (E 171). The TEM images of titanium dioxide from the food products or commercial E 171 showed the presence of spherical particles with a diameter range from 30 to 250 nm. Athinarayanan et al. (2015) also reported that the analysis by DLS showed an average particle size of TiO_2 of 152 nm, whereas Periasamy et al. (2015) reported that the average particle size of TiO_2 was 42 nm.

The Panel noted that determination of the fraction of TiO_2 nanoparticles in the food additive (E 171) is method-dependent. In addition, the Panel noted that according to the data provided by industries and from the literature, TiO_2 (E 171) as a food additive would not be considered as a nanomaterial according to the EU Recommendation on the definition of a nanomaterial.[8]

For the sake of comparison, the particle size characteristics of the substances used in the major toxicological studies described in 'Section 3' are given in Table 4.

Overall, the Panel noted that the great majority of the data indicates that in aqueous media, TiO_2 is present in the form of agglomerates and/or aggregates.

The Panel noted that the information on the percentage of nanoparticles by mass was limited (Doc. provided to EFSA n. 6 and 19). According to CEFIC (2011b; Doc. provided to EFSA n. 6), the weight percentage of particles with a size below 100 nm ranged from 0% to 3.2% (maximum value analysed by TEM). The percentage of particles by mass with a size below 100 nm was also provided in the TDMA (2015) report (Doc. provided to EFSA n. 19) when DLS HD and XSDC HD were used (from non-detected to 2%).

For the purpose of estimating the exposure to TiO_2 nanoparticles from the use of TiO_2 (E 171) as a food additive, the Panel considered that the highest reported percentage value of 3.2% of nanoparticles (< 100 nm) by mass, could reasonably be used to address in a conservative way a preliminary estimate.

[8] In Commission Recommendation of 18 October 2011 on the definition of nanomaterial, 2011/696/EU nanomaterials are defined as follows: 'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm.

Table 4: Data on the particle size of TiO_2 as used in toxicological studies

Reference	Warheit et al. (2015b)[a]			US National Cancer Institute (NCI, 1979)	Warheit et al. (2015a)		Tassinari et al. (2014)	Mohammadipour et al. (2014)
Type of study	Acute oral toxicity	28-day study rat	Subchronic 90-day study rat (OECD TG 408)	Carcinogenicity study in mice and rat	Developmental toxicity study in pregnant rats (OECD 414)		Short-term oral exposure to rats	Developmental toxicity study in pregnant rats
TiO_2 grade	Ultrafine Alumina and silica coated	Uncoated pigment grade	Alumina-coated pigment grade	Purity 98%, unitane 0–220	Three samples of ultrafine	Three samples of pigment grade	< 25 nm (Sigma-Aldrich, UK)	Nanopowder purchased from Nano Lima, Co. (Iran) Purity: 99% Particle size: 10 nm
Crystal form	79% Rutile 21% Anatase	Rutile	Rutile	Anatase	Anatase and/or rutile		Anatase	Anatase
Analytical method used	TEM[b]				TEM[b]		TEM and SEM	TEM
d_{50} (number %)	73	173	145		19–23	120–165		
Number % particles < 100 nm	73	11	21		100	11–27	13	
Other parameters				Arithmetic mean diameter 180–320 nm			The size distribution was dominated by agglomerates with mean diameter up to 1.6 µm 48% of particles were in the range of 100–300 nm Average diameter of the size distribution: 284 nm SSA = 45–55 m^2/g	Particle diameters were in the nanosize range SSA > 150 m^2/g

OECD TG: Organisation for Economic Co-operation and Development Testing Guidelines; TEM: transmission electron microscopy; SEM: scanning electron microscopy; SSA: specific surface area.
(a): For further details on the synthesis and characterisation, reference is made to Hu et al. (2011a,b) and Yang et al. (2014).
(b): Detailed particle size data are included in the papers using different analytical methods. For comparison purpose, only TEM results are reported in this table.

2.2. Specifications

Specifications have been defined in Commission Regulation (EU) No 231/2012 laying down specifications for food additives and by JECFA (2012).

The purity of TiO_2 (E 171), on a dry basis, is specified as not less than 99% on an alumina (aluminium oxide)- and silica (silicon dioxide)-free basis; the total content of aluminium oxide and/or silicon dioxide is not more than 2%, either alone or combined [Commission Regulation (EU) No 231/2012; JECFA, 2012].

Table 5 shows the specifications for TiO_2 (E 171) according to Commission Regulation (EU) No 231/2012 and JECFA (2012).

Table 5: Specifications for TiO_2 (E 171) according to Commission Regulation (EU) No 231/2012 and JECFA (2012)

	Commission Regulation (EU) No 231/2012	**JECFA (2012)**
Definition	Titanium dioxide consists essentially of pure anatase and/or rutile titanium dioxide, which may be coated with small amounts of alumina and/or silica to improve the technological properties of the product The anatase grades of pigmentary titanium dioxide can only be made by the sulfate process, which creates a large amount of sulfuric acid as a by-product. The rutile grades of titanium dioxide are typically made by the chloride process Certain rutile grades of titanium dioxide are produced using mica (also known as potassium aluminium silicate) as a template to form the basic platelet structure. The surface of the mica is coated with titanium dioxide using a specialised patented process Rutile titanium dioxide, platelet form is manufactured by subjecting titanium dioxide (rutile)-coated mica nacreous pigment to extractive dissolution in acid followed by an extractive dissolution in alkali. All of the mica is removed during this process and the resulting product is a platelet form of rutile titanium dioxide	Produced by either the sulfate or the chloride process. Processing conditions determine the form (anatase or rutile structure) of the final product In the sulfate process, sulfuric acid is used to digest ilmenite ($FeTiO_3$) or ilmenite and titanium slag. After a series of purification steps, the isolated titanium dioxide is finally washed with water, calcined and micronised In the chloride processes, (a) titanium-containing mineral is reacted with chlorine gas under reducing conditions to form anhydrous titanium tetrachloride, which is subsequently purified and converted to titanium dioxide either by direct thermal oxidation or by reaction with steam in the vapour phase; (b) titanium-containing mineral is reacted with concentrated hydrochloric acid to form a solution of titanium tetrachloride, which is further purified and hydrolysed to get titanium dioxide. The compound is filtered, washed and calcined. Commercial titanium dioxide may be coated with small amounts of alumina and/or silica to improve the technological properties of the product
Assay	Content not less than 99% on an alumina and silica-free basis	Not less than 99.0% on the dried basis and on an aluminium oxide and silicon dioxide-free basis
Description	White to slightly coloured powder	White to slightly coloured amorphous powder
Identification		
Solubility	Insoluble in water and organic solvents. Dissolves slowly in hydrofluoric acid and in hot concentrated sulfuric acid	Insoluble in water, hydrochloric acid, dilute sulfuric acid and organic solvents. Dissolves slowly in hydrofluoric acid and hot concentrated sulfuric acid
Colour reaction		Add 5 mL sulfuric acid to 0.5 g of the sample, heat gently until fumes of sulfuric acid appear, then cool. Cautiously dilute to about 100 mL with water and filter. To 5 mL of this clear filtrate, add a few drops of hydrogen peroxide; an orange-red colour appears immediately

	Commission Regulation (EU) No 231/2012	JECFA (2012)
Purity		
Loss on drying	Not more than 0.5% (105°C, 3 h)	Not more than 0.5% (105°C, 3h)
Loss on ignition	Not more than 1.0% on a volatile matter-free basis (800°C)	Not more than 1.0% (800°C) on the dried basis
Aluminium oxide and/or silicon dioxide	Total not more than 2.0%	Not more than 2%, either singly or combined
Acid-soluble substances/ matter soluble in 0.5 N HCl	Not more than 0.5% on an alumina and silica-free basis and, in addition, for products containing alumina and/or silica, not more than 1.5% on the basis of the product as sold	Not more than 0.5%; Not more than 1.5% for products containing alumina or silica Suspend 5 g of the sample in 100 mL of 0.5 N hydrochloric acid and place on a steam bath for 30 min with occasional stirring. Filter through a Gooch crucible fitted with a glass fibre filter paper. Wash with three 10 mL portions of 0.5 N hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight
Water-soluble matter	Not more than 0.5%	Not more than 0.5%
Antimony	Not more than 2 mg/kg after an extraction with 0.5 N HCl	Not more than 2 mg/kg (impurities soluble in 0.5 N hydrochloric acid)
Arsenic	Not more than 1 mg/kg after an extraction with 0.5 N HCl	Not more than 1 mg/kg (impurities soluble in 0.5 N hydrochloric acid)
Cadmium	Not more than 1 mg/kg after an extraction with 0.5 N HCl	Not more than 1 mg/kg (impurities soluble in 0.5 N hydrochloric acid)
Lead	Not more than 10 mg/kg after an extraction with 0.5 N HCl	Not more than 10 mg/kg (impurities soluble in 0.5 N hydrochloric acid)
Mercury	Not more than 1 mg/kg after an extraction with 0.5 N HCl	Not more than 1 mg/kg (impurities soluble in 0.5 N hydrochloric acid)

The International Agency for Research in Cancer (IARC, 2010) stated that natural rutile and anatase contain impurities of up to ~ 2% including iron, chromium, vanadium, aluminium, niobium, tantalum, hafnium and zirconium. It further stated that, as most commercial titanium dioxide is manufactured from natural material by dissolution of the parent mineral and reprecipitation as fine particles with the structure of anatase or rutile, most but not all of these chemical impurities are generally removed (IARC, 2010). However, the Panel recommends that limits for these elements should be included in the EU specifications for TiO_2 (E 171). JECFA specifications for TiO_2 were set in 2012 (JECFA, 2012). The JECFA specifications in 2004 referred only to the sulfate process for the production of TiO_2, whereas both the sulfate and chloride processes are mentioned in the 2006, 2009, 2010 and 2012 specifications (JECFA, 2006a, 2009, 2010, 2012).

The Panel noted that, according to the EU specifications for TiO_2 (E 171), impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to concentrations of 1, 10, 1 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012).

The Panel noted that there are no set limits for the particle size of TiO_2 in the EU specifications (Commission Regulation (EU) No 231/2012), and therefore characterisation of the particle size in the food additive E 171 should be included among the specifications. The full characterisation should include the particles size distribution, together with determination and quantification of any nanoparticulate material.

The Panel noted that the manufacturing process for powdered or particulate food additives resulted in material with a range of sizes. Although the median size of the particles is generally significantly greater than 100 nm, a small fraction will always be, and has been, with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nanofraction. The test

requirements stipulated in the current EFSA guidance documents and the European Commission guidelines for the intended use in the food/feed area apply in principle to unintended nanoforms, as well as to engineered nanomaterials. Therefore, the Panel considers that, in principle, for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive, including its nanoparticulate fraction. The Panel considers that for the re-evaluation of food additives, this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014 (EFSA, 2015).

2.3. Manufacturing process

The principal raw materials for manufacturing TiO_2 include ilmenite (iron titanium oxide, $FeTiO_3$), naturally occurring rutile (TiO_2) or titanium slag. TiO_2 (E 171) is manufactured to obtain either the anatase or the rutile crystal structures (Commission Regulation (EU) No 231/2012).

Titanium pigment is extracted from the raw material via either the sulfate process or the chloride process.

- In extraction via the sulfate process, there are three main stages. The ore (usually ilmenite) is dissolved in sulfuric acid to form a mixture of sulfates. Most of the TiO_2 from the ore is solubilised as a titanium oxysulfate. Iron is removed from the solution in view of the required white colour of the final product. The titanyl oxysulfate is then hydrolysed in solution to give insoluble, hydrated TiO_2. The isolated TiO_2 is washed with water, calcined and micronised. However, due to environmental issues (i.e. the production of a large amount of sulfuric acid as a by-product) and also cost issues associated with the sulfate process, currently, the chloride process predominates (Kirk-Othmer, 1997, 2006).
- In extraction via the chloride process, there are two main stages. In a first step, the dry ore is reacted with chlorine to produce titanium tetrachloride. In a second step, titanium tetrachloride is oxidised by burning it in oxygen with another combustible gas (often carbon monoxide). By adding seed crystals, the TiO_2 is formed as a fine solid in a gas stream and is filtered out of the gases. The reaction products are cooled by mixing with chlorine gas. The product is further washed, calcined, milled and coated (Kirk-Othmer, 1997, 2006).

Both anatase and rutile TiO_2 can be produced by the sulfate process depending on the specific processing conditions. To produce anatase specifically, titanium oxysulfate is hydrolysed and neutralised under alkaline conditions. Rutile is typically produced by the chloride process (Kirk-Othmer, 2006).

The rutile form can be formed into platelets on a mica (potassium aluminium silicate) template, which is removed by extractive dissolution in acid and then alkali. The specific properties of the TiO_2 are determined by the thickness of the TiO_2 layer and the process used to coat the mica substrate (EFSA, 2005).

2.4. Methods of analysis in food

Leone (1973) used a spectrophotometric method described by Kolthoff and Sandell (1952) to determine TiO_2 in cheese.

Hamano et al. (1990) described a colorimetric procedure for the determination of small amounts of TiO_2 (10–100 mg TiO_2/kg) in processed cheese, chocolate and chewing gum.

Lomer et al. (2000) used inductively coupled plasma optical emission spectrometry to determine TiO_2 in 25 foodstuffs, including confectionery, cheese, chewing gum, sauces and dressings, mustard and beverage whiteners. The limits of detection were 2–7.5 μg/kg, depending on spectral integration times, and the signal was linear up to 5 mg/kg.

Scotter (2011) describes a number of methods for the determination of TiO_2 in food and feed, but stresses that there are very few literature references to the determination of TiO_2 in foods.

2.5. Reaction and fate in food

TiO_2 (E 171) is highly stable to heat, light, oxygen and pH, making it unaffected by almost any food processing (Scotter, 2011). In any food application, its role is as an insoluble whitening agent (Emerton, 2008).

2.6. Case of need and proposed uses

Maximum levels of TiO_2 (E 171) have been defined in Annex II to Regulation (EC) No 1333/2008[1] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, TiO_2 (E 171) is an authorised food additive in the EU at quantum satis[9] (QS) in all 51 foods. TiO_2 (E 171) as such is permitted to be used in seaweed-based fish analogues, in fish paste and crustacean paste, in precooked crustaceans and in smoked fish. TiO_2 (E 171) is also included in Group II of food colours authorised at QS.

Table 6 summarises foods that are permitted to contain TiO_2 (E 171) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 6: Maximum permitted levels of TiO_2 (E 171) in foods according to Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Group II		QS
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	Except unflavoured products	QS
01.6.3	Other creams	Group II	Only flavoured creams	QS
01.7.1	Unripened cheese, excluding products falling in category 16	Group II	Only flavoured unripened cheese	QS
01.7.3	Edible cheese rind	Group II		QS
01.7.4	Whey cheese	Group II		QS
01.7.5	Processed cheese	Group II	Only flavoured processed cheese	QS
01.7.6	Cheese products, excluding products falling in category 16	Group II	Only flavoured unripened products	QS
01.8	Dairy analogues, including beverage whiteners	Group II		QS
03	Edible ices	Group II		QS
04.2.4.1	Fruit and vegetable preparations, excluding compote	Group II	Only *mostarda di frutta*	QS
04.2.4.1	Fruit and vegetable preparations, excluding compote	E 171	Only seaweed-based fish roe analogues	QS
04.2.5.3	Other similar fruit or vegetable spreads	Group II	Except *crème de pruneaux*	QS
05.2	Other confectionery including breath-refreshening microsweets	Group II		QS
05.3	Chewing gum	Group II		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group II		QS
06.3	Breakfast cereals	Group II	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	QS
06.5	Noodles	Group II		QS
06.6	Batters	Group II		QS
06.7	Precooked or processed cereals	Group II		QS
07.2	Fine bakery wares	Group II		QS
08.2.3	Casings and coatings and decorations for meat	Group II	Except edible external coating of pastourmas	QS

[9] Article 3 (2) of Regulation (EC) No 1333/2008 'quantum satis' shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled'.

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
09.2	Processed fish and fishery products, including molluscs and crustaceans	Group II	Only surimi and similar products and salmon substitutes	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	E 171	Only fish paste and crustacean paste	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	E 171	Only precooked crustacean	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	E 171	Only smoked fish	QS
09.3	Fish roe	Group II	Except sturgeons' eggs (caviar)	QS
12.2.2	Seasonings and condiments	Group II	Only seasonings, for example curry powder, tandoori	QS
12.4	Mustard	Group II		QS
12.5	Soups and broths	Group II		QS
12.6	Sauces	Group II	Excluding tomato-based sauces	QS
12.7	Salads and savoury-based sandwich spreads	Group II		QS
12.9	Protein products, excluding products covered in category 1.8	Group II		QS
13.2	Dietary foods for special medical purposes defined in Directive 1999/ 21/EC, excluding products from food category 13.1.5	Group II		QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group II		QS
14.1.4	Flavoured drinks	Group II	Excluding chocolate milk and malt products	QS
14.2.3	Cider and perry	Group II	Excluding *cidre bouché*	QS
14.2.4	Fruit wine and made wine	Group II	Excluding *wino owocowe markowe*	QS
14.2.5	Mead	Group II		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	Except spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1 14 of Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	QS
14.2.7.1	Aromatised wine-based products as defined by Regulation (EEC) No 1601/ 91	Group II	Except Americano, bitter vino	QS
14.2.7.2	Aromatised wine-based drinks	Group II	Except bitter soda, sangria, claria, zurra	QS
14.2.7.3	Aromatised wine-product cocktails	Group II		QS

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group II		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II		QS
15.2	Processed nuts	Group II		QS
16	Desserts, excluding products covered in categories 1, 3 and 4	Group II		QS
17.1	Food supplements supplied in a solid form, including capsules and tablets and similar forms, excluding chewable forms	Group II		QS
17.2	Food supplements supplied in a liquid form	Group II		QS
17.3	Food supplements supplied in a syrup-type or chewable form	Group II		QS

MPL: maximum permitted level; QS: quantum satis.

2.7. Reported use levels or data on analytical levels of TiO_2 (E 171) in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a level lower than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call for concentration data (usage and/or analytical data) on TiO_2 (E 171).[7]

In response to this public call, updated information on the actual use levels of TiO_2 (E 171) in foods was made available to EFSA by industry and the Member States (MSs).

2.7.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 61) of TiO_2 (E 171) in foods for 14 of the 51 food categories in which TiO_2 is authorised.

Updated information on the actual use levels of TiO_2 in foods was made available to EFSA by FoodDrinkEurope (FDE) (Doc. provided to EFSA n. 10), the International Chewing Gum Association (ICGA) (Doc. provided to EFSA n. 13), the Association of the European Self-Medication Industry (AESGP) (Doc. provided to EFSA n. 1) and Capsugel (Doc. provided to EFSA n. 3).

Appendix A provides data on the use levels of TiO_2 (E 171) in foods, as reported by industry.

2.7.2. Summarised data on concentration levels in foods from the Member States

In total, 28 analytical results were reported to EFSA by one country (Austria) for foods intended for particular nutritional uses (FCS Category 13) and food supplements (FCS Category 17). Foods were sampled between 2007 and 2012. Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, but all samples were derived from accredited laboratories.

Foods classified in the FCS 13 (n = 2) were described as foods for sports people without further detail, and could not be used in the current assessment.

Appendix B shows the analytical results for TiO_2 (E 171) in foods as reported by MSs (full set of reported data and positive samples only).

2.8. Summarised data extracted from the Mintel GNDP database

Mintel's Global New Products Database (GNPD) is an online database, which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over two million food and beverage products of which more than 800,000 are or have been available on the European food market. Mintel started covering the EU's food markets in 1996, having 20 out of its 28 member countries presented in the GNPD.[10]

For the purpose of this Scientific Opinion, GNPD[11] was used for checking the labelling of products containing TiO_2 (E 171) within the EU's food products as GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, TiO_2 (E 171) is labelled on more than 6,500 products. The use of TiO_2 increased constantly until 2014. In the last 5 years, TiO_2 has been labelled on more than 3,500 foods or drinks, mainly in chewing gums, cakes and pastries, and confectionary (pastilles, gums, jellies and chews).

Appendix C presents the percentage of food products labelled with TiO_2 (E 171) between 2011 and 2015, out of the total number of food products per food subcategories according to Mintel food classification.

2.9. Information on existing authorisations and evaluations

TiO_2 was evaluated by JECFA in 1969 (JECFA, 1970), the SCF in 1975 and 1977, and by EFSA in 2004. It was also reviewed by TemaNord in 2002. The British Industrial Biological Research Association (BIBRA) issued a toxicity profile on TiO_2 in 1990.

In 1969, JECFA did not establish a limit on the intake of TiO_2 (anatase and rutile forms were not distinguished), considering that the available information indicated '…that it is free from toxic effects on account of its insolubility and inertness'. An acceptable daily intake (ADI) 'not limited except for good manufacturing practice,' was allocated (JECFA, 1970).

In 1975, the SCF did not establish an ADI for TiO_2 because they 'felt able to accept the use of this colouring matter for the surface and mass colouring of sugar confectionary only, without the need for further investigations'. In a later SCF evaluation (1977), it was indicated that new information on other potential uses and specifications had been presented to the Committee, and subsequently, they included TiO_2 in the category 'colours for which an ADI was not established but which could be used in food'.

In 2004, the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and materials in Contact with Food (AFC Panel) evaluated the safety in use of platelet forms of rutile TiO_2 as an alternative to the permitted anatase form. The AFC Panel concluded that the bioavailability of these forms was essentially the same, that the toxicological database would, therefore, be applicable to either form and that the platelet forms of rutile TiO_2 could be used to replace anatase TiO_2 in any of its current applications (EFSA, 2005).

In 2000, the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP)[12] evaluated TiO_2 as a cosmetic product. The SCCNFP concluded that TiO_2 is photocatalytic in UV light, but that it did not give rise to concern for human use (SCCNFP, 2000). The SCCS issued an Opinion on TiO_2 (nano form) in 2013, and a commentary on this Opinion was released in 2015. The aim of these reports were to provide an answer to the question of the European Commission on whether the use of TiO_2 in its nanoform as a UV filter in cosmetic products (e.g. sunscreens), at a concentration up to maximum 25.0%, was safe for the consumers.

In 2002, TemaNord reviewed TiO_2 and concluded that 'the available data do not currently meet requirements. However, the inertness of the substance and the lack of absorption and tissue storage does not warrant further testing or a re-evaluation of the safety in use of this compound'.

In the USA, a platelet form of rutile TiO_2 is currently permitted for use in aqueous film coating systems for food and drug use under Code of Federal Regulations Title 21CFR73.575. This regulation states that TiO_2 may be used as a food colour provided that it does not exceed 1% of the weight of the food (Food and Drug Administration (FDA), 2002). In 2006, the FDA amended the colour additive regulation to allow the use of TiO_2-coated mica-based pearlescent pigments (identified as the colour additive 'formed by depositing titanium salts onto mica, followed by heating to produce TiO_2 in mica')

[10] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[11] http://www.gnpd.com/sinatra/home/ accessed on 19/5/2016.
[12] Presently called 'Scientific Committee on Consumer Products (SCCP)'.

as a colour additive for foods (FDA, 2006). TiO_2-coated mica-based pearlescent pigments are authorised for use up to 1.25% by weight in the following food categories: 'cereals, confections and frostings, gelatin desserts, hard and soft candies (including lozenges), nutritional supplement tablets and gelatin capsules and chewing gum' (FDA, 2006).

In Japan, TiO_2 is used without limitations other than for certain food categories in which it is not permitted (JECFA, 2006b). In India, TiO_2 is only authorised for use in chewing gum and bubble gum at not more than 1%, and in powdered concentrate mixes for fruit drinks at not more than 100 mg/kg (Kuznesof, 2006).

In 2010, IARC re-evaluated TiO_2 and revised the classification as 'possibly carcinogenic to humans (Group 2B)' based on an excess incidence of lung tumours in inhalation studies. It was stated that 'No increases were observed among mice and hamsters exposed intratracheally. Other studies that used different routes of administration did not observe excesses in tumour incidence' (IARC, 2010).

In 2015, the Organisation for Economic Co-operation and Development (OECD) published different Series on the Safety of Manufactured Nanomaterials, among which there is a dossier on titanium dioxide (TiO_2) manufactured nanomaterials. Detailed information on results and tests performed can be found in the technical dossiers of the particular TiO_2 nanomaterials (OECD, 2015).

In the very recent Scientific Report by the Food Standard Agency of New Zealand (FSANZ) (2016) on 'The potential health risks associated with nanotechnologies in existing food additives', it is reported that all forms of TiO_2 (nano- and microsized) in the diet are poorly absorbed from the gastrointestinal tract. There are few studies investigating the toxicity of TiO_2 by dietary exposure (grade or particle size not specified) reporting no evidence of carcinogenicity or systemic toxicity. Nevertheless, there is some evidence that oral exposure to nano-TiO_2 (non-food-grade) by gavage can result in small increases in tissue titanium potentially associated with a range of tissue effects. Overall, this review concluded that there is limited information available to support a contemporary risk assessment of nano-TiO_2 in food. There are no epidemiology studies available regarding possible associations with adverse health outcomes. However, the long history of use has not given rise to reports of adverse effects.

The Panel is aware that the European Chemical Agency (ECHA) is carrying out an evaluation for a proposal for harmonised classification and labelling (CLH) on TiO_2, for which the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) is the Rapporteur on behalf of the French Member State Competent Authority. ANSES prepared a report in which concluded that TiO_2 should be considered as being potentially carcinogenic to humans when inhaled and thus be classified Carc. Cat 1B – H350i. However, it also concluded that there was no carcinogenic concern after oral or dermal administration. A public consultation on this report is currently underway.[13]

2.10. Exposure

2.10.1. Food consumption data used for exposure assessment

2.10.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment'; EFSA, 2011a). New consumption surveys recently added to the Comprehensive Database were also taken into account in this assessment.[14,15]

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

[13] http://echa.europa.eu/harmonised-classification-and-labelling-consultation/-/substance-rev/13832/term; http://echa.europa.eu/web/guest/harmonised-classification-and-labelling-consultation

[14] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

[15] Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Food consumption data from the following population groups, infants, toddlers, children, adolescents, adults and the elderly, were used for the exposure assessment. For the current assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 7).

Table 7: Population groups considered for the exposure estimates of TiO_2 (E 171)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the EFSA guidance on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

2.10.1.2. Food categories considered for the exposure assessment of TiO_2

The food categories in which the use of TiO_2 (E 171) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at a detailed level (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database and therefore could not be taken into account in the current estimate. This might result in an underestimation of the exposure. The food categories that were not taken into account are described below (in ascending order of the FCS codes):

- 01.7.3. edible cheese rind,
- 01.7.6. cheese products (excluding products falling in category 16), only flavoured unripened products,
- 04.2.4.1. fruit and vegetable preparations, excluding compote, only *mostarda di frutta*,
- 04.2.4.1. fruit and vegetable preparations, excluding compote, only seaweed-based fish analogue,
- 05.4. decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4, only decorations, coatings and sauces, except fillings and only fillings,
- 06.6. batters,
- 06.7. precooked or processed cereals,
- 08.2.3. casings and coatings and decorations for meat,
- 14.2.4. fruit wine and made wine,
- 14.2.5. mead.

It has to be mentioned that these food categories could be country-specific products (*mostarda di frutta*) or could be included in other food categories taken into account with the EFSA Comprehensive

Database (edible cheese rind with the ripened cheeses) or should represent minor food consumption amounts (seaweed-based fish analogue, batters, mead, etc.).

In addition, food categories for which no or inadequate reported use/analytical levels were available were not considered in the exposure assessment. This concerns 25 food categories, which are presented in Appendix C.

The Panel noted that if TiO_2 is nevertheless used in those food categories for which reported use/analytical levels were not available, the calculated refined exposure assessment might result in an underestimation of the exposure to TiO_2. The current exposure assessment takes into consideration a percentage of the foods in which TiO_2 is authorised and that is dependent on the individuals. The Panel calculated that between 60% and 80% of food (by weight), authorised to contain TiO_2 according to Annex II, was reported to potentially contain TiO_2 as a food additive

Overall, during the current exposure estimate, 10 out of 51 food categories were not taken into account because they are not referenced in the EFSA Comprehensive Database and 25 food categories were not included in the exposure assessment due to a lack of data. Thus, in the current exposure estimate, 35 out of 51 food categories are not taken into account.

2.10.2. Exposure to TiO_2 (E 171) from its use as a food additive

The Panel estimated chronic exposure to TiO_2 (E 171) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to TiO_2 (E 171) was calculated by multiplying TiO_2 (E 171) concentrations for each food category (Appendix D) by their respective consumption amount per kilogram of body weight (bw) for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 7). Based on these distributions, the mean and 95th percentiles of exposure were calculated per survey and per population group. High percentile exposure was calculated only for those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the current assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Assessment of exposure to TiO_2 (E 171) was carried out by the ANS Panel based on the maximum levels of data provided to EFSA (defined as the maximum level exposure assessment scenario), and reported use levels (defined as the refined exposure assessment scenario), as provided by industry and the MSs.

2.10.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 6. As TiO_2 (E 171) is authorised according to QS in all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry or high level of analytical data provided by the MSs, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014), whichever was highest or available. This exposure scenario can consider only food categories for which data were available to the Panel.

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the consumer will be continuously (over a lifetime) exposed to TiO_2 (E 171) present in food at maximum reported use levels/high level of analytical data.

2.10.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on reported use levels by industry and analytical results submitted to EFSA by the MSs. This exposure scenario can only consider food categories in which the above data were available to the Panel.

Appendix D summarises the concentration levels of TiO_2 (E 171) used in the refined exposure assessment scenario. Based on the available dataset, the Panel calculated two estimates based on different model populations:

1) The brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to the food additive present at the maximum reported use/analytical levels for one food category. This exposure estimate is calculated as follows:
   - combining food consumption with the maximum of the maximum reported use levels or the maximum of the analytical results, whichever was highest or available, for the main contributing food category at the individual level;
   - using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for the remaining food categories.
2) The non-brand-loyal consumer scenario: It was assumed that the population is exposed long term to the food additive present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results for all food categories.

In the two refined exposure assessment scenarios, the concentration levels considered by the Panel were extracted from the whole dataset (i.e. reported use levels and analytical results). To consider left-censored analytical data (i.e. analytical results below the limit of detection (LOD) or below the limit of quantification (LOQ)), the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010) was used. In the current Opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (medium bound). Subsequently, per food category, the mean or median, whichever is highest, medium bound concentration was calculated.

If both reported use levels and analytical results were available for the same food category, the most reliable value was used.

2.10.2.3. Dietary exposure to TiO_2 (E 171)

Table 8 summarises the estimated exposure to TiO_2 (E 171) from its use as a food additive for all six population groups (Table 7). Detailed results by population group and survey are presented in Appendix E.

Table 8: Summary of dietary exposure to TiO_2 (E 171) from its use as a food additive using the maximum level exposure assessment scenario and refined exposure scenarios, in six population groups (min–max across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks – 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (> 65 years)
Maximum level exposure assessment scenario						
Mean	0.4 1.9	1.2 9.2	1.8 10.4	0.8 6.7	0.6 6.8	0.4 4.5
95th percentile	1.4 9.6	4.0 19.3	4.9 32.4	3.1 23.5	2.2 15.0	1.2 10.7
Refined estimated exposure scenario						
Brand-loyal scenario						
Mean	0.4 1.8	1.1 7.6	1.5 8.8	0.7 5.9	0.5 5.7	0.4 3.9
95th percentile	1.2 9.2	3.6 14.7	4.1 30.2	2.5 21.2	1.9 13.6	1.1 9.2
Non-brand-loyal scenario						
Mean	0.2 0.8	0.6 4.6	0.9 5.5	0.4 4.1	0.3 4.0	0.2 2.8
95th percentile	0.7 3.9	2.0 6.8	2.4 14.8	1.3 10.8	1.1 9.7	0.5 7.0

For the maximum level exposure assessment scenario, at the mean, the exposure estimates ranged from 0.4 mg/kg bw per day for infants and the elderly to 10.4 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 1.2 mg/kg bw per day for the elderly to 32.4 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the brand-loyal scenario, the exposure estimates ranged at the mean from 0.4 mg/kg bw per day for infants and the elderly to 8.8 mg/kg bw per day

for children. At the 95th percentile, exposure estimates ranged from 1.1 mg/kg bw per day for the elderly to 30.2 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the non-brand-loyal scenario, the exposure estimates ranged at the mean from 0.2 mg/kg bw per day for infants and the elderly to 5.5 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.5 mg/kg bw per day for the elderly to 14.8 mg/kg bw per day for children.

For the purpose of providing an indicative estimate of nanoparticles of titanium dioxide from the use of E 171 as a food additive, the Panel considered that the highest reported weight percentage value of 3.2% of nanoparticles by mass could reasonably be used in a conservative way to address this issue. Table 9 summarises the estimated exposure to nanoparticles from the use of TiO_2 as a food additive for all six population groups.

Table 9: Summary of exposure to nanoparticles (present at a level of 3.2% by weight in TiO_2 (E 171)) from the use of TiO_2 as a food additive using the maximum level exposure assessment scenario and refined exposure scenarios in six population groups (min–max across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks – 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (> 65 years)
Maximum level exposure assessment scenario						
Mean	0.01 0.06	0.04 0.30	0.06 0.33	0.03 0.21	0.02 0.22	0.01 0.14
95th percentile	0.04 0.31	0.13 0.62	0.16 1.04	0.10 0.75	0.07 0.48	0.04 0.34
Refined estimated exposure scenario						
Brand-loyal scenario						
Mean	0.01 0.06	0.03 0.24	0.05 0.28	0.02 0.19	0.02 0.18	0.01 0.12
95th percentile	0.04 0.29	0.11 0.47	0.13 0.97	0.08 0.68	0.06 0.44	0.03 0.29
Non-brand-loyal scenario						
Mean	0.01 0.03	0.02 0.15	0.03 0.18	0.01 0.13	0.01 0.13	0.01 0.09
95th percentile	0.02 0.13	0.06 0.22	0.08 0.47	0.04 0.35	0.04 0.31	0.02 0.23

For the maximum level exposure assessment scenario, at the mean, the exposure estimates to nanoparticles ranged from 0.01 mg/kg bw per day for infants and the elderly to 0.33 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.04 mg/kg bw per day the infant and elderly to 1.04 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the brand-loyal scenario, the exposure estimates ranged at the mean from 0.01 mg/kg bw per day for infants and the elderly to 0.28 mg/kg bw per day for children. At 95th percentile, exposure estimates ranged from 0.03 mg/kg bw per day for the elderly to 0.97 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the non-brand-loyal scenario, the exposure estimates ranged at the mean from 0.01 mg/kg bw per day for infants, adolescents, adults and the elderly to 0.18 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.02 mg/kg bw per day for infants and the elderly to 0.47 mg/kg bw per day for children.

2.10.3. Main food categories contributing to exposure to TiO_2 (E 171) using the maximum level exposure assessment scenario

Table 10 summarises the main food categories contributing to exposure to TiO_2 (E 171) using the maximum level exposure scenario for all six population groups.

Table 10: Main food categories contributing to exposure to TiO_2 (E 171) using maximum levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Foods	Range of % contribution to the total exposure (number of surveys)[a]					
		Infants	Toddlers	Children	Adolescents	Adults	The elderly
03	Edible ices	20.9 (1)	5.7 14.9 (7)	6.3 29.9 (18)	5.3 31.1 (12)	5.1 18.2 (7)	9 13.8 (2)
05.2	Other confectionery, including breath-refreshening microsweets	14.3 (1)	7.3 37.4 (8)	5.5 61.2 (18)	7.2 71.9 (15)	5.2 38.8 (10)	5.2 25.4 (7)
05.3	Chewing gum			6.7 9.6 (3)	7.2 13.3 (3)	12.9 (1)	10.6 (1)
07.2	Fine bakery wares	5.6 81.3 (4)	7.5 43.4 (8)	6.1 34.0 (16)	5.5 28.1 (15)	5.2 20.5 (13)	5.9 20.3 (13)
12.5	Soups and broths	40.0 (1)	5.2 10.5 (3)	8.7 8.8 (2)	7.7 (1)	7.2 11.6 (3)	6.7 17.7 (6)
12.6	Sauces	18.0 66.6 (5)	12.8 58.9 (9)	11.6 53.4 (16)	6.4 58.1 (17)	12 58.3 (17)	11 57.8 (14)
12.7	Salads and savoury-based sandwich spreads			7.8 44.9 (4)	10.2 41.6 (3)	5.3 54.4 (6)	6 48.1 (3)
14.1.4	Flavoured drinks	13.6 (1)	5.8 12.4 (5)	5.6 11.8 (12)	5.8 22.8 (12)	5.1 16.4 (9)	5.7 13.2 (3)
15.2	Processed nuts	5.6 24.6 (3)	7 24.4 (4)	5.4 11.8 (8)	5.1 14.4 (11)	5.5 54.1 (16)	5.9 50.9 (12)
16	Desserts, excluding products covered in categories 1, 3 and 4	7.9 15.6 (2)	5.3 12.5 (3)	5.3 5.6 (2)			
17	Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants and young children	7.5 81.0 (3)	7.9 50.5 (4)	5.4 9.8 (4)	6.5 (1)	6.0 21.0 (8)	11.9 42.4 (6)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

2.10.4. Main food categories contributing to exposure to TiO_2 (E 171) using the refined exposure assessment scenarios

Table 11 summarises the main food categories contributing to exposure to TiO_2 (E 171) using the brand-loyal refined exposure scenario for all six population groups.

Table 11: Main food categories contributing to exposure to TiO_2 (E 171) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Foods	Range of % contribution to the total exposure (number of surveys)[a]					
		Infants	Toddlers	Children	Adolescents	Adults	The elderly
03	Edible ices	21.4 (1)	7.4 14.8 (6)	5.3 32.9 (18)	5.2 34.5 (7)	5.6 18.9 (5)	7.7 14.4 (2)
05.2	Other confectionery, including breath-refreshening microsweets	12.6 (1)	6.7 40.2 (8)	5.1 70.3 (18)	5.8 81.5 (15)	6.0 42.4 (8)	5.2 26 (7)
05.3	Chewing gum			6 8.5 (2)	6.8 11.4 (2)	13.4 (1)	11.4 (1)
07.2	Fine bakery wares	8.9 81.8 (3)	5.9 44.0 (7)	5.3 32.8 (14)	5.1 26.6 (11)	5.0 18.4 (8)	5.8 18.3 (11)
12.5	Soups and broths	42.6 (1)	5.9 12.8 (3)	5.6 10.6 (3)	5.1 8.9 (2)	5.0 13.5 (4)	7.6 19.8 (6)
12.6	Sauces	15.2 69.6 (5)	10.5 63.4 (9)	9.7 58.3 (16)	6.5 65.0 (16)	10.0 63.2 (17)	8.7 62.3 (14)
12.7	Salads and savoury-based sandwich spreads			9.0 50.4 (4)	11.7 46.7 (3)	6.0 59.4 (6)	6.3 54.7 (3)
14.1.4	Flavoured drinks	13.2 (1)	5.2 11.4 (3)	5.1 9.5 (11)	6.2 21.2 (10)	6.4 14.5 (6)	5.0 11.9 (3)
15.2	Processed nuts	5.7 26.9 (3)	7.8 30.3 (4)	5.7 12.3 (9)	5.2 16.5 (12)	5.5 58.5 (15)	5.2 53.5 (13)

Food category number	Foods	Range of % contribution to the total exposure (number of surveys)[a]					
		Infants	Toddlers	Children	Adolescents	Adults	The elderly
16	Desserts, excluding products covered in categories 1, 3 and 4	7.2 15.8 (2)	7.3 13.7 (2)	5.2 5.4 (2)			
17	Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants and young children	7.7 83.9 (3)	8.5 52.9 (4)	6.8 10.8 (3)	7.8 (1)	6.8 22.2 (8)	12 45.6 (6)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

Table 12 summarises the main food categories contributing to exposure to TiO_2 (E 171) using the non brand-loyal refined exposure scenario for all six population groups.

Table 12: Main food categories contributing to exposure to TiO_2 (E 171) following the non-brand-loyal exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Foods	Range of % contribution to the total exposure (number of surveys)[a]					
		Infants	Toddlers	Children	Adolescents	Adults	The elderly
03	Edible ices	21.8 (1)	5.7 17.3 (8)	6.2 30 (18)	5.2 31.2 (14)	5.5 17.3 (7)	5.8 13.2 (3)
05.2	Other confectionery including breath-refreshening microsweets	7.1 (1)	5.1 24.2 (7)	6.8 46.7 (14)	5.7 61.1 (13)	6.2 24.1 (5)	10.1 16 (3)
05.3	Chewing gum				5.0 7.5 (2)	7.0 (1)	5.4 (1)
07.2	Fine bakery wares	6.2 82.6 (4)	7.6 50.6 (8)	5.4 38.2 (16)	6.5 28.4 (14)	5.6 19.6 (14)	6.0 19.4 (13)
09.2	Processed fish and fishery products, including molluscs and crustaceans			6.2 (1)	5.4 (1)		
12.5	Soups and broths	9.5 59.3 (2)	6.6 21 (6)	5.4 18.5 (6)	5.9 12.1 (6)	7.4 22.3 (6)	8.0 33.5 (7)
12.6	Sauces	12.8 52.7 (5)	9.1 46.7 (9)	8.4 44.8 (16)	9.5 47.6 (16)	8.2 47.4 (17)	7.5 48.6 (14)
12.7	Salads and savoury-based sandwich spreads			15.3 56.3 (4)	8.0 54.3 (4)	6.2 66.3 (7)	10.5 61.1 (3)
14.1.4	Flavoured drinks	16.3 (1)	5.4 16.9 (7)	5.7 15.3 (13)	5.6 27.0 (15)	5.4 18.3 (10)	8.3 15 (3)
15.2	Processed nuts	6.1 28.6 (3)	10.2 28.7 (4)	6.5 14.2 (9)	5.8 17.2 (12)	5.1 58.4 (16)	5.1 55.9 (13)
16	Desserts excluding products covered in categories 1, 3 and 4	12.2 22.8 (2)	5.5 19.0 (5)	5.4 9.3 (3)	6.1 (1)		
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	8.9 85.9 (3)	9.4 53.7 (4)	7.0 16.8 (4)	8.7 9.1 (2)	7.5 26.5 (8)	13 48.7 (6)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

2.10.5. Uncertainty analysis

Uncertainties in the exposure assessment of TiO_2 (E 171) have been discussed above. In accordance with the guidance provided in the EFSA Opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 13.

Table 13: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainty	Direction
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties about which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 10/51 food categories)	–
Food categories included in the exposure assessment: data not available for certain food categories which were excluded from the exposure estimates (n = 35/51 food categories)	–
Concentration data: • levels considered applicable for all items within the entire food category, • not representative of foods on the EU market (coming from one Member State)	+ +/–
Maximum level exposure assessment scenario: – exposure calculations based on the maximum (reported use from industries or analytical data from Member States)	+
Refined exposure assessment scenarios: – exposure calculations based on the maximum or mean levels (reported use from industries or analytical data from Member States)	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Exposure to nanoparticles: uncertainties on the percentage of nanoparticles	+

+: uncertainty with potential to cause over-estimation of exposure; –: uncertainty with potential to cause underestimation of exposure; EU: European Union.

Overall, the Panel noted that not all the food categories in which use of TiO_2 (E 171) is authorised were taken into account in the current exposure estimate. The Panel, therefore, considered that the uncertainties identified would, in general, result in an underestimation of the exposure to TiO_2 (E 171) if all food categories according the regulation had the reported uses. The Panel noted that the usage data submitted by industries for food categories and considered in its estimates were for some of them confirmed, when comparing with the qualitative information as described in the Mintel database.

The Panel also noted that the uncertainties identified in its estimates of exposure to nanoparticles that could be present in TiO_2 used as a food additive, would result in an overestimation because in these estimates it was assumed that nanoparticles were present in all considered food categories at the maximum reported percentage value (3.2% by mass).

3. Biological and toxicological data

In their review, Walkey and Chan (2012) indicated that when small particles, such as nanomaterials enter a physiological environment, they rapidly adsorb proteins from the biological fluids forming a protein 'corona'. This protein corona alters the size, aggregation state and interfacial composition of a nanomaterial, giving it a biological identity that is distinct from its synthetic identity. The biological identity determines the physiological response, including signalling, kinetics, transport, accumulation, and toxicity. The structure and composition of the protein corona depends on the synthetic identity of the nanomaterial (size, shape and composition), the nature of the physiological environment (blood, interstitial fluid, cell cytoplasm, etc.) and the duration of exposure. The Panel considered that these elements should be taken into account when interpreting the biological and toxicological data on nano- and microsized materials after oral intake. However, the Panel wants to emphasise that E 171 is not an (engineered) nanomaterial. The Panel was aware of the extensive database on TiO_2 nanomaterials,

however, most of these data were not considered relevant to the evaluation of TiO_2 as the food additive (E 171) in this opinion. Therefore, the Panel considered these data could not be directly applied to the evaluation of the food additive.

A large number of animal experimental studies (80 publications in PubMed) has been published from the Medical College of Soochow University (Suzhou, China) describing effects of nanosized TiO_2 on various organ systems. The Panel noted that the publications of Gui et al. (2013); Zhao et al. (2014); Hu et al., 2011b) were retracted from the journals by the Editor due to deficiencies and inadequate reporting of the data (Hu et al., 2011a; Gui et al., 2015; Zhao et al., 2015). These deficiencies were the use of the same 5% standard deviation or standard error for all measured values and thus the real variation and statistical significance of the results cannot be evaluated. The Panel noted that the same data handling was also found in other publications on TiO_2 nanoparticles from this group (e.g. Hu et al., 2010; Cui et al., 2011; Gui et al., 2011; Gao et al., 2012, 2013; Sheng et al., 2013, 2014; Sang et al., 2014). The Panel evaluated these publications but did not consider them as appropriate for risk assessment in the present evaluation.

References of the toxicological studies with coated nanoparticles considered by the Panel are given in Appendix F.

3.1. Absorption, distribution and excretion

Numerous studies on the absorption, distribution and excretion of inhaled TiO_2 particles from animal experiments and human exposure are available in the literature. However, the Panel considered that this route of exposure was not directly relevant to the safety evaluation of TiO_2 as a food additive and therefore further details on exposure via inhalation were not considered in this Opinion. The general consensus is that small amounts of TiO_2, when under a nanoform, can enter the systemic circulation from the lungs (Jin and Berlin, 2008).

3.1.1. Absorption

Reports in the literature on studies with animals indicate that a primary port of entry into the body for orally absorbed micro- and nanoparticulates from the undamaged intestine was the gut-associated lymphoid tissue (GALT), represented by Peyer's patches and the follicle-associated epithelium overlying Peyer's patches. Follicle-associated epithelium contains a population of phagocyte cells (M cells) that are responsible for absorbing particulates. Uptake also takes place, but to a limited extent, across normal epithelial cells (enterocytes) and by paracellular means. Quantitative models have shown that particle binding to the apical membrane of M cells was followed by rapid internalisation (Florence, 1997; Hussain et al., 2001; des Rieux et al., 2006; Emond, 2011).

In general, smaller particles, < 1 μm (1,000 nm), lead to higher absorption rates. Particles > 1 μm were effectively trapped in the Peyer's patches. At this size, the particles were not translocated into the systemic circulation. Oral absorption was influenced by different particle characteristics (e.g. diameter, surface chemistry, surface ligands, shape and elasticity, physical and chemical stability) (Hussain et al., 2001). Particles > 3 μm (3,000 nm) were phagocytosed and stayed sequestered in the gastrointestinal tract cells (Emond, 2011).

3.1.1.1. *In vitro*

In a study by McCracken et al. (2013), TiO_2 nanoparticles (particle size 21 nm; surface area 35–65 m^2/g; purity > 99.5%) were dispersed in simulated digestion media and placed in contact with a Caco-2 cell monolayer (C2BBe1) isolated from a human colon cancer. The nanoparticles were added to the cells at a dose of 10 $\mu g/cm^2$. Aggregates of negatively charged particles appeared in the culture media, but the charge became positive in the presence of pepsin (pH 2). The same particles became strongly negative in a simulated intestinal digestive solution, whereas a corona made of bile salts/proteins was identified on the particles. TEM indicated the internalisation of TiO_2 particles to occur. The authors indicated that, based on assays on necrosis, apoptosis, membrane damage and mitochondrial activity, no toxicity was exhibited by TiO_2 particles suspended in the media at loading levels of 10 $\mu g/cm^2$. The authors further indicated that although no toxicity was exhibited, internalisation of the particles by the epithelial cells may result in the circulation and migration of the particles to other parts of the body.

In a study by Chaudhry et al. (2013; Doc. provided to EFSA n. 7) (published as MacNicoll et al., 2015), the potential of microsized TiO_2 and of TiO_2 nanoparticles to cross the gastrointestinal–epithelial barrier was tested. A coculture of human enterocytes (Caco-2 cells) and M cells was used as test

model system. Translocation of TiO_2 nano- and microparticles, dispersed in ovalbumin solution was studied in a transwell system. For comparison, the smallest particles were also tested without ovalbumin in the medium in agglomerated form (dispersed by sonication in water). The integrity of the cell monolayer, the viability of the cells and the translocation of TiO_2 were determined. The TiO_2 particles were characterised using TEM and limited DLS analysis.

Three sizes of TiO_2 particles obtained from one producer and a 25-nm TiO_2 nanomaterial from a second producer were tested.

Table 14: Characteristics of tested TiO_2 materials (Chaudhry et al., 2013; Doc. provided to EFSA n. 7)

Material	Description	Measured particle size	Use in tests
TiO_2-anatase; purity 99.7%	Nominal particle size: < 25 nm	~ 15 nm (~ 250 400 nm when in agglomerated form)	*In vitro*
TiO_2-rutile; purity 99.5%	Nominal particle size: < 100 nm	~ 40 50 nm (submicron-sized when in agglomerated form)	*In vitro* and *in vivo*
TiO_2-rutile; purity 99.5%	Nominal particle size: < 5,000 nm (< 5 μm)	Up to 5 μm	*In vitro* and *in vivo*
TiO_2 mixture: anatase (80%)/rutile (20%); purity 99.5%	Nominal particle size: 23.9 nm	~ 25 nm (~ 125 nm when in agglomerated form)	*In vitro*

The characteristics of the test materials are given in Table 14.

The authors concluded that TiO_2 nanoparticles are very agglomerative in nature; it was not straightforward to obtain, or keep, the nanoparticles within narrow size ranges. The study provided evidence of a lack of any significant TiO_2 translocation above the limit of detection across the gut epithelium model whether it was in the micro- or nanosized forms. The TiO_2 particles seemed to settle between or in the cells, because analytical measurements showed titanium in the cell fractions, but not in the basolateral fraction.

3.1.1.2. *In vivo*

Studies in the mouse

Gu et al. (2015) orally administered 64 mg microsized TiO_2/kg bw per day (> 100 nm in size) to CD-1 mice, and examined the effects on plasma glucose levels. They showed that titanium levels were not changed in blood, liver and pancreas. No histopathological changes in liver or pancreas were observed. The authors concluded that their results indicated that microsized TiO_2 cannot be absorbed after oral administration and consequently, cannot affect plasma glucose levels in mice.

Studies in the rat

In a study by Fournier (1950) (cited by JECFA, 1970), rats (species, sex and number of animals not stated) given a diet of either 0.2, 1 or 2% TiO_2 (not further specified) (equivalent to 236, 1,180 and 2,360 mg TiO_2/kg bw per day, respectively)[16] for 7 days did not appear to absorb TiO_2 from the gastrointestinal tract. In the same study, it was reported that no titanium was found in the blood, liver, kidney and urine of rats given 660 mg TiO_2/kg bw per day for 15 days (sensitivity of analysis 10 μg).

Jani et al. (1994) investigated the uptake of rutile TiO_2 particles (particle size 500 nm) from the rat gastrointestinal tract. Six adult female Sprague–Dawley rats (average weight: 150 g; age: 12 14 weeks) were administered 12.5 mg TiO_2/kg bw per day (0.1 mL of a 2.5% w/v suspension) by oral gavage for 10 days. Organs and tissues, such as Peyer's patches, small intestine, colon, mesentery network and nodes, peritoneal tissue, liver, spleen, heart and kidney, were removed for histological examination, SEM and spectrometric analysis for titanium using inductively coupled plasma emission spectroscopy. Histopathological examination showed the presence of particles, proved to contain TiO_2 by chemical analysis, in all major tissues of the GALT, and demonstrated that TiO_2 particles (500 nm) were translocated to systemic organs such as the liver and the spleen. TiO_2 particles were also found in the lung and peritoneal tissues, but were not detected in the heart or kidney. The authors calculated, based on inductively coupled plasma measurements of titanium levels, that 6.5% of the total dose of TiO_2 particles (size range of 500 nm) administered orally over 10 days was taken up. The authors concluded that the uptake of rutile TiO_2 particles occurs primarily via Peyer's patches and that

[16] Calculated by the Panel according to EFSA Scientific Committee (2012).

the particles subsequently translocate to the mesentery network where they accumulate in the mesenteric lymph nodes. Some particles then entered the general circulation and were taken up by the liver and the spleen.

Onishchenko et al. (2012) studied the penetration of TiO_2 nanoparticles (rutile; physical characteristics not given) into enterocytes, after administration of water dispersions of the test material (rutile dispersion; 50 mg/cm^3) into an isolated loop of Wistar rat small intestine. Penetration was shown *in vivo* using TEM. After 3-h exposure using electron diffraction, rutile nanoparticles were identified in the apical regions of the cells under plasma membranes and in deeper parts of the cytoplasm as solitary objects or small aggregations. The data indicated that the rutile TiO_2 nanoparticles, administered into the gastrointestinal tract, penetrated the small intestinal epithelial barrier.

In a study by Chaudhry et al. (2013; Doc. provided to EFSA n. 7) cited above (published as MacNicoll et al., 2015), the absorption of TiO_2 was further studied in rats bred, fed and maintained in titanium-controlled environment (strain not given; five groups/six rats per type of material) receiving a single oral dose of TiO_2 (4.6 mg TiO_2/kg bw) in the form of nanosized particles (two anatase and one rutile) and microsized particles (rutile). The characteristics of the test materials are given in Table 14. Following oral administration of TiO_2, samples of blood, urine and faeces were collected at appropriate time intervals. When the particles were submitted to pH values mimicking gastrointestinal tract biological conditions, no appreciable dissolution (titanium release) was observed. No significant difference in the amounts of titanium in the urine from the control (microsized) and treated (nanosized) groups was found during the 96 h post-treatment period. The bulk of the titanium (not quantified) was found in the faeces. Titanium concentrations in blood, urine or tissues were not significantly increased. It was concluded by the authors that absorption/translocation to blood, urine and faeces, and distribution to various organs (liver, kidney, spleen, heart, brain, gastrointestinal tract) was very limited.

Cho et al. (2013) studied the fate of spherical nanoparticles (80% anatase, 20% rutile) after oral administration to Sprague Dawley rats. The measured particles size (using SEM) was 26.4 + 6.1 nm and the hydrodynamic particle size was 37.8 ± 0.4 nm. Samples were administered for 13 weeks (7 days/week) at doses of 0, 260, 521 and 1,042 mg TiO_2/kg bw per day. The durability of the particles under gastrointestinal-mimicking conditions was demonstrated. Samples of blood, tissues (liver, kidneys, spleen and brain), urine and faeces were obtained at necropsy. The absorption of TiO_2 nanoparticles was shown to be extremely low. Tissue distribution data showed that TiO_2 nanoparticles were not significantly increased in sampled organs, even in the group receiving the highest dose (1,042 mg/kg bw per day). Titanium concentrations were not significantly increased in the urine. Very high concentrations of titanium were detected in the faeces.

In a study by Geraets et al. (2014) on the tissue distribution, elimination and oral absorption of different TiO_2 nanoparticles in Wistar rats, five different TiO_2 samples were tested (NM-100, NM-101, NM-102, NM-103 and NM-104) after oral or intravenous administration. The characteristics of the test materials used are given in Table 15.

Table 15: Characteristics of materials obtained from the JRC Nanomaterials (NM) Repository (JRC, 2011)

NM code	Type of material	Mean particle size (nm)	Primary particle (nm)	Specific area (mm^2/g)
NM-100	TiO_2	267	42 90	10
NM-101	TiO_2	38	6	320
NM-102	TiO_2, anatase	132	20	90
NM-103	TiO_2, thermal hydrophobic	186	20	60
NM-104	TiO_2, thermal hydrophilic	67	20	60

Animals were dosed either orally (gavage) or intravenously (injection, tail vein) once (three males per group, four TiO_2 nanomaterials and controls) or during five consecutive days (three males per group, four TiO_2 nanomaterials and controls); in addition, for the NM-101 test material, three females per group and controls were dosed.

For the oral route study, the single dose groups received a dose of 2.3 mg TiO_2/animal (calculated by the authors to be equivalent to 6.8–8.6 mg TiO_2/kg bw depending on the actual weight of the (male) animals). The repeated dose groups received five consecutive daily doses (day 1 5) of 2.3 mg TiO_2/mL per animal, resulting in a cumulative dose range of 34.1–42.4 mg TiO_2/kg bw for males and 54.5–59.9 mg TiO_2/kg bw for females.

The rats were killed and tissue sampling was carried out 24 h after the last exposure (day 2 or 6). Liver, spleen and mesenteric lymph nodes were selected as target tissues for titanium analysis.

For the intravenous study, the single-dose groups received a dose of 8.4–9.8 or 12.4–14.1 mg TiO_2/kg bw, for male and female animals, respectively. The repeated intravenously treated animals received five consecutive daily doses (day 1–5) for a cumulative dose range (taking into account the actual weight of the animals) of 42.3–49.4 and 61.2–71.9 mg TiO_2/kg bw for male and female animals, respectively.

Blood and tissue samples were collected from day 2 to day 90 after administration.

From the data on absorption, it was concluded that titanium levels in liver and spleen could only be measured above the limit of detection (30 ng/g tissue) in some rats. Titanium could be detected in the mesenteric lymph nodes, although the levels were very low. When compared with data from non-exposed animals it was shown that some minor, but very limited, absorption occurred in the gastrointestinal tract. No increase in titanium levels was observed in the other tissues.

3.1.2. Distribution

3.1.2.1. Studies in the mouse

Wang et al. (2007b) compared the biodistribution of different sized TiO_2 particles ((25, 80 and 155 nm (fine) in CD-1 (ICR) mice (40 males/40 females). The animals were randomly divided into four groups: one control and three experimental groups receiving a single oral (gavage) dose of the different particles sizes at a level of 5 g TiO_2 suspension/kg bw. Two weeks after treatment, titanium concentrations were analysed in different tissue samples from female mice only.

Titanium accumulated mainly in the liver: 3970 ± 1670 ng titanium/g in the 80 nm group, 106 ± 8 ng titanium/g in the 25 nm group, and 107 ± 25 ng titanium/g in the 155 nm (fine) group. In the kidneys, the titanium concentrations were as follows: ~ 440 ng titanium/g in the 80 nm group (statistically significantly different from control; $p < 0.05$), ~ 375 ng titanium/g in the 25 nm group, ~ 170 ng titanium/g in the 155 nm (fine) group and ~ 150 ng titanium/g in the control group. Titanium concentrations for animals receiving the 155 nm particle suspension were highest in the spleen ($p < 0.05$ compared with control), followed by the lung and brain ($p < 0.05$ compared with control). In the red blood cell, titanium concentrations were ~ 130 ng titanium/g for the 25 nm group, ~120 ng titanium/g for the 80 nm and 155 nm (fine) groups and ~ 80 ng titanium/g for the control group.

As regards biodistribution, the experiment showed that TiO_2 is mainly retained in the liver, spleen, kidneys and lungs, indicating that TiO_2 particles can be transported to other tissues and organs after uptake via the gastrointestinal tract. Furthermore, a basal level of TiO_2 of 150 ng/g TiO_2 in kidney and 80 ng/g in the red blood cell was demonstrated in control animals.

3.1.2.2. Studies in the rat

Lloyd et al. (1955) tested TiO_2 (particle size not given) as an index material for determining the digestibility of a rat diet. Albino male rats (n = 30, 60 days old) were fed a diet containing 0.25% TiO_2 (equivalent to ~ 295 mg TiO_2/kg bw per day) for 6 days.[16] Another group of rats (n = 30) were fed a diet containing 0.25% chromium(III) oxide, but there was no control group. The faeces of 10 of the 30 rats receiving the diet for 6 days were collected individually and daily for 13 days after the initial consumption. Twenty other rats were divided equally into four groups and after the 6-day feeding period; total faeces per group were collected for 7 days (total food consumption was also noted for this 7-day period). For the 30 rats on the TiO_2 diet, an average of 92% of the administered TiO_2 was recovered. The authors noted that some TiO_2 (8%) was unaccounted for which they treated as absorbed and hypothesised that delayed excretion could be due to accumulation of titanium in some part of the gastrointestinal tract.

West and Wyzan (1963) (as reported in IPCS, 1982) fed male and female rats (no further details given) a diet containing TiO_2 (100 g TiO_2/kg diet; particle size not given) for ~ 32 days. A statistically significant amount of titanium was found only in the muscles (0.06 mg/kg wet weight in males and 0.11 mg/kg wet weight in females); no retention was observed in the liver, spleen, kidney, bone, plasma or erythrocytes.

A bioavailability study (Colorcon, 2003 as reported by EFSA in 2004) performed in Sprague-Dawley (Cr:CD® BR) rats using four test substances of TiO_2 (no information on particle size given): rutile TiO_2 (thick platelet), rutile TiO_2 (thin platelet), rutile TiO_2 (amorphous) and anatase TiO_2 (amorphous). Groups (three animals/sex per time-point, aged 6–10 weeks) were fed *ad libitum* either a control diet

or a diet containing one of the four types of TiO_2, which were given at a concentration of 200 mg TiO_2/kg diet (equivalent to ~ 30 mg TiO_2/kg bw per day).[17] These TiO_2-containing diets were fed to the rats for seven consecutive days and were then replaced by the control diet for a maximum of 72 h before sacrifice. The control diet administered during the treatment phase contained a mean concentration of 9 mg TiO_2/kg wet weight and the control diet administered after the treatments contained a mean concentration of 7 mg TiO_2/kg wet weight. Groups of animals were killed at 1, 24 and 72 h after withdrawal from the treatment diet, and the titanium contents of the liver, kidneys, muscle, whole blood, urine and faeces were determined. The main route of titanium excretion was via the faeces. Faecal excretion in each collection interval (0–24, 24–48, 48–72 h) was similar for all TiO_2-treated groups. The mean total amounts of titanium excreted in the faeces during 0–72 h after withdrawal of the TiO_2-treated diet were in the range of 1.4–2.2 mg/animal for male rats and 1.1–1.3 mg/animal for female rats, accounting for means of 39–63% of the daily dose. Urinary excretion of titanium was equivalent to < 2% daily dose/L of urine for all groups and was generally below the limit of quantification (< 0.04 mg/L). Whole-blood concentrations of titanium from all groups were < 0.04 mg/L and concentrations of titanium in liver, kidney and muscle were generally below the LOD(< 0.1 to < 0.2 mg/kg wet weight) or in the range of 0.1–0.3 mg/kg wet weight for most animals treated with either the control diet or a diet containing TiO_2. The bioavailability study showed that there was no difference in the systemic absorption of the four forms of TiO_2 following dietary administration at a nominal concentration of 200 mg TiO_2/kg (based on a LOQ < 0.04 mg/L for urinary excretion).

In the study by Onishchenko et al. (2012) cited above, the effect of the administration of water dispersions of TiO_2 nanoparticles with an anatase structure (not further specified) and of micron-sized TiO_2 particles (food additive E 171; crystal structure not indicated) at low (1 mg/kg) and high (100 mg/kg) doses for 28 days was studied in Wistar rats. Titanium in basal amounts, characteristic of a great number of biological objects, was present in the liver tissue of rats fed a standard semisynthetic diet. Administration of the water dispersions induced no appreciable increase in these basal values. A similar result was observed in animals treated with rutile nanoparticles at the low dose (1 mg/kg). However, the titanium concentration in the liver increased significantly (almost doubling) in rats receiving intragastric water dispersions of rutile nanoparticles at the high dose (100 mg/kg), which, according to the authors, could indicate its penetration through the intestinal barrier. The Panel noted that the authors did not reveal the size characteristics of the nanoparticulate test material.

In a study by Chaudhry et al. (2013; Doc. provided to EFSA n. 7) (published as MacNicoll et al., 2015) described in Section 3.1.1, rats were administered by gavage a single dose of different TiO_2 nano- and larger particles dispersed in water (see Table 14). Animals were killed at different time intervals during the 96 h post-treatment and tissues (liver, brain, heart, kidney and spleen) were sampled. Based on titanium determination (LOD = 1 ng/g), no translocation of TiO_2 was observed in any of the treatments applied and tissues selected.

Tassinari et al. (2014) studied the effect of short-term oral exposure to TiO_2 nanoparticles in Sprague–Dawley rats with a focus on the reproductive and endocrine systems and spleen. In the study, anatase nanoparticles with two different morphologies were used, i.e. spherules with primary sizes ranging from 20 to 60 nm and irregular-shaped particles ranging from 40 to 60 nm. Moreover, large agglomerates and chains of spherules were also observed to be present. The test materials were administered, by gavage, for five consecutive days at doses of 0, 1 and 2 mg TiO_2/kg bw. An increase in the titanium concentration was found in the spleen and ovaries of treated animals compared with controls, even though the titanium tissue levels remained low (control, 0.036 ng/g fresh weight; 1 mg/kg bw dose, 0.040 ng/g fresh weight; 2 mg/kg bw dose, 0.046 ng/g fresh weight) and were similar to the levels reported in controls and were within the values reported by Wang et al. (2007a). In the spleen of treated animals, TiO_2 aggregates of 200–400 nm (in high-dose females) were identified and quantified ($2–3 \times 10^4$ particles/mm^2 vs $< 1 \times 10^4$ particles/mm^2 in controls).

Geraets et al. (2014) concluded that after both single and repeated intravenous exposure, titanium (not further specified in terms of purity, nanosized distribution) is rapidly distributed from the systemic circulation to all tissues evaluated (i.e. liver, spleen, kidney, lung, heart, brain, thymus and reproductive organs). Liver was identified as main target tissue, followed by spleen and lung. Total recovery (expressed as % nominal dose), measured 24 h after single or repeated exposure, ranged from 64% to 95%. Based on calculations using different scenarios (i.e. using LOD or half the LOD for

[17] A grade of sufficient purity to meet or exceed requirements of the United States National Formulary (NF) (merged with the United States Pharmacopeia, USP-NF).

the non-detects; correcting tissue levels for background levels; using only the positive liver titanium levels), the authors estimated that ~ 0.02% of the administered dose of TiO_2 was distributed in the tissues. The Panel agreed with this conclusion of the authors.

3.1.3. Excretion

From the Geraets et al. (2014) study (see above), it was concluded that, following intravenous administration, a decrease in titanium in the investigated organs was observed over the 90-day period, although > 50% of the administered dose was still present at the end, indicating a long half-life (28–248 days for the liver). Titanium levels in liver, the tissue exhibiting the highest levels, showed a decrease during that period for all nanoparticles tested, together with a concomitant increase in spleen, in which the final titanium level was higher than in liver. Only minor differences in kinetic profiles were observed, both after single and repeated exposure.

The authors of the study further indicated that the titanium levels measured in the faeces of intravenously treated (single and repeated dose) animals revealed no clear differences between TiO_2-exposed animals and vehicle-treated controls. Furthermore, no increase in titanium levels in urine was observed.

At day 90 post-exposure, titanium levels in spleen were higher than in liver (expressed as µg/g tissue). This would be consistent with a redistribution of the TiO_2 nanoparticles between liver and spleen and slow elimination. The Panel noted that there were only a few sampling times during the post-exposure period.

The Panel also noted that although tissue half-life was estimated, it was not possible to determine the excretion pathway.

3.1.4. Human studies

West and Wyzan (1963) (as reported in International Programme on Chemical Safety (IPCS), 1982), gave five male volunteers 5 g of National Formulary grade TiO_2 suspended in milk on three consecutive days.[18] Urine samples were collected for 5 days after the start of ingestion. No detectable change in urinary titanium levels was detected, which suggests the absence of any significant absorption of the titanium ion, although accumulation in the body cannot be excluded.

Böckmann et al. (2000) measured blood titanium levels in males (24–66 years old) after oral administration of TiO_2 (23 or 46 mg) either as anatase (median particle size, 160 nm) in gelatin capsules or as a powder (median particle size, 380 nm). Pretreatment background blood levels had titanium levels ranging from 6 to 18 µg/L. After TiO_2 administration, blood samples were taken over 24 h (i.e. 0, 15 and 30 min and 1, 2, 4, 8, 12 and 24 h) and the titanium level in the blood was measured. The authors reported concentration–time data, from which the Panel calculated the area under the blood concentration/time course (AUC) as a measure of absorbed amount. The AUC of five subjects (median: 17,573.25 µg/L × min) having taken the gelatin capsules (160 nm particles) was higher than for the two subjects (AUC: 9,384 and 10,519.5 µg/L × min) having taken the powder (380 nm particles). According to these authors, this indicates that there might be an influence of particle diameter on the extent of absorption of TiO_2, however, the Panel noted the median particle size of both particles studied were greater than 100 nm. The authors reported that the blood concentration/time correlation showed the type of curve characteristic of a persorption mechanism of absorption.

In a study by Jones et al. (2015), human volunteers (four males and five females; aged 30–56 years) received a 5 mg/kg bw single oral dose of TiO_2 (particle sizes: 15 nm (anatase; ~ 100% by number < 50 nm), 100 nm (rutile; 95% by number between 48 and 154 nm) and < 5000 nm (rutile; 100% by number > 100 nm) dispersed in water. Doses were administered at least 4 weeks apart. All urine samples were collected in timed collections over a 4-day period starting 24 h before dosing and ending 72 h post-dose, and analysed for titanium content after hydrolysis. Blood samples were collected before dosing and at 2, 4, 24 and 48 h after dosing and analysed for titanium content, full blood count and liver function tests. The study demonstrated that very little TiO_2 at all nanosizes tested was absorbed gastrointestinally after an oral dose at a maximum estimate of 0.1% of the administered dose. There was no demonstrable difference in absorption for any of the three particle sizes tested. Because of the very low absorption and the variable endogenous titanium levels, no classic absorption and elimination curve was observed in any of the studies. A dose of 5 mg/kg bw was well tolerated (both clinically and biochemically) by all volunteers for all particle sizes.

[18] 'NF grade' is the purity standard as defined in the US National Formulary.

In another recent published study (Pele et al., 2015), seven human volunteers were given a single oral dose of 100 mg TiO_2 (particle size (d_{50}), 260 nm). Venous blood was sampled up to 10 h post-administration, TiO_2 particles were identified by dark field microscopy (reflectance) and ^{47}Ti was measured by inductively coupled plasma mass spectrometry. An unquantified fraction of TiO_2 particles was detected in blood with a peak of absorption observed at 6 h, which paralleled the titanium concentration in blood (~ 10 µg/mL, which decreased to 5 µg/mL at 10 h). Whether the particles are transported within or outside immune cells requires confirmation.

Based on the above dataset, the Panel considered that:

- TiO_2 was chemically stable under physicochemical conditions that mimic the gastrointestinal situation. No release of titanium ions was shown to occur.
- The vast majority of orally administered TiO_2 was excreted in the faeces.
- TiO_2 particles did not cross the gastrointestinal–epithelial barrier models by diffusion *in vitro*, but there was minimal translocation into the cells, which varied with the model system used.
- Nano/microsized TiO_2 particles were absorbed to a limited extent from the gastrointestinal tract (bioavailability estimated at 0.02–0.1%), essentially through the GALT. However, there were uncertainties regarding the real physical state (primary size, aggregation/agglomeration, protein corona) of the absorbed particles and estimates were based on measurements of titanium ion. Furthermore, evaluating the data overall, the Panel considered that there were no differences in the extent of absorption related to particle size. The Panel noted that the very low bioavailability and variable background basal levels of TiO_2 in tissues not only made it difficult to interpret the results, but also prevented accurate determination of kinetic parameters such as elimination half-life.
- Absorbed nano/microsized TiO_2 particles were distributed in different organs, by order of decreasing concentration: mesenteric lymph nodes, liver, spleen, kidney, lungs, heart and reproductive organs (testes and ovaries).
- After intravenous administration of nano- and microsized TiO_2 particles, studying four different particles, titanium was poorly excreted via urine and higher titanium concentrations were observed in tissues than in blood. After repeated intravenous dosing over 6 days, titanium concentrations in tissues were higher than after single intravenous administration. A long-term redistribution of titanium from the liver to the spleen has been shown to occur, which emphasises the role of the mononuclear phagocyte system in particle processing. The decline of titanium concentrations in the tissues was slow; the authors calculated half-lives of between 28 and 650 days, depending on the TiO_2 particles and tissue. The Panel noted that titanium absorbed after oral TiO_2 administration would have the same kinetic pattern as TiO_2 administered by repeated i.v. The Panel also noted that after oral administration, direct evidence for higher concentrations in the tissues was lacking, which may be due to the low bioavailability, high variability of intake and high background (basal) tissue levels of titanium. The slow elimination of titanium after intravenous administrations indicates the potential for a low but steady increase in titanium tissue levels with time for absorbed titanium after oral administration.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The acute oral median lethal dose (LD_{50}) value for TiO_2 was > 10 g TiO_2/kg bw per day for mice and > 25 g/kg bw per day for rats (Hallagan et al., 1995; SCCNFP, 2000).

Three different TiO_2 particle sizes (25, 80 and 155 nm) were administered by gavage with a single dose of 5,000 mg TiO_2/kg bw in CD-1 (ICR) mice in accordance with OECD 420, by oral gavage (Wang et al., 2007b). After 2 weeks, TiO_2 particles showed no obvious acute toxicity. Female animals exposed to nanosized TiO_2 showed hepatotoxicity characterised by changes in aspartate amino transferase/alanine amino transferase ratio and lactate dehydrogenase activity, and hydropic degeneration around the central vein and focal necrosis of hepatocytes. In addition, nephrotoxicity (increased blood urea nitrogen levels) was also observed in these groups. These changes were not seen in mice treated with TiO_2 particles of 155 nm.

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Studies in the mouse

The US National Cancer Institute (NCI, 1979) performed a subchronic toxicity (90-day) dose range-finding study in B6C3F1 mice to estimate the maximum tolerated doses of TiO_2 (anatase; particle size not given) to be used in a carcinogenesis study in the mouse. Doses of 6,250, 12,500, 25,000, 50,000 or 100,000 mg TiO_2/kg diet were administered (equivalent to 1,344, 2,688, 5,375, 10,750 or 21,500 mg TiO_2/kg bw per day for female mice and 1,056, 2,113, 4,225, 8,450 or 16,900 mg TiO_2/kg bw per day for male mice, respectively).[16] TiO_2 had a purity of minimum 98%. Ten males and 10 females were administered the test substance at each dose, and 10 males and 10 females received basal diets for 13 consecutive weeks. There were no deaths, and dosed animals had mean bw gains that were comparable with those of the controls. No gross or microscopic pathology was found that could be related to the administration of anatase in the mice.

The Panel noted that the study was only briefly described in the NCI (1979) report and that no haematological parameters and no biochemical parameters in urine and blood were measured.

3.2.2.2. Studies in the rat

West and Wyzan (1963) (as reported in JECFA, 1970) fed a group of 10 male and 10 female rats (strain not given) 100 mg National Formulary Grade TiO_2/kg diet for 30–34 days.[17] A second, untreated group was used as a control. All animals remained healthy and normal. Weight gain and food intake were comparable for the two groups. At autopsy, no relevant gross pathology was observed. No evidence of an increase in titanium content was found in any of the seven different tissues analysed (no further details) except muscle, where the increase was 0.1 mg/kg compared with tissues from the control animals.

In a study that was in line with OECD Test Guideline 407 for 'Repeated Dose 28-Day Oral Toxicity Study in Rodents', three groups of five young male Sprague Dawley Crl:CD(SD) rats were given daily gavage doses of either pure water (control) or 24,000 mg/kg bw of one of two similar non-coated pigment-grade forms of rutile with a d_{50} of 173 nm; one form was described as 'research grade' and the other was 'commercial grade'. One rat from each of the test groups died prematurely due to misdosing (perforation of the oesophagus). There were no treatment-related effects on food intake, body weight, clinical signs, haematology, serum clinical chemistry, organ weights, gross pathology or histopathology. Particles found in intestinal lymphoid tissue were not regarded as an adverse effect. There were no differences in response to the two forms of the test material. The no observable adverse effect level (NOAEL) for the study was 24,000 mg/kg bw per day for both forms of TiO_2 tested. Although this study was not performed with TiO_2 (E 171), its results are useful as supporting evidence in the assessment of the use of TiO_2 as a colouring agent for food and feeds (Warheit et al., 2015b).

The NCI (1979) performed a subchronic toxicity (90-day) dose range-finding study in Fischer 344 rats to estimate the maximum tolerated doses of TiO_2 (anatase; particle size not given) to be used in a carcinogenesis study in the rat. Doses of 6,250, 12,500, 25,000, 50,000 or 100,000 mg TiO_2/kg diet were administered (equivalent to 569, 1,138, 2,275, 4,550 or 9,100 mg TiO_2/kg bw per day for female rats and 506, 1,013, 2,025, 4,050 or 8,100 mg TiO_2/kg bw per day for male rats, respectively).[16] TiO_2 had a purity of minimum 98%. Ten males and 10 females were administered the test substance at each dose, and 10 males and 10 females received basal diets for 13 consecutive weeks. There were no deaths, and dosed animals had mean body weight gains that were comparable with those of the controls. No gross or microscopic pathology was found that could be related to the administration of the test substance in the rats.

The Panel noted that the study was described only briefly in the NCI (1979) report and that no haematological parameters and no biochemical parameters in urine and blood were measured.

The Panel noted that there was rather limited information available on the short-term and subchronic toxicity on the food additive TiO_2 (E 171).

In a well-performed 28-day gavage study in rats with non-coated pigment-grade TiO_2 (rutile form; d_{50} 173 nm) at a dose of 24,000 mg TiO_2/kg bw, no treatment-related effects were observed (Warheit et al., 2015b). Particles found in intestinal lymphoid tissue were not regarded as an adverse effect. The NOAEL for the study was 24,000 mg/kg bw per day. Although the study was not performed with food-grade TiO_2, the Panel considered the results useful as supporting evidence in the assessment of the use of TiO_2 as food additive (E 171) colouring agent for food and feeds.

In a 90-day feeding study, doses up to 16,900 mg TiO_2/kg bw per day for male mice and up to 8,100 mg TiO_2/kg bw per day for male rats did not result in differences in body weight or in relevant gross or microscopic pathology compared with the control (NCI, 1979). However, no haematological parameters and no biochemical parameters in urine and blood were measured.

3.2.3. Genotoxicity

3.2.3.1. *In vitro*

In an early study, TiO_2 was reported to be negative in a rec-assay with *Bacillus subtilis* for genotoxicity using a M45 recombination-deficient strain (Kada et al., 1980). The Panel noted that such a test system has not been validated and considered this information not relevant for risk assessment.

In a screening study of 63 carcinogenic and non-carcinogenic chemicals, TiO_2 (CAS Registry number 13463-67-7, particle size not specified) was tested for mutagenicity in a bacterial reverse mutation assay using the plate-incorporation procedure in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and *Escherichia coli* WP2 *uvrA*, in the absence and in the presence of exogenous metabolic activation S9 liver preparations from uninduced and Aroclor 1254-induced F344 rats, B6C3F1 mice and Syrian hamsters. Dose levels were selected at half-log intervals and never exceeded 10 mg/plate. Clear negative results were observed for TiO_2 (Dunkel et al., 1985). The Panel noted that in this, and in the other genotoxicity assays performed within the validation exercise coordinated by the National Toxicological Programme (NTP) (Dunkel et al., 1985; Tennant et al., 1987; Ivett et al., 1989; Myhr and Caspary, 1991; Shelby et al., 1993; Shelby and Witt, 1995), the sample of TiO_2 tested was received from the NTP repository of the chemicals tested in carcinogenicity bioassays. According to the NCI-CG-TR 97, the sample was an anatase TiO_2 white pigment designated Unitane® 0.220.

Tennant et al. (1987) assayed TiO_2 (particle size not given, see above) in the Ames *Salmonella*/microsome mutagenicity assay, in the assays for chromosomal aberrations and sister chromatid exchanges (SCEs) in a Chinese hamster ovary (CHO) cell line, and in the mouse lymphoma L5178Y cell mutagenicity assay. Standard protocols developed by the NTP of the NCI were used for the selected assays. Negative results for TiO_2 were reported in any of the four short-term tests (STTs) selected. The highest negative dose levels assayed were as follows: 10,000 μg/plate in the Ames test, 25 μg/mL in the assays for chromosomal aberrations and SCEs, and 1.6 μg/mL in the mouse lymphoma L5178Y cell mutagenesis assay.

Ivett et al. (1989) studied the genotoxicity of TiO_2 (particle size not given, see above) in a SCE assay and in a chromosomal aberration assay in CHO cells, both in the absence and presence of rat liver S9. Cells were exposed for 25 and 2 h in the SCE assay and for 8 and 2 h in the chromosomal aberration assay in the absence and presence of rat liver S9, respectively. In both assays, a top-dose level of 25 μg/mL (equivalent to 313 μM) was selected based on the solubility of the test material. Reported results indicated that TiO_2 did not induce SCE or chromosomal aberrations in mammalian cells *in vitro*.

Myhr and Caspary (1991), in a following screening study on 31 coded compounds, tested TiO_2 (particle size not given, see above) for its mutagenicity in an *in vitro* mammalian cell gene mutation assay in L5178Y mouse lymphoma cells at the thymidine kinase (TK) locus in both the absence and presence of S9. The test compound was administered for 48 h at dose levels ranging from 1.56 to 50 μg/mL. Negative results were reported in any treatment conditions.

In the study by Miller et al. (1995), TiO_2 (particle size not given) was assessed for its genotoxic potential in an *in vitro* micronucleus assay in CHO cells in both the absence and presence of rat S9. Dose levels ranged from 0.025 to 10 μg/mL in the absence of S9 and from 0.25 to 10 μg/mL in its presence, and treatment times were 48 and 3 h, respectively. Top-dose levels were selected according to cytotoxic effects, which were based on a reduction of cell density by at least 25% of concurrent control values. However, precipitation of TiO_2 was observed at concentrations of ≥ 0.5 and ≥ 1.0 μg/mL in the absence and in the presence of S9, respectively. Micronuclei were scored in at least 1,000 mononucleated cells from each culture. Results obtained indicated that TiO_2 was not able to induce micronuclei in CHO cells.

Linnainmaa et al. (1997) assessed the induction of micronuclei in a rat liver epithelial cell line by two ultrafine (UF1 and UF2) TiO_2 preparations. The test material consisted of uncoated anatase (UF1, average particle size 20 nm), rutile coated with aluminium hydroxide and stearic acid (UF2, average particle size 20 nm), and pigmentary TiO_2 (average particle size 170 nm). Treatments were conducted for 21 h alone or in combination with UV irradiation (365 nm). Dose levels evaluated

ranged from 5 to 20 μg/cm^2. Cytochalasin B (1 μg/mL) was added to the culture for the last 20 h. The reported results indicate that TiO_2 (pigmentary or ultrafine) alone or in combination with UV light did not induce chromosomal damage measured as induction of micronuclei. However, the Panel noted that the spontaneous frequencies of micronuclei in the untreated controls were markedly high (53 71 micronuclei/1,000 binucleated cells) indicating elevated genomic instability of the cell line employed, and on this basis, the Panel considered the results reported in this study of limited relevance for risk assessment.

Nakagawa et al. (1997) investigated the photogenotoxicity of TiO_2 particles in a single-cell gel electrophoresis Comet assay with mouse lymphoma L5178Y cells, a microbial mutation assay with *S.* Typhimurium, a mammalian cell mutation assay with L5178Y cells and a chromosomal aberration assay with Chinese hamster CHL/IU cells. The following TiO_2 particles were tested in the single-cell gel electrophoresis assay: anatase-p-25, (average size 21 nm) and anatase-WA, (average size 255 nm); rutile-WR, (average size 255 nm) and rutile-TP-3, (average size 420 nm). In the TiO_2 Comet assay WA, WR and TP-3 were tested at concentrations from 250 to 2,000 μg/mL and p-25 was tested at five concentrations from 2.1 to 800 μg/mL. In the chromosomal aberration assay in CHL cells, only p-25 was tested at concentrations from 25 to 800 μg/mL, in the absence of UV radiation and at concentration from 0.78 to 28.5 μg/mL in the presence of UV radiation. In bacteria (*S.* Typhimurium strains TA100, TA98 and TA102), only p-25 TiO_2 particles were tested from 6,750 to 54,000 μg/plate with and without UV radiation. Results obtained showed that p-25 and TP-3 induced primary DNA damage only when UV irradiated (minimum effective concentrations of 12.5 and 200 μg/mL, respectively); WA particles (50 3,200 μg/mL) were also positive without irradiation, but only at the highest tested dose, whereas WR particles were negative in the same dose range. Negative results were observed with p-25 nanoparticles in bacteria (500 4,000 μg/plate) and in the L5178Y mouse lymphoma gene mutation assays (250 2,000 μg/mL). Positive results were obtained with p-25 in an *in vitro* chromosomal aberration assay in Chinese hamster cells (minimum effective concentration 12.5 μg/mL), only in the presence of UV irradiation.

Lu et al. (1998) studied the effect of TiO_2 (particle size not indicated) for the induction of SCE and micronuclei in CHO-K1 cells. TiO_2 was administered for 24 h, at dose levels of 1, 2 and 5 μM for SCE and at 5, 10, 15, 20 μM for 18 and 24 h in the conventional and cytokinesis-block micronuclei analysis, respectively. Selection of top-dose levels was based on a reduction in colony-forming ability. Results obtained indicated that TiO_2 induced dose-related and statistically significant increases in SCE compared with concurrent untreated control cultures. Dose-related and statistically significant increases were also observed for induction of micronuclei both in the conventional micronuclei analysis and in the cytokinesis-block micronuclei analysis. However, higher levels of micronuclei (2.5- to 3-fold increases) were observed with and without the cytokinesis-block micronuclei.

Rahman et al. (2002) reported the effects of ultrafine TiO_2, particle size < 20 nm, and fine TiO_2, particle size > 200 nm, on chromosomal damage in Syrian hamster embryo cells (SHE) monitored by the formation of micronuclei. Cells were treated on coverslips at concentrations of 0.5, 1, 5 and 10 μg/cm^2 for 12, 24, 48, 66 and 72 h. DNA was stained with bisbenzimide at 1 μg/mL and micronuclei scored at ×630 magnification under a fluorescence microscope. For further micronuclei analyses, kinetochores were stained with CREST serum to allow discrimination of clastogenic effects from aneuploidy. Results obtained revealed significant increases in micronuclei induction by ultrafine TiO_2 at a dose of 1 μg/cm^2 at sampling times for 24, 48, 66 and 72 h, whereas fine TiO_2 did not induce significant increases in micronuclei. Furthermore, kinetochore analyses revealed no significant increases in the kinetochore-positive micronuclei compared with micronuclei in the untreated control, indicating that induced micronuclei arise mainly from clastogenic and not aneugenic events.

Wang et al. (2007a) evaluated the cytotoxic and genotoxic activity of ultrafine TiO_2 particles (particle size not specified) in human lymphoblastoid WIL2-NS cells. Cells were incubated for 6, 24 and 48 h with 0, 26, 65 and 130 μg/mL ultrafine TiO_2; cytotoxicity was evaluated by the methyl tetrazolium cytotoxicity (MTT) assay, apoptosis assay by the flow cytometry, and genotoxicity by the cytokinesis block micronucleus assay, by the Comet assay and by the hypoxanthine–guanine phosphoribosyltransferase gene mutation assay. Significant decreases in viability and proliferation, and increase in apoptosis were seen at the highest doses. In genotoxicity assays, increased incidence of micronuclei (~ 2.5-fold at 130 μg/mL), olive tail moment (~ 5-fold increases at 65 μg/mL) and hypoxanthine guanine phosphoribosyltransferase mutations (~ 2.5-fold increases at 130 μg/mL) were observed in cells following exposure to ultrafine TiO_2.

Türkez and Geyikoğlu (2007) evaluated the potential genotoxic effects of TiO_2 (particle size not indicated) in human whole-blood cultures. Blood samples were obtained from four young non-smoking

and healthy donors, and pooled for treatment. SCE and micronuclei were scored as genetic endpoints. Dose levels of 1, 2, 3, 5, 7.5 and 10 μM were administered to blood cultures for 72 h. For SCE, 5-bromo-2′-deoxiuridine at 20 μM was added from the beginning of culture. For micronuclei analyses, cytochalasin B (6 μg/mL) was added 44 h from the beginning of culture. Results obtained showed dose-related and statistically significant increases in both SCE and micronuclei, indicating the potential genotoxicity of TiO_2. These results were confirmed in a second study (Turkez, 2011), in which the role of oxidative stress was suggested based on the observed reduction in TiO_2 genotoxicity in presence of ascorbic acid.

Warheit et al. (2007) tested TiO_2 particles (79% rutile, 2% anatase; median particle sizes of 140 nm) for mutagenicity in a bacterial reverse mutation test in *S.* Typhimurium strains TA98, TA100, TA1535 and TA1537, and in *E. coli* strain WP2uvrA in the absence and presence of metabolic activation (Aroclor-induced rat liver S9). Negative results were reported up to 5,000 μg/plate. The same test item was also negative in a chromosome aberrations test in CHO cells in the absence and presence of metabolic activation (Aroclor-induced rat liver S9). The test item was analysed without S9 up to 2,500 and 100 μg/mL in the 4- and 20-h treatment, respectively, whereas with S9, the top dosage was 250 μg/mL.

Karlsson et al. (2009) compared the toxicity of nano- and micrometre particles of some metal oxides, and nano- and micrometre particles of TiO_2 (average particle size 63 nm and 1 μm, respectively) by assessing DNA damage and DNA oxidative lesions in the human alveolar type II-like cell line A549. To study DNA damage in forms of DNA strand breaks and alkali labile sites, the alkaline version of the Comet assay was used. For analyses of oxidative DNA lesions, mainly oxidised purines, the enzyme formamidopyrimidine DNA glycosylase was applied to the Comet assay. When A549 cells were treated with nano- and micrometre particles of TiO_2 for 4 h at 40 and 20 μg/cm^2, statistically significant increases in DNA damage compared with untreated controls were observed for both nano- and micrometre particles. However, micrometre particles caused markedly higher levels of DNA damage compared with nanoparticles. By contrast, for oxidative DNA damage, no significant increases in oxidised purines were observed for both nano- and micrometre particles.

Xu et al. (2009) assessed the genotoxicity of TiO_2 particles of different size distributions (anatase form, size 5 nm, 40 nm and 325 mesh, applied in the dose range 0.1 30 μg/mL) using gpt delta transgenic mouse primary embryo fibroblasts. Mutation frequencies were investigated at redBA and gam loci, sensitive to kilobase deletion mutations. TiO_2 nanoparticles (both 5 and 40 nm) significantly increased mutation yield at 0.1 μg/mL and above, with no clear relation with the dose applied. The effect was abrogated by the concurrent treatment with the endocytosis inhibitor Nystatin.

Bhattacharya et al. (2009) evaluated the genotoxicity and oxidative stress induced by TiO_2 nanoparticles (anatase; size < 100 nm) in human lung fibroblasts (IMR-90) and human bronchial epithelial cells (BEAS-2B). TiO_2 nanoparticles (2–50 μg/cm^2) did not induce detectable DNA damage, as evaluated by Comet assay, although they increased both oxidative damage (8-hydroxy 2′-deoxyguanosine (8-OH-dG)) and the intracellular generation of reactive oxygen species (ROS).

In the study by Falck et al. (2009), the *in vitro* genotoxicity of nanosized TiO_2 rutile and anatase was assessed in comparison with fine TiO_2 rutile in human bronchial epithelial BEAS-2B cells using the single-cell gel electrophoresis (Comet) assay and the cytokinesis-block micronucleus test. BEAS-2B cells were exposed to eight doses (1 100 μg/cm^2) of titanium oxide nanosized rutile (99.9% < 5 nm), nanosized anatase (99.7%; < 25 nm) or fine rutile (99.9%; < 5 μm) for 24, 48 and 72 h. Fine rutile reduced cell viability at lower doses than nanosized anatase, which was more cytotoxic than nanosized rutile. In the Comet assay, nanosized anatase and fine rutile induced DNA damage at several doses for all treatment times. The lowest doses inducing DNA damage were 1 μg/cm^2 for fine rutile and 10 μg/cm^2 for nanosized anatase. Nanosized rutile showed a significant induction in DNA damage only at 80 and 100 μg/cm^2. Only nanosized anatase could elevate the frequency of micronucleated BEAS 2B cells, producing a small but significant increase at 10 and 60 μg/cm^2 (with no dose dependency).

Di Virgilio et al. (2010) analysed the cytotoxicity and genotoxicity of titanium oxide nanoparticles (20 + 7 nm) on CHO-K1 cells using the Neutral Red and MTT assays, and by the SCE and micronuclei assays. Results showed a dose-related cytotoxic and genotoxic effects, with micronuclei frequencies significantly increased at 0.5 and 1 μg/mL, and SCE significantly increased at 1–5 μg/mL TiO_2. Cytotoxicity, evidenced also by the absence of metaphases, was observed at higher concentrations.

Landsiedel et al. (2010) investigated the genotoxicity of coated rutile TiO_2 nanoparticles (size 10 × 50 nm) in standard OECD *in vitro* and *in vivo* test systems. No genotoxicity was observed *in vitro* in the *Salmonella* gene mutation test (at 20 5000 μg/plate) and in the V79 micronucleus test

(at 75–300 μg/mL), or *in vivo* in Comet assays on alveolar lavage cells from rats exposed by inhalation 6h/day for 5 days to 10 mg/m^3 TiO_2 nanoparticles.

Using a Hep-2 cell line, Osman et al. (2010), evaluated the cytotoxicity and genotoxicity of TiO_2 nanoparticles using the MTT and Neutral Red assays, and the Comet and the cytokinesis-block micronucleus assays, respectively. Concentration- and time-dependent cytotoxicity and increases in DNA and cytogenetic damage were observed (no further details available).

Shukla et al. (2011) evaluated the cytotoxic and genotoxic activity of TiO_2 nanoparticles (anatase; average diameter 50 nm) in the human epidermal cell line (A431). A mild cytotoxic response of TiO_2 nanoparticles was observed using the MTT and Neutral Red uptake assays after 48 h of exposure. A statistically significant ($p < 0.05$) induction in DNA damage was observed using the formamidopyrimidine DNA glycosylase-modified Comet assay in cells exposed to 0.8 μg/mL TiO_2 nanoparticles (2.20 ± 0.26 vs control 1.24 ± 0.04) and higher concentrations for 6 h. A significant ($p < 0.05$) induction in micronucleus formation was also observed at the above concentration (14.7 ± 1.2 vs control 9.3 ± 1.0). TiO_2 nanoparticles elicited a significant cytotoxicity, evaluated using the MTT and Neutral Red assays, and reduced glutathione level with a concomitant increase in lipid hydroperoxides and ROS.

Wang et al. (2011) examined oxidative stress as well as cyto- and genotoxicity induced by TiO_2 nanoparticles (100% anatase; < 25 nm) in CHO-K1 cells following 60 days of continuous exposure at 0, 10, 20 or 40 μg/mL. The results of the study showed that oxidative stress increased in a concentration-dependent manner in short-term (2 days) cultures, whereas long-term cultures had lower levels of oxidative stress. The primary ROS appeared to be superoxide, because ROS indicators were lowered on addition of superoxide dismutase. No cyto- or genotoxic effects were apparent using the MTT, Trypan Blue exclusion and colony-forming assays for viability, and the Comet and *hprt* gene mutation assays for genotoxicity. According to the authors, CHO cells appear to adapt to chronic exposure to nano-TiO_2 and to detoxify excess ROS, possibly through upregulation of superoxide dismutase in addition to reduction of particles uptake.

Hackenberg et al. (2011) evaluated the *in vitro* geno- and cytotoxicity of TiO_2 anatase nanoparticles (diameter 15–30 nm) in peripheral blood lymphocytes from 10 male donors. TEM was performed to describe particle morphology and size, the degree of particle aggregation, and their intracellular distribution. Cells were exposed to nanoparticles in increasing concentrations of 20, 50, 100 and 200 μg/mL for 24 h. Cytotoxic effects were analysed by the Trypan Blue exclusion test and the single-cell microgel electrophoresis (Comet) assay was applied to detect DNA strand breaks, alkali labile sites and repair intermediates. Particles displayed a strong tendency to form aggregates, despite dispersive treatments. The Trypan Blue exclusion test did not show any decrease in lymphocyte viability, and there was no evidence of genotoxicity in the Comet assay for any of the tested concentrations, despite particles being detected in the cytoplasm as well as in the nucleus of treated cells.

Jugan et al. (2012) characterised the genotoxic potential of TiO_2 nanoparticles of different sizes and crystalline phases in the human lung cell line A549. Test material consisted of spherical anatase (nano) particles with average diameters of 12, 25 and 140 nm (A12, A25 and A140), and spherical rutile nanoparticles with average diameters of 20 and 68 nm (R20 and R68). Cells were exposed for various lengths of time (4, 24 and 48 h), and cytotoxicity, oxidative stress and genotoxicity were evaluated using a set of complementary techniques (MTT and clonogenic assays for cytotoxicity, Comet and micronuclei assays and γ-H2AX immunostaining for genotoxicity, and 8-OH-dG analysis, titration of intracellular ROS, glutathione content, antioxidant enzyme activities for oxidative stress). Mild cytotoxicity was observed after 48 h treatment with nanoparticles (A12, A25, R20 at 1–100 μg/mL), whereas no or borderline toxicity was elicited by R68 and A140. Increased intracellular ROS levels and genotoxicity were observed in the Comet assays with all particles after 4 h treatment (100 μg/mL), which decreased at later times. At the same dose, increased 8-OH-dG levels were observed in cells treated with A12, A25, A68 and R20, but not with A140. Negative results were obtained with all particles in micronucleus and γ-H2AX assays (50, 100 and 200 μg/mL). In conclusion, this work showed that TiO_2 particles with different sizes and crystalline phases could elicit oxidative stress and induce the formation of transient DNA lesions detectable by Comet assay – but not with the γ-H2AX immunostaining specific for DNA double-strand breaks – which did not result in clastogenic or aneugenic events visualised as micronuclei.

The lung adenocarcinoma epithelial cell line A549 was also used by Toyooka et al. (2012) in an *in vitro* study on the genotoxicity of TiO_2 anatase microparticles (diameter 5000 nm) and nanoparticles (diameter 5 nm). Genotoxicity elicited by treatments (1 100 μg/mL) was evaluated based on the

phosphorylation of the histone H2AX (γ-H2AX). Both TiO_2 particles generated γ-H2AX foci, which was more remarkable with the smaller particles. The flow cytometric analysis showed that γ-H2AX generation was independent of cell-cycle phase, and cells that incorporated larger amounts of TiO_2 particles had more γ-H2AX foci. Low levels of intracellular ROS were detected, even if large amounts of TiO_2 particles were taken up. By contrast, the generation of γ-H2AX was attenuated by coating the surface of TiO_2 particles with bovine serum albumin. According to the study authors, these results suggested that smaller TiO_2 particles were easy to incorporate into cells and generated cell-cycle phase-independent γ-H2AX, which was dependent on the condition of the TiO_2 surface, but not on the formation of ROS.

TiO_2 nanoparticles induced cytotoxicity and DNA damage in human amnion epithelial (WISH) cells, as investigated by Saquib et al. (2012). Crystalline, polyhedral rutile TiO_2 nanoparticles (diameter 30 nm) were characterised using X-ray diffraction, UV–visible spectroscopy, Fourier transform infrared spectroscopy and TEM analyses. The Neutral Red uptake and MTT assays revealed a concentration-dependent cytotoxic effect of TiO_2 nanoparticles over a concentration range of 0.625–10 μg/mL. Cells exposed to TiO_2 nanoparticles (10 μg/mL) exhibited a significant reduction (46.3% and 34.6%; $p < 0.05$) in catalase activity and glutathione level, respectively. Treated cells showed a 1.87-fold increase in intracellular ROS generation and a 7.3% ($p < 0.01$) increase in G_2/M cell-cycle arrest compared with the untreated control. Cells treated with TiO_2 nanoparticles also demonstrated the formation of DNA double-strand breaks with a 14.6-fold ($p < 0.05$) increase in the Olive tail moment value at 20 μg/mL concentration (highest dose tested), under neutral Comet assay conditions.

Woodruff et al. (2012) assessed the genotoxicity of 10 nm uncoated sphere TiO_2 nanoparticles with an anatase crystalline structure using the *Salmonella* reverse mutation assay (Ames test) and the single-cell gel electrophoresis (Comet) assay in TK6 cells. For the Ames test, *Salmonella* strains TA102, TA100, TA1537, TA98 and TA1535 were preincubated with eight different concentrations of TiO_2 nanoparticles for 4 h at 37°C, ranging from 0 to 4,915.2 μg per plate. No mutation induction was found. TEM and energy-dispersive X-ray spectroscopy analyses showed that the TiO_2 nanoparticles were not able to enter the bacterial cell. For the Comet assay, TK6 cells were treated with 0–200 μg/mL TiO_2 nanoparticles for 24 h at 37°C. Although the TK6 cells did take up TiO_2 nanoparticles, no significant induction of DNA breakage or oxidative DNA damage was observed in treated cells using the standard alkaline Comet assay and the endonuclease III and human 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified Comet assay, respectively.

Guichard et al. (2012) studied the *in vitro* cytotoxicity and genotoxicity of commercially available nanosized and microsized anatase TiO_2 and rutile TiO_2 in SHE cells. Samples had the following characteristics: anatase, 14 ± 4 nm; anatase, 160 ± 48 nm; rutile, 62 ± 24 nm; and rutile, 530 ± 216 nm. The particle concentrations in the different tests varied between 0.5 and 200 μg/cm^2. In acellular assays, TiO_2 particles were able to generate ROS. At the same mass dose, all nanoparticles produced higher levels of ROS than their microsized counterparts. Measurement of particle size in the SHE culture medium showed that primary nanoparticles and microparticles are present in the form of micrometric agglomerates of highly polydispersed size. Uptake of primary particles and agglomerates by SHE exposed for 24 h was observed for all samples. TiO_2 samples were found to be cytotoxic, anatase TiO_2 and rutile TiO_2 nanoparticles being found to induce higher cytotoxicity than their microparticle counterparts after 72 h of exposure. Over this treatment time, anatase TiO_2 nanoparticles also produced more intracellular ROS compared with the microparticles. However, similar levels of DNA damage were observed in the Comet assay after 24 h of exposure to anatase nanoparticles and microparticles. Rutile microparticles were found to induce more DNA damage than the nanoparticles. None of the samples tested showed significant induction of micronuclei formation after 24 h of exposure. In agreement with previous size-comparison studies, the authors suggested that *in vitro* cytotoxicity and genotoxicity induced by metal oxide nanoparticles are not always higher than those induced by their bulk counterparts.

Magdolenova et al. (2012) investigated the effect of dispersion on the cytotoxicity and genotoxicity of TiO_2 nanoparticles (rutile/anatase; particle size, 15–60 nm). Two protocols giving TiO_2 nanoparticle dispersions with different stability and agglomeration states were assessed: TK6 human lymphoblast cells, EUE human embryonic epithelial cells and Cos-1 monkey kidney fibroblasts were used to assess cytotoxicity (by Trypan Blue exclusion, proliferation activity and plating efficiency assays) and genotoxicity (Comet assay). DNA strand breaks were detected by the alkaline Comet assay. DNA oxidation lesions (especially 8-oxo-7,8-dihydroguanine) were measured using a modified Comet assay including incubation with the specific repair enzyme formamidopyrimidine DNA glycosylase. TiO_2 nanoparticle dispersion with large agglomerates (3-min sonication and no serum in stock solution)

induced DNA damage in all three cell lines, whereas TiO_2 nanoparticles dispersed with agglomerates < 200 nm (fetal serum in stock solution and sonication for 15 min) had no effect on the genotoxicity. An increased level of DNA oxidation lesions detected in Cos-1 and TK6 cells indicated that the leading mechanism by which TiO_2 nanoparticles trigger genotoxicity was most likely oxidative stress. The results showed that the dispersion method used could influence the results of toxicity studies. Therefore, according to the authors, at least two different dispersion procedures should be incorporated into assessment of cyto- and genotoxic effects of nanoparticles.

Demir et al. (2013a) evaluated the genotoxic activity of TiO_2 nanoparticles (anatase; spherical shape with average diameter 2.3 nm) in human peripheral blood lymphocytes and cultured human embryonic kidney (HEK293) cells by means of a modified alkaline Comet assay with/without formamidopyrimidine DNA glycosylase and endonuclease III in order to detect also oxidised DNA bases. Both human peripheral blood lymphocytes and cultured embryonic kidney cells were incubated with TiO_2 nanoparticles at concentrations of 1, 10, or 100 μg/mL. In both cell types, a significant induction in DNA damage (similar with/without endonuclease III and formamidopyrimidine DNA glycosylase) was only observed at the highest concentration of 100 μg/mL. The ionic form of TiO_2 was completely inactive.

The same author (Demir et al., 2013b) reported the results of a study with TiO_2 nanoparticles (anatase; mean diameter 2.3 nm) and microparticles in the wing somatic mutation and recombination assay in *Drosophila melanogaster*. Larvae were fed TiO_2 particles at concentrations ranging from 0.1 to 10 mM. The results obtained did not show any significant increases in the frequency of wing spots, indicating that exposure to TiO_2 nanoparticles by feeding was unable to elicit genotoxicity detectable by the wing spot assay of *D. melanogaster*.

The influence of medium composition on the physicochemical characteristics and genotoxicity of TiO_2 nanoparticles (86% anatase, 14% rutile; size 27.5 nm) was assessed in a study by Prasad et al. (2013). In this work, the influence of TiO_2 nanoparticle agglomeration, cellular interaction and cell-cycle stage on the induction of genotoxicity was evaluated in human lung epithelial cells using three different nanoparticle-treatment media: keratinocyte growth medium (KGM) plus 0.1% bovine serum albumin (KB); a synthetic broncheoalveolar lavage fluid containing phosphate-buffered saline, 0.6% bovine serum albumin and 0.001% surfactant (DM); or KGM with 10% fetal bovine serum (KF). The Comet assay showed that TiO_2 nanoparticles (10–100 μg/mL) induced similar amounts of DNA damage in all three media, independent of the amount of agglomeration, cellular interaction or cell-cycle changes. By contrast, TiO_2 nanoparticles induced micronuclei only in KF, which is the medium that facilitated the lowest amount of agglomeration, the greatest amount of nanoparticle cellular interaction, and the highest population of cells accumulating in the S phase.

Setyawati et al. (2013) investigated the potential cytotoxicity and genotoxicity of TiO_2 nanoparticles (73–85% anatase; diameter 22 nm) in the human skin fibroblast cell line (BJ). The nanoparticles were first characterised by size, morphology and surface charge, and cytotoxicity was evaluated by monitoring the proliferation of treated BJ cells. Genotoxicity was evaluated based on the induction of phosphorylation of histone H2AX, a cellular marker of DNA double-strand break recognition and repair. TiO_2 nanoparticles induced dose-dependent cytotoxicity (dose range 10–1,000 μg/mL) and genotoxicity (at both 10 and 500 μg/mL, the two doses assayed) in this test system.

Shukla et al. (2013) evaluated the genotoxicity of TiO_2 nanoparticles (anatase; size range 30–70 nm) in the human liver cell line HepG2. Treatment with TiO_2 nanoparticles induced significant ($p < 0.05$) DNA damage in Comet assay at 10 μg/mL and above, with a possible increase in oxidative (formamidopyrimidine DNA glycosylase-dependent) damage even at the lowest dose of 1 μg/mL. Increased micronucleus frequency was observed at 20 μg/mL. The genotoxicity observed was attributed by the study authors to the generation of ROS, with concomitant reduced glutathione levels and increase in lipid peroxidation. Increased expression of p53, BAX, Cyto-c, Apaf-1, caspase 9 and caspase 3, and a decreased level of Bcl-2 were also observed by immunoblotting, indicating that TiO_2-induced apoptosis occurs via the caspase-dependent pathway.

Srivastava et al. (2013) evaluated apoptosis, oxidative stress and genotoxicity induced by TiO_2 particles (< 25 nm) in the human lung cancer cell line A549. Tetrazolium bromide salt and lactate dehydrogenase release assays were used to measure cytotoxicity. Genotoxicity was evaluated by the cytokinesis block micronucleus assay and apoptosis was assessed by the formation of apoptotic bodies and altered expression of p53, p21, Bax, Bcl-2 and cleaved caspase 3. Cells exposed to TiO_2 particles (10 and 50 μg/mL) for 6–24 h showed dose-related induction of cytotoxicity, oxidative stress (as shown by increase intracellular ROS and lipid peroxidation, and decrease catalase and glutathione activity), apoptotic bodies (up to twofold) and micronuclei (up to threefold).

Tavares et al. (2014) evaluated the genotoxicity of a set of TiO_2 nanoparticles in human lymphocytes using the cytokinesis-blocked micronucleus assay. Four TiO_2 nanoparticles were assessed: NM-102 (anatase; size 28 nm), NM-103 (rutile; size 22 nm), NM-104 (rutile; size 19 nm) and NM-105 (85% anatase, 15% rutile; size 20 nm. The morphology and size of the nanoparticles were characterised using TEM, whereas the hydrodynamic particle size distributions were determined by DLS. Particles were dispersed using a standardised procedure and applied up to the limit allowed by the dispersibility in the vehicle (0.5% ethanol and bovine serum albumin in water), corresponding to a final concentration of 250 μg/mL. Additional lower doses of 125, 45, 15 and 5 μg/mL were tested. Statistical comparison of the results showed weak (two- to threefold), but significantly increased frequencies of micronuclei for NM-102 at a dose of 125 μg/mL, for NM-103 at 5 and 45 μg/mL, and for NM-104 at 15 and 45 μg/mL; no significant effect was observed for NM-105. None of the tested TiO_2 NMs induced a dose-dependent effect. Cell viability and cell-cycle progression, assessed by RI and cytokinesis-block proliferation indices were not affected by treatments. The study authors highlight as differential genotoxicity was observed for closely related NMs, indicating the need for investigating the toxic potential of each NM individually, instead of assuming a common mechanism and equal genotoxic effects for a set of similar NMs.

3.2.3.2. *In vivo*

Shelby et al. (1993), in a survey study, tested 49 chemicals in a mouse bone marrow micronucleus test via three daily exposures by intraperitoneal injection. TiO_2 (particle size not specified) was tested for its clastogenicity in an *in vivo* mouse bone marrow micronucleus test. B6C3F1 mice were administered, for three consecutive days, doses of 250, 500 and 1,000 mg TiO_2/kg bw on the first trial, and 500, 1,000 and 1,500 mg TiO_2/kg bw on the second trial. Mice were killed 24 h after the third injection. Micronuclei were analysed in bone marrow and peripheral blood erythrocytes in the first trial and in bone marrow erythrocytes in the second trial. The initial test was positive by trend analysis in the bone marrow cells at 1,000 mg TiO_2/kg bw, showing significantly elevated levels of micronuclei at this dose level. The repeat study was trend negative, as were results from scoring blood samples in the first trial. However, due to the elevated levels of micronucleated immature erythrocytes at 1,000 mg TiO_2/kg both in the peripheral blood samples and in the repeat bone marrow test, the overall results were considered positive. Trend analyses performed following decoding of slides and excluding the upper dose level from the repeat bone marrow study showed significant effects ($p = 0.002$) at 1,000 mg TiO_2/kg bw. However, although the available data showed significant increases and a linear trend, the effect is not marked and the highest mean value obtained for induction of micronuclei falls within historical range values for untreated controls, and therefore this result should be considered equivocal or of uncertain biological relevance.

In a further study, aiming to compare induction of chromosomal aberrations and micronuclei in the bone marrow of mice using 65 chemicals, Shelby and Witt (1995) also tested TiO_2 (particle size not specified). For the micronucleus test, B6C3F1 mice were administered TiO_2 for three consecutive days at doses of 250, 500 and 1,000 mg TiO_2/kg bw on the first trial, and 500, 1,000 and 1,500 mg TiO_2/kg bw on the second trial. Mice were killed 24 h after the third injection. In the bone marrow chromosomal aberration test, B6C3F1 mice were administered with TiO_2 once by intraperitoneal injection at doses of 625, 1,250 and 2,500 mg TiO_2/kg bw. Mice were killed at sampling times of 17 and 36 h. Animals received colchicine by intraperitoneal injection to accumulate cells in metaphase 2 h before sampling. For the 17 h sampling time, animals were subcutaneously implanted with 5-bromo-2′-deoxyuridine tablets (18 h before the scheduled sampling) to allow selection of first metaphase for scoring. In the first trial for the induction of micronuclei, a significant trend was obtained with the effect significantly elevated at the highest dose. In the second trial for the induction of micronuclei, effects of a similar magnitude were observed, a single-dose level group (1,000 mg TiO_2/kg) was significantly elevated, and the trend test was significant when the high-dose level group was excluded from analysis. Results on chromosomal aberrations were clearly negative at both sampling times.

The Panel noted that the data on micronuclei in bone marrow erythrocytes in the Shelby and Witt (1995) study are identical to the data presented in the earlier Shelby et al. (1993) study.

Trouiller et al. (2009) investigated the genotoxicity, oxidative DNA damage and inflammation of nano-TiO_2 in an *in vivo* study in male and female mice (C57Bl/6Jp^{un}/p^{un}). The test material was a mixture of 75% anatase and 25% rutile TiO_2 with a primary particle size of 21 nm and a mean, agglomerated, particle size of 160 nm. Groups of five male mice were dosed for 5 days with drinking water supplemented with 60, 120, 300 and 300 μg TiO_2/mL, corresponding to 0, 50, 100, 250 and 500 mg TiO_2/kg bw per day. Pregnant dams were dosed in drinking water with 500 mg TiO_2/kg bw

per day for 10 days at gestation days from 8.5 to 18.5 post coitum. In males, a marginal increase of tail moment in peripheral blood cells (~ 0.010 vs 0.013 μm as average, from the graphical representation of data), and a twofold increase in micronuclei in peripheral blood normochromatic erythrocytes, were observed in mice treated with the highest dose tested (500 mg/kg bw per day). At this dose, a slight but significant increase of oxidative DNA damage (8-hydroxy-2'-deoxyguanosine levels) was observed in the liver (~ 4.2 vs 6.4 8-OH-dG/10^6 dG), and the increased expression of proinflammatory cytokine in peripheral blood. A dose-related increase in γ-H2AX positive cells (i.e. with more than four foci) was observed at all tested doses in bone marrow. *In utero* exposure of fetuses via the mothers (five animals per group) was associated with a slight increase in large deletions in offspring (6.42 ± 1.47 vs 8.13 ± 1.70 eyespots in the offspring of control and treated mice, respectively). The authors concluded that TiO_2 nanoparticles were genotoxic and clastogenic *in vivo* in mice, possibly as a consequence of a secondary mechanism associated with inflammation and/or oxidative stress.

The Panel noted, however, that in the above study, the methods implemented had some shortcomings and that therefore their reliability was limited because:

- For the micronucleus assay, the study protocol applied is not appropriate to detect micronuclei in mature (normochromatic) erythrocytes. Micronuclei in mature erythrocytes can be used as endpoint only when the treatment period exceeds the lifespan of erythrocytes, e.g. 4 weeks or more in the mouse (OECD TG474, 2014). In this work, a far shorter treatment period was applied (5 days), with no positive control to demonstrate the efficacy of treatment. Thus, the results reported were not considered a reliable indication of a treatment-related effect.
- The alkaline Comet assay performed in peripheral blood did not include the evaluation of cytotoxicity, which is mandatory in this assay (OECD TG489, 2014). Moreover, due to the exiguity of the difference between treated and control groups, the biological significance of the effect reported should be evaluated based on the distribution of historical control values, which were not available in this study.
- The assessment of genotoxicity in developing embryos was based on method developed in-house, which has not been validated.

Overall, the Panel concluded that this study cannot be used for risk assessment.

Sycheva et al. (2011) treated CBAB6F1 male mice by oral gavage with TiO_2 particles (anatase; microsized, 160 nm; nanosized, 33 nm) at doses of 40, 200 and 1000 mg/kg bw per day, for 7 days. Genotoxic effects were analysed by Comet assay in the cells of brain, liver and bone marrow of mice treated with 40 and 200 mg/kg bw, by the micronucleus assay in bone marrow, and in cells of forestomach, colon and testis with a poly-organ karyological assay (analysis of micronuclei, nuclear protrusions, atypical nuclei, multinucleated cells, mitotic and apoptotic index) in mice treated with 40, 200 and 1,000 mg/kg bw. In Comet assays, an increase of DNA damage was reported in bone marrow cells at both tested doses (40 and 200 mg/kg bw) with both micro- and nanosized TiO_2, and in liver at 200 mg/kg bw with nanoparticles only. An increase in micronuclei was observed in the bone marrow of mice administered 1,000 mg/kg microsized TiO_2, (the highest dose tested), but not with nanoparticles. This increase of less than twofold was considered statistically significant. In the karyological assay, micro- and nanosized TiO_2 increased the mitotic index in forestomach and colon epithelia, the frequency of spermatids with two and more nuclei, and apoptosis in forestomach (only nanosized TiO_2) and testis. According to the authors, this study demonstrated that micro- and nanosized TiO_2 were genotoxic *in vivo* in mice, possibly through an indirect genotoxic mechanism associated with inflammation and/or oxidative stress because no genotoxic effect was observed at the site of direct contact with the particles (forestomach, colon).

However, the Panel noted that some of the methods implemented had some shortcomings and that, therefore, their reliability was limited:

- The micronucleus assay was performed with a limited protocol, based on the analysis of 1,000 immature erythrocytes per animal instead of the 4,000 recommended (OECD 474, 2014); moreover, the statistical analysis of the experimental results, performed by the chi-square test, is incorrect because it does not consider the animal as a statistical unit, as recommended. Finally, the biological significance of the small and not dose-related relative increase in micronucleated cells in treated animals compared with controls should be evaluated based on the distribution of historical control values, which were not available in this study.

- The 'poly-organ karyological assay' is not a validated assay for risk assessment. Moreover, the parameters evaluated, i.e. mitotic index, apoptosis and nuclear abnormalities of spermatids, are not adequate to evaluate genotoxicity.

Overall, the Panel concluded that this study cannot be used for risk assessment.

Sadiq et al. (2012) conducted *in vivo* micronucleus and Pig-A (phosphatidylinositol glycan, class A gene) mutation assays to evaluate the genotoxicity of TiO_2 nanoparticles (anatase; 10 nm) in mice. Groups of five, 6–7-week-old male B6C3F1 mice were treated intravenously for three consecutive days with 0.5, 5.0 and 50 mg TiO_2/kg bw for the two assays. Mouse blood was sampled 1 day before the treatment and on day 4, and weeks 1, 2, 4 and 6 after the beginning of the treatment. Pig-A mutant frequencies were determined at day 1 and weeks 1, 2, 4 and 6, whereas per cent micronucleated reticulocyte frequencies were measured on Day 4 by flow cytometry in 2×10^{e4} CD71-positive reticulocytes/animal. Additional animals were treated intravenously with three daily doses of 50 mg TiO_2/kg bw for the measurement of titanium levels in bone marrow 4, 24 and 48 h after the last treatment. The measurement indicated that the accumulation of nanoparticles reached a peak in the tissue 4 h after the administration, and the levels were maintained for a few days. No increase in either Pig-A mutant frequency or the frequency of per cent micronucleated reticulocytes was detected, although the per cent micronucleated reticulocytes were reduced in the treated animals on day 4 in a dose-dependent manner indicating cytotoxicity of TiO_2 nanoparticles in the bone marrow. A marked positive response was elicited in both the Pig-A and micronucleus assays by the positive control substance ethylnitrosourea. These results suggest that although TiO_2 nanoparticles can reach the mouse bone marrow inducing measurable cytotoxicity, no genotoxic effect detectable by the micronucleus or Pig-A gene mutation assays is elicited.

Xu et al. (2013) reported negative results in a bone marrow micronucleus test on ICR mice administered intravenously with TiO_2 nanoparticles (0, 140, 300, 645 and 1,387 mg/kg bw) 14 days before sacrifice (Xu et al., 2013). However, the Panel noted that the sampling time applied in this study (14 days after treatment) is not appropriate for the test method applied, and considered this study not relevant for risk assessment.

In another recent *in vivo* study (Louro et al., 2014), transgenic C57B1/6 mice harbouring a plasmid containing the bacterial *lacZ* reporter gene were exposed to TiO_2 nanoparticles (anatase; average diameter 22 nm) with two daily intravenous injections at 10 and 15 mg/kg bw. Top dose was the maximum achievable based on concentration of stable nanoparticle dispersion and the administered volume. Micronuclei in reticulocytes were scored in blood smears prepared 42 h after last treatment; gene mutations in *lacZ* and DNA strand breaks (by Comet assay) were assessed in liver and spleen 28 days after treatment. No genotoxic effect was detected, although TEM and light microscopy highlighted the accumulation of nanoparticles and a mild inflammatory response in liver at the time of sacrifice. A marked positive response was elicited in both the Pig-A and micronucleus assays by the positive control substance ethylnitrosourea.

Chen et al. (2014) administered TiO_2 nanoparticles (anatase; 75 + 15 nm) intragastrically to Sprague Dawley rats at 0, 10, 50 and 200 mg/kg bw every day for 30 days. DNA damage in bone marrow was evaluated by the micronucleus assay and immunofluorescence detection of histone H2AX phosphorylation. In the same study, the genotoxicity of TiO_2 nanoparticles was assessed with *in vitro* Comet and gene mutation (*hprt* locus) assays in V79 cells treated at 0, 5, 10, 20, 50 and 100 μg/mL. A significant and dose-related increase in γ-H2AX foci in bone marrow cells was observed at the end of treatment, with no concurrent increase in micronuclei in polychromatic erythrocytes (PCE), or deviation in the polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) ratio. *In vitro*, TiO_2 nanoparticles induced a slight increase in tail moment after 24 h treatment with the highest dose, and a significant and dose-related increase of *hprt* gene mutations.

Dobrzynska et al. (2014) injected male Wistar rats intravenously with 5 mg/kg bw TiO_2 nanoparticles (anatase/rutile powder, average size 21 nm). Animals were killed either 24 h, 1 or 4 weeks later, and genotoxicity was evaluated in bone marrow cells by Comet and micronucleus assays. No genotoxicity was detected in bone marrow leukocytes by Comet assays at any sampling time. A significant (threefold) increase in micronucleated polychromatic erythrocytes stained with the conventional May–Grunwald and Giemsa stains was observed at the first sampling time (i.e. 24 h after treatment), but not at later times. However, the Panel noted that the authors also reported no increase in the number of micronuclei in bone marrow reticulocytes stained with Acridine Orange. Because both PCEs and reticulocytes represent the same cell type, i.e. immature erythrocytes detected with different staining procedures, this raises doubts about the biological significance of the positive result reported. Overall, the Panel concluded that this study cannot be used for risk assessment.

El-Ghor et al. (2014) exposed male Swiss Webster mice to nanosized TiO_2 (rutile and anatase; size 45 nm) by intraperitoneal injection once a day for 5 days at 500, 1,000 and 2,000 mg/kg bw. Animals were killed 24 h after last treatment and the genotoxic effect of treatment evaluated by the micronuclei assay in bone marrow PCEs, by Comet assays in bone marrow, brain and liver, and by the single-strand conformation polymorphisms analysis in p53 exons 5 8 (as a surrogate of gene mutation). Moreover, the oxidative stress induced by TiO_2 administration was evaluated by measuring hepatic malondialdehyde level and glutathione, superoxide dismutase, catalase and glutathione peroxidase levels. The results showed a highly significant ($p < 0.001$) and dose-dependent increase in micronuclei in PCEs and Comet parameters (tail length, % DNA and tail moment) in bone marrow, brain and liver cells, and an increased frequency of mutations in p53 exons in brain and liver of treated animals. TiO_2 treatment also resulted in significantly increased ($p < 0.001$) liver malondialdehyde and significantly decreased ($p < 0.001$) hepatic glutathione, superoxide dismutase, catalase and glutathione peroxidase. Coadministration with chlorophyllin (40 mg/kg bw per day) effectively suppressed both oxidative stress and genotoxicity biomarkers, indicating a mechanistic link between ROS generation and TiO_2-induced genotoxicity. The Panel noted that for the micronucleus test, a distinct genotoxic activity of nanosized TiO_2, even greater than the concurrent positive control cyclophosphamide at 25 mg/kg, is described in this paper. No comparable effect has been observed in any other *in vivo* micronucleus test, including those performed by intravenous administration. For the Comet assay, highly significant and dose-dependent increases in tail length, % DNA and tail moment were obtained in the absence of adequate measurements of cytotoxicity, and organ collection was performed 24 h from the last administration and not at 2 6 h as recommended by the relevant OECD Guideline No. 489, which strongly limit the reliability of the test. Furthermore, the screening of mutations in exons 5–8 of the p53 gene is not considered an actual genotoxicity test and has not received adequate validation. Overall, the Panel concluded that the reliability of this study is limited. The Panel also noted that the intraperitoneal route of administration applied in this study is not recommended by OECD guidelines, as non-physiological, and that study results obtained with this route have no relevance for oral risk assessment.

Donner et al. (2016) evaluated three pigment grades (size range 153–213 nm) and three nanoscale (size range 43–47 nm) TiO_2 particle samples (both anatase and/or rutile) in an *in vivo* micronucleus test performed in compliance with OECD Guideline No. 474 (2014) and Good laboratory Practice (GLP). The materials were administered to groups of five male and female rats once by gavage at the doses of 0, 500, 1,000 or 2,000 mg/kg bw. Concurrent control groups received water (vehicle) or cyclophosphamide (positive control). The effect of treatment on micronucleus induction in bone marrow was evaluated by analysing 20,000 peripheral blood reticulocytes by flow cytometry at ~ 48 and 72 h after treatment. No increases in the frequency of micronucleated reticulocytes, and no reduction in the ratio of reticulocytes to total erythrocytes (indicative of cytotoxicity to bone marrow) was detected in rats administered TiO_2. According to the authors, no increase in titanium content was detected by inductively coupled plasma mass spectrometry in blood and liver of rats treated with the highest dose of both nano- and pigment-grade TiO_2 (one sample of each). The Panel noted that the very low intestinal absorption of TiO_2 is consistent with the lack of systemic genotoxicity reported in this study.

Mohamed (2015) investigated the toxic and genotoxic effects of TiO_2 nanoparticles (77% rutile, 22% anatase; average size 46 nm) on the gastric mucosa of orally treated male mice. Five animals per experimental group were orally administered 0, 5, 50 or 500 mg/kg bw TiO_2 nanoparticles in distilled water for five consecutive days and killed 24 h, 1 or 2 weeks after the last treatment. No positive control group was included in the study.

The author reported that the titanium content in gastric cells (measured by inductively coupled plasma mass spectrometry) showed a dose-dependent increase and remained stable over 2 weeks. Treatments caused a remarkable local cytotoxic effect at all dose levels. The histopathological examination revealed, already at the low dosage of 5 mg/kg bw, submucosal oedema after 24 h that developed to ulcerations and mucosal necrosis after 1 and 2 weeks, respectively. The severity of the effects reported in the two other treatment groups (50 or 500 mg/kg bw) was even higher. Several indicators of oxidative stress, as well as of apoptosis (analysed by the colorimetric diphenylamine assay and by laddered DNA fragmentation assay) and DNA damage (measured by comet assay) of gastric cells were found to be increased in a dose- and time-dependent manner.

The Panel noted that the toxic findings reported in this study are clearly in conflict with the results reported by the US NCI carcinogenicity study (NCI, 1979), in which male mice receiving up to 6,500 mg TiO_2/kg bw per day (anatase; particle size not specified; purity 98%) for 103 consecutive weeks did not show at histopathological examination any alteration in a wide range of organs, including stomach.

Moreover, the Panel noted that the reported relatively high and constant concentration of TiO_2 in gastric cells is not consistent with the high turnover of gastric epithelium. Concerning the genotoxicity findings, the Panel noted that the reported DNA fragmentation was observed in conditions associated with evident cytotoxicity, and as such cannot be taken as an evidence of genotoxicity. The secondary origin of DNA damage is also supported by its relative increase with longer intervals after last treatment, which parallels the exacerbation of local toxicity. As to the other genotoxicity results, the Panel noted that the modest increase in single-strand conformation polymorphism of the p53 exons 3 and 8 cannot be taken as an evidence of mutagenicity without confirmatory sequencing data.

Overall, due to these remarkable uncertainties, the Panel concluded that this work should not be considered for risk assessment.

In addition to the above, a few *in vivo* studies were performed using inhalational or intratracheal routes of administration. The Panel noted that such studies, especially when assessing genotoxicity at site of direct contact with nanoparticles, have limited relevance for the safety assessment of oral exposure to TiO_2.

Driscoll et al. (1997) evaluated the role of pulmonary inflammation in driving mutagenesis in rat lungs after *in vivo* instillation of different particles. These included a fine anatase TiO_2 sample (180 nm median diameter, 8.8 m^2/g). Mutagenicity was studied by *hprt*-analysis of lung epithelial cells isolated from the lungs of female SPF F334 Fischer rats, 15 months after intratracheal instillation of particles at 10 or 100 mg/kg. Enhanced *hprt*-mutagenesis was observed with 100 mg/kg, the dose that also elicited persistent lung inflammation, but not with the 10 mg/kg dose. The inflammatory cells obtained by broncheoalveolar lavage from the particle-treated animals were found to induce *hprt*-mutagenesis in a rat lung epithelia cell line *in vitro*.

Rehn et al. (2003) also investigated oxidative DNA damage induction by two samples of TiO_2 in rat lungs after intratracheal instillation at dosages of 0, 0.15, 0.3, 0.6 and 1.2 mg/kg bw per day. The samples used were an untreated TiO_2 and a trimethoxyoctylsilane-treated TiO_2 sample, both ~ 20 nm. Oxidative damage induction was determined after 90 days by immunohistochemical analysis of lung sections using an 8-oxoguanine antibody. Enhanced oxidative DNA damage was not observed with the untreated or silanised TiO_2 nanoparticles. Analysis of markers of pulmonary inflammation and toxicity at 3, 21 and 90 days indicated only mild inflammatory effects.

Lindberg et al. (2012) examined whether inhalation of freshly generated nanosized TiO_2 (74% anatase, 26% brookite; 5 days, 4 h/day) at 0.8, 7.2 and 28.5 mg/m^3 (the highest concentration allowing stable aerosol production) could induce genotoxic effects in C57BL/6J mice locally in the lungs or systematically in peripheral PCEs. DNA damage was assessed by the Comet assay in lung epithelial alveolar type II and Clara cells sampled immediately following the exposure. Micronuclei were analysed by Acridine Orange staining in blood PCEs collected 48 h after the last exposure. A dose-dependent deposition of titanium in lung tissue was seen. Although the highest exposure level produced a clear increase in neutrophils in BAL fluid, indicating an inflammatory effect, no significant effect on the level of DNA damage in lung epithelial cells or micronuclei in PCEs was observed, suggesting no genotoxic effects by the 5-day inhalation exposure to nanosized TiO_2 anatase.

In the work by Saber et al. (2012), DNA-damaging activity and inflammogenicity (pulmonary cell composition and mRNAs) were determined in mice 24 h after intratracheal instillation of a single dose (54 μg) of three TiO_2-based particles (two coated rutile, size 288 and 20 nm; one uncoated anatase; size 12 nm). The coated TiO_2 induced DNA damage, as detected by Comet assay, in lung lining fluid cells. The uncoated TiO_2 was not DNA damaging by the same assay 24 h after exposure despite being highly inflammogenic, suggesting that inflammation is not a prerequisite for the induction of DNA damage in lung cells by TiO_2-based products.

Naya et al. (2012) evaluated the *in vivo* genotoxicity of anatase TiO_2 nanoparticles using the Comet assay after a single or repeated intratracheal instillation in Sprague Dawley rats. The nanoparticles were instilled at a dosage of 1 or 5 mg/kg bw (single instillation group) and 0.2 or 1 mg/kg bw once a week for 5 weeks (repeated instillation group). Macrophages and neutrophils were detected at sacrifice in the alveolus of the lung in the 1 and 5 mg/kg TiO_2 groups. In the Comet assay, there was no increase in % tail DNA in any of the TiO_2 groups.

Summary of genotoxicity data

In summary, numerous genotoxicity studies with TiO_2 particles of different specifications are available in the literature. The overall results obtained with particles of different size can be summarised as follows:

Microsized TiO_2 particles – in vitro *and* in vivo

A set of *in vitro* and *in vivo* studies, coordinated by the NTP, was performed with a TiO_2 anatase (Unitane® 0-220) with undefined particle size distribution. This material was not genotoxic in gene mutation tests in bacteria and in mammalian cells, in cytogenetic assays *in vitro* (chromosomal aberrations and SCE) (Dunkel et al., 1985; Tennant et al., 1987; Ivett et al., 1989; Myhr and Caspary, 1991) and *in vivo* (micronuclei and chromosomal aberrations in mouse bone marrow by intraperitoneal) (Shelby et al., 1993; Shelby and Witt, 1995). The Panel noted that the same material was non-carcinogenic in the NCI mouse and rat bioassays.

Microsized TiO_2, with a defined size > 100 nm or designed as 'fine rutile or anatase' also produced mixed results in genotoxicity tests *in vitro*: negative in Comet assays in CHL cells (both anatase and rutile, 255 nm, Nakagawa et al., 1997), chromosomal aberrations in CHO cells (anatase; 140 nm, Warheit et al., 2007), micronuclei in SHE cells and in human bronchial epithelial (BEAS-2B) cells (fine particles, Rahman et al., 2002; Falck et al., 2009), micronuclei and H2AX phosphorylation in human lung adenocarcinoma A549 cells (anatase, 140 nm, Jugan et al., 2012).

Conversely, positive results were reported by other authors in Comet assays with A549 cells (anatase, 140 nm, Jugan et al., 2012; fine TiO_2, 1 μm size, Karlsson,2009), BEAS-2B cells (fine rutile; Falck et al., 2009), SHE cells (anatase 160 nm and rutile 530 nm, Guichard et al., 2012), and for H2AX phosphorylation in A549 cells (anatase, 5 μm, Toyooka et al., 2012).

Nanosized TiO_2 particles – in vitro

Both positive and negative results have been reported in the numerous *in vitro* investigations on the genotoxicity of TiO_2 nanoparticles in a variety of experimental systems. As for microsized TiO_2, the crystalline phase and nanoparticle size do not seem to be important determinants of TiO_2 genotoxicity in experimental systems.

Anatase nanoparticles (with various diameters) were tested with negative results in Comet assays in rodent (Nakagawa et al., 1997; Wang et al., 2011) and human cells (Bhattacharya et al., 2009; Hackenberg et al., 2011; Jugan et al., 2012; Vales et al., 2015), gene mutation in rodent cells (Nakagawa et al., 1997; Wang et al., 2011), and micronuclei in rodent (Guichard et al., 2012) and human cells (Jugan et al., 2012; Vales et al., 2015).

However, positive results have been reported from a number of other studies covering also similar genetic endpoints, i.e. Comet assays in various cell types (Falck et al., 2009; Shukla et al., 2011, 2013; Guichard et al., 2012; Jugan et al., 2012; Magdolenova et al., 2012; Demir et al., 2013a; Prasad et al., 2013), micronucleus induction (Falck et al., 2009; Shukla et al., 2011, 2013; Prasad et al., 2013; Tavares et al., 2014) and H2AX phosphorylation (Toyooka et al., 2012; Setyawati et al., 2013).

A similar picture can be drawn for rutile nanoparticles, for which, however, fewer studies are available: negative in micronuclei tests in rodent (Landsiedel et al., 2010; Guichard et al., 2012) and human cells (Falck et al., 2009; Jugan et al., 2012), and in the γH2AX assay in A549 cells (Jugan et al., 2012); positive in Comet assays in rodent (Falck et al., 2009; Guichard et al., 2012) and human cells (Jugan et al., 2012), and in a micronuclei test with human lymphocytes (Tavares et al., 2014).

Additional positive results have been reported from studies with nanosized TiO_2 particles in an undefined crystalline phase. These consist of *in vitro* Comet, micronuclei, SCE and *hprt* assays with various cell lines (Rahman et al., 2002; Wang et al., 2007a; Karlsson et al., 2009; Di Virgilio et al., 2010; Osman et al., 2010; Magdolenova et al., 2012; Prasad et al., 2013; Srivastava et al., 2013).

Overall, the Panel noted that variable results have been obtained in genotoxicity tests *in vitro* with both nano- and microsized TiO_2. The observed discrepancies cannot be explained based on the crystalline phase or size of tested material, or on the specificity of the endpoint of the test system, but are more likely to be related to the variable experimental conditions applied, which greatly affect the aggregation status, availability and ensuing biological activity of particles (see Magdolenova et al., 2012).

Nanosized TiO_2 particles – in vivo

Fewer *in vivo* studies are available, with mixed results. Some evidence of genotoxicity in liver and bone marrow was reported following oral administration of both nano- and microsized TiO_2 particles (Trouiller et al., 2009; Sycheva et al., 2011). The Panel, however, noted a series of shortcomings in these studies, which cast doubts on the reliability of these results.

In another oral *in vivo* study, the intragastric administration of TiO_2 nanoparticles for 30 days to rats resulted in an increase in H2AX phosphorylated loci in bone marrow (an indication of double-strand break DNA repair), with no concurrent increase of chromosome breaks (micronuclei) (Chen et al., 2014).

Negative results were also obtained in a micronucleus assay on rat blood cells after administration by gavage of acute doses of both nano- and microsized TiO_2 particles (Donner et al., 2016).

Other *in vivo* studies have used other routes of exposure. Negative results in gene and chromosomal mutation tests were obtained in rats injected intravenously (Sadiq et al., 2012; Louro et al., 2014). A mild increase in micronuclei in bone marrow, with no concurrent DNA damage detectable by Comet assay, was reported in another recent intravenous study (Dobrzynska et al., 2014), but the Panel noted some inconsistencies in these results which are regarded of questionable biological significance.

Recently, the repeated intraperitoneal administration of TiO_2 nanoparticles has been reported to induce oxidative stress and genotoxicity in mice. The Panel noted that these results are not corroborated by any other *in vivo* study, by intraperitoneal or other routes, and concluded that these results should be considered with caution.

Unspecified particles size

Another commercial TiO_2, with unspecified particle size distribution, provided variable results in cytogenetic assays *in vitro*: positive in the micronucleus assay in human lymphocytes (Türkez and Geyikoğlu, 2007), either negative (Miller et al., 1995) or positive (Lu et al., 1998) in the micronuclei test in CHO cells, and positive in the SCE assay in CHO cells (Lu et al., 1998).

Conclusion on genotoxicity

The Panel concluded that the available mixed results provide some evidence of *in vitro* genotoxicity for TiO_2 micro- and nanoparticles. The Panel noted that most positive results have been reported under experimental conditions associated with the induction of oxidative stress (as shown by increased 8-OH-dG, lipid peroxidation and ROS generation), and that the genotoxic effects observed mainly concern indicator assays (comet and H2AX histone phosphorylation), which in some studies were shown not to be associated with permanent chromosome damage such as chromosome breaks visualised as micronuclei (Falck et al., 2009; Jugan et al., 2012).) In this respect, the Panel noted that the reliability of Comet assay for evaluating nanoparticle-induced genotoxicity has been questioned because of the possible secondary induction of DNA damage by nanoparticles during sample processing (Karlsson et al., 2015). Indeed, comparing the results obtained in intact cells and isolated nuclei, Ferraro et al. (2016) recently demonstrated that most DNA damage elicited by TiO_2 nanoparticles in human epithelial cells was produced during the assay performance (*ex post* damage) rather than during treatment (*ex ante* damage), through the direct interaction of cytoplasm-internalised nanoparticles with DNA in nucleoids.

In vivo, overall negative results have been obtained in genotoxicity studies with microsized TiO_2 pigment. Limited evidence of genotoxicity, if any, is provided by studies with orally administered TiO_2 nanoparticles. Limited or no indication of the genotoxicity of TiO_2 nanoparticles is provided by studies using an intravenous route of administration, which allows maximum exposure of target tissues.

Overall, the Panel concluded that the use of TiO_2 (E 171) as a food additive does not raise a concern with respect to genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

JECFA (1970) evaluation on TiO_2 reported a study by Lehmann and Herget (1927) in which two guinea pigs, two rabbits, two cats and one dog were fed technical-grade TiO_2 (assay of ≥ 99%) for 390 days. From the diets, the dog received 9 g/day (equivalent to 900 mg TiO_2/kg bw per day),[16] the rabbits received a total amount of 1170 g (equivalent to 1.5 g/kg bw per day),[16] the cats received 3 g/day (equivalent to 1.5 g TiO_2/kg bw per day)[16] and the guinea pigs received 0.6 g/day (equivalent to 800 mg/kg bw per day).[16] Two additional cats received 3 g TiO_2 daily for 175 and 300 days, respectively. No adverse effects were seen and histopathological examination revealed no abnormality. Less than 5 mg of titanium was detected in the bile, heart, spleen and skeletal muscle (no further information was available).

The US NCI (NCI, 1979) conducted a carcinogenicity study in groups of both Fischer 344 rats and B6C3F1 mice (50 animals/sex). These studies are summarised below.

3.2.4.1. Mice

Groups of B6C3F1 mice (50 animals/sex) were administered, in the diet, TiO_2 (anatase; particle size not specified, purity 98%) at doses of 0, 25,000 and 50,000 mg/kg diet (equivalent to 0, 3,250,

6,500 mg TiO_2/kg bw per day and 0, 4,175, 8,350 mg TiO_2/kg bw per day for male and female mice, respectively).[16] The study was conducted for 103 consecutive weeks and animals then observed for an additional week. All surviving animals were killed at week 104. A full histopathological evaluation was done and the following tissues were examined microscopically: brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons), pituitary, spinal cord (if neurological signs were present), eyes (if grossly abnormal), oesophagus, trachea, salivary glands, mandibular lymph node, thyroid, parathyroid, heart, thymus, lungs and main stem bronchi, liver, gallbladder, pancreas, spleen, kidney, adrenal, stomach, small intestine, colon, urinary bladder, prostate or uterus, testes or ovaries, sternebrae, femur, or vertebrae including marrow, mammary gland, tissue masses, and any gross lesion. At the end of the study, the test compound had not affected the survival rates of male mice; 80% of the high-dose males survived until the end of the 104-week study, compared with 64% survival in the controls. In female mice, there was a statistically significant dose-related trend for decreased survival ($p = 0.001$, Tarone test). It was reported that in female mice fed 50,000 mg TiO_2/kg diet (equivalent to 8,350 mg TiO_2/kg bw per day),[16] 66% survival was reported until the end of the 104-week study, in comparison with 90% survival in the controls. There was a slight increase in the incidence of hepatocellular carcinomas in high-dose male mice compared with controls, but this was not increased compared with historical control data. Tumour incidences in the dosed groups were not significantly higher than in controls. The study authors concluded that TiO_2 administered orally was not carcinogenic in B6C3F1 mice.

From this study, the Panel identified a NOAEL of 50,000 mg/kg diet, equivalent to 6,500 and 8,350 mg TiO_2/kg bw per day, for male and female mice, respectively, the highest doses tested.

3.2.4.2. Rats

Groups of Fischer 344 rats (50 animals/sex) were administered in the diet TiO_2 (anatase; particle size not specified, purity 98%) at doses of 0, 25,000 and 50,000 mg/kg diet (equivalent to 0, 1,125, 2,250 mg/kg bw per day and 0, 1,450, 2,900 mg/kg bw per day for male and female rats, respectively).[16] The study was conducted for 103 consecutive weeks and the animals were then observed for an additional week. All surviving animals were killed at week 104. A full histopathological evaluation was done and the following tissues were examined microscopically: brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons), pituitary, spinal cord (if neurological signs were present), eyes (if grossly abnormal), oesophagus, trachea, salivary glands, mandibular lymph node, thyroid, parathyroid, heart, thymus, lungs and main stem bronchi, liver, pancreas, spleen, kidney, adrenal, stomach, small intestine, colon, urinary bladder, prostate or uterus, testes or ovaries, sternebrae, femur, or vertebrae including marrow, mammary gland, tissue masses, and any gross lesion. At the end of the study, the test compound had not affected survival rates of male and female rats. Tumour incidences in the dosed groups were not significantly higher than in controls. The study authors concluded that TiO_2 administered orally was not carcinogenic in Fischer 344 rats.

From this study, the Panel identified a NOAEL of 50,000 mg/kg diet, equivalent to 2,250 and 2,900 mg TiO_2/kg bw per day, for male and female rats, respectively, the highest doses tested.

The US National Sanitation Foundation (NSF) International (2005) evaluated non-cancer oral toxicity data for TiO_2, and calculated an oral reference dose of 3 mg/kg per day based on the NCI study (1979) reported above, in which no adverse effects were observed in Fischer 344 rats or B6C3F1 mice fed TiO_2 for 2 years at concentrations up to 50,000 mg/kg. US NSF International applied a composite uncertainty factor of 1,000 (10 each for inter- and intraspecies extrapolation and for database deficiencies) to a NOAEL of 2,680 mg/kg bw per day in rats.

The IARC Monograph (IARC, 2010) concluded that: 'there was inadequate evidence from epidemiological studies to assess whether titanium dioxide causes cancer in humans', but that 'there is sufficient evidence in experimental animals for the carcinogenicity of titanium dioxide' and overall concluded that 'titanium dioxide is possibly carcinogenic to humans (Group 2B)'. However, this conclusion was based on an excess incidence of lung tumours in male and female rats in inhalation studies (Lee et al., 1985a,b, 1986; Trochimowicz et al., 1988; Heinrich et al., 1995; as cited in IARC, 2010). However, the same report noted that in other studies using different routes of administration, like oral, no excesses in tumour incidence were observed (IARC, 2010).

The Panel noted that there was one carcinogenicity study in rats and one in mice (NCI, 1979), performed with TiO_2 administered via the oral route, and that the outcome of this study was reported to be negative for both mice and rats. These negative findings are supported by negative results from earlier studies reported in the JECFA (1970) evaluation in which similar doses were tested in various animal species but for a shorter duration (~ 56 weeks).

Initiation and promotion studies

In a recent study by Urrutia-Ortega et al. (2016), the authors investigated the effects of intragastric administration of TiO_2 (E 171) in a chemically colitis-associated colorectal cancer (CAC) model in mice. Balb/c male mice (n = 24) were divided in the following 4 groups: (a) control; (b) 5 mg/kg bw food grade TiO_2 (E 171; 99% pure) by gavage, 5 days/week for 10 weeks; (c) the chemically colitis-associated cancer (CAC) group received a single i.p. dose of 12.5 mg/kg bw azoxymethane (AOM) and 2% dextran sulfate sodium (DSS) in the third, sixth and ninth week in water *ad libitum*; (d) the CAC + TiO_2 (E 171) group: AOM, DSS and TiO_2 (E 171). After 11 weeks, mice were necropsied and colon, kidneys, liver, spleen and lungs were collected. TiO_2 (E 171) in combination with the initiator increased the expression of markers of tumour progression including COX2, Ki67 and β-catenin. TiO_2 (E 171) alone did not show any enhancing effect on tumour markers. The Panel noted that further research is needed and that the study cannot be used for risk assessment of TiO_2 (E 171) as a food additive.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Reproduction toxicity studies

No reproductive (one- or two-generation toxicity) studies with TiO_2 (as the food additive, micro- or nanosized) performed according to the OECD guidelines were available for evaluation.

Jia et al. (2014) studied the effects of TiO_2 (crystal anatase; size 25 nm) in mice. Four-week-old male mice (n = 15/group) were daily administered by gavage with vehicle (phosphate-buffered saline with 0.5% Tween 80), or nano-TiO_2 solution at a dose of 10, 50 or 250 mg/kg bw for 42 days. There was a decrease in body weight gain in the 250 mg/kg bw group (only presented in a graph, body weight values not presented). Sperm abnormalities were increased in the mid- and high-dose groups (mean ~ 21 and 29 vs 13 in the control group). However, it should be noted that the number of abnormalities in the control group was also high. The figures were given for between six and nine animals. No differences in sperm counts were observed. Mean serum testosterone was decreased in all treated groups. The figures were given for between five and seven animals. Testes from the control and the 10 mg/kg groups showed no histopathological changes. Vacuoles were observed in the seminiferous tubules of mice treated with 50 and 250 mg TiO_2/kg bw per day. In the high-dose group, decreased layers of spermatogenic cells were observed. Two randomly selected animals per group were used for this examination and the number of abnormalities was not presented. Real-time quantitative polymerase chain reaction analysis (n = 3) and western blot analysis (n = 4–5) showed differences in the testis messenger RNA expression levels and protein expression levels of the 50 and/or 250 mg/kg bw groups. The results showed downregulation of CYP17 and 17βHSD and upregulation of CYP19 both in gene and protein expression, which may explain the found decreased testosterone levels (Jia et al., 2014).

The results of this study (Jia et al., 2014) pointed to an effect of nanosized TiO_2 on the reproductive system. However, it is not known whether the indicated effects are induced by the nanoparticles themselves or to the TiO_2. In addition, contradictory results on testosterone levels were reported by Tassinari et al. (2014) as described below. The Panel noted that, further research is needed and, this study cannot be used for risk assessment of TiO_2 (E 171) as a food additive.

Tassinari et al. (2014) (described in Section 3.1.2) investigated the possible reproductive and endocrine effects of short-term (5 days) oral exposure to anatase TiO_2 particles (0, 1, and 2 mg/kg bw per day) in Sprague–Dawley rats (n = 7/sex per group). Particles were characterised by SEM and TEM (average particle diameter 284 ± 43 nm, with 10% particles < 100 nm, 48% of particles between 100 and 300 nm, and 87% of particles between 30 and 900 nm). Most of the particles were agglomerates up to 1.6 μm in diameter. TEM analysis showed two different shapes for primary nanoparticles: spherules of 20–60 nm and irregular shapes of 40–60 nm. Their presence in spleen, a target organ for bioaccumulation, was investigated using single-particle inductively coupled plasma mass spectrometry and SEM/energy-dispersive X-ray analysis. Analyses included serum hormone levels (testosterone, 17β-oestradiol and triiodothyronine) and histopathology of thyroid, adrenals, ovary, uterus, testis and spleen. In addition, the spleen was examined by electron microscopy (SEM/energy-dispersive X-ray analysis) for the deposition of TiO_2 nanoparticles. In males from the 2 mg/kg bw per day group, feed intake was significantly decreased. Increased total titanium tissue levels were found in spleen and ovaries. Sex-related histological alterations were observed at both dose levels (i.e. 1 and 2 mg/kg bw per day) in thyroid, adrenal medulla, adrenal cortex (females) and ovarian granulosae, without general

toxicity. Altered thyroid function was indicated by reduced triiodothyronine (T3) (males). Testosterone levels increased in high-dose males and decreased in females. Estradiol levels were not affected by treatment. In the spleen of treated animals, TiO_2 aggregates and increased white pulp (high-dose females) were detected, even though titanium levels in tissue remained low, reflecting the low doses and short exposure time. The authors suggested that their results should prompt a comprehensive assessment of endocrine and reproductive effects of nanomaterials. The Panel agreed that further research is necessary preferentially following OECD guidelines considering the low levels of exposure (1 and 2 mg/kg bw/day) at which effects were reported in this study.

3.2.5.2. Developmental toxicity studies

Mohammadipour et al. (2014) exposed pregnant Wistar rats (n = 6) by gavage to 0 or 100 mg TiO_2 nanoparticles (particle size 10 nm, area > 150 m^2/g, purity 99%, suspended in distilled water) from gestation day 2 to gestation day 21. On post-natal day 1, pups were killed and brains were collected. The titanium content in the hippocampus of the pups in the test group was increased. In addition, reduced cell proliferation was observed in the hippocampus. On post-natal day 60, learning and memory was tested in 12 male pups per group and was found to be impaired in the test group. Although the results of the study point to effects on hippocampus and learning and memory, the Panel noted the limitations of the study such as small group size (only six females per group were used), only one dose level tested, and no information on the (random) selection of the pups. Therefore, according to the Panel, further research is needed before the results of this study can be used for risk assessment.

Warheit et al. (2015a) evaluated three pigment-grade (pg-1, pg-2 and pg-3) and three ultrafine (uf-1, uf-2 and uf-3)/nanoscale (anatase and/or rutile) TiO_2 particulates in prenatal developmental toxicity studies in pregnant rats, according to OECD TG 414. All six test particles contained > 95 wt % TiO_2. Primary particle sizes and surface were characterised as follows: pg-1, pg-2, pg-3 (d_{50} = 153–213 nm and Brunauer–Emmett–Teller = 50–82 m^2/g) and uf-1, uf-2, uf-3 (d_{50} = 43–47 nm and Brunauer–Emmett–Teller = 7–17 m^2/g). The test substances were formulated in sterile water. In three studies, time-mated pregnant Sprague–Dawley, Crl:CD(SD), rats (n = 22/group) were exposed to TiO_2 particulates (uf-1, uf-3 and pg-1) by oral gavage daily on gestation days 6–20. In three additional studies, pregnant Wistar rats (n = 22–23/group) were exposed to TiO_2 particulates (uf-2, pg-2 and pg-3) by oral gavage daily from gestation days 5–19. The dose levels used in the studies were 0, 100, 300 or 1,000 mg/kg bw per day. The dose volume was 5 mL/kg bw per day. Clinical signs were recorded at least daily. Body weight and feed intake were measured at regular intervals. Sprague–Dawley rats were killed for a caesarean section on gestation day 21 and Wistar rats on gestation day 20. Gross necropsy included gross examination of the dam, counting of the number of corpora lutea, implantation sites, resorptions, live and dead fetuses, fetal sex and weight. Fetal pathological external, visceral and skeletal examinations were performed in order to detect abnormalities. At 1,000 mg uf-1/kg per day, mean fetal sex ratio and the means for male and female fetuses per litter were statistically significantly different from the control group means. The mean number of male fetuses was 7.2 compared with 5.5 male fetuses for the concurrent control group; the test facility historical control group data ranges from 5.2 to 7.4. The mean number of female fetuses was 4.8 compared with 6.7 for the concurrent control group; the test facility historical control group data ranges from 5.8 to 8.3. Mean fetal sex ratio of the 1,000 mg uf-1/kg bw per day group was 60% (males/females) compared with a sex ratio of 46% in the concurrent control group; the test facility historical control group data ranges from 43% to 53%. Apart from some incidental changes in body weight and feed intake, no other changes were observed in the dams or the fetuses in these studies. The authors concluded that there were no significant toxicological or developmental effects in females or fetuses at any of the dose levels or compounds tested, and considered the NOAEL for each compound to be 1,000 mg/kg bw per day. The Panel agreed with this conclusion.

Overall, the Panel noted that prenatal developmental studies with three pigment-grade (pg-1, pg-2 and pg-3) and three ultrafine (uf-1, uf-2 and uf-3)/nanoscale (anatase and/or rutile) TiO_2 particulates performed according to the OECD guidelines (TG 414) did not give concern for maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg bw per day). However, the Panel noted that reproductive toxicity studies performed according to the OECD guidelines using TiO_2, meeting the food additive specifications were not available. Furthermore, the Panel noted that results from other reproductive and developmental studies with titanium nanoparticles (Jia et al., 2014 and Tassinari et al., 2014) showed contradictory results in the change in hormone levels. Because of

deficiencies in the study designs and inadequate data reporting, the Panel considered that the relevance of these findings is currently uncertain for the risk assessment of TiO_2 as a food additive.

3.2.6. Hypersensitivity, allergenicity, intolerance

Numerous studies are available on the effects of TiO_2 nanoparticles on the immune system. Some have been reviewed recently (Smith et al., 2014; Lappas, 2015; Luo et al., 2015).

3.2.6.1. Immunotoxicity

In vitro

Nuuja et al. (1982) investigated the effects of six different TiO_2 pigments (particle sizes not given) on the phagocytic capacity of mouse peritoneal macrophages. Male NMRI mice (4–6 weeks old) were given a single intraperitoneal injection TiO_2 (called TiO_2 pigments by the authors) in 1 mL of 0.9% aqueous NaCl solution. Compared with controls, the phagocytotic activity of mouse peritoneal cells treated with TiO_2 (98%) was reported to increase by < 10% within 2 days after intraperitoneal administration, but in a second set of experiments, the increase was up to 30% at days 7 and 15.

Kumazawa et al. (2002) studied the effect of soluble and particulate titanium (particle sizes 1–3 and 10 μm, 99.9% pure) on the function, morphology and cytotoxicity of human neutrophils. Neutrophils were mixed with titanium in Hanks' balanced salt solution (2 and 10 mg/kg) and incubated at 37°C for 30 min. Compared with the control (Hanks' balanced salt solution), there was no effect of titanium particles on cell survival (2 and 10 mg titanium/kg) or lactate dehydrogenase release (10 mg titanium/kg), but there was a significant effect of 2 mg titanium/kg (1–3 μm particle size) on superoxide anion production ($p < 0.05$), and an effect on tumour necrosis factor (TNF)-α production (1–3 μm particle size). In addition, 1–3 μm titanium particles were inserted subcutaneously into the abdominal cavity of Wistar rats aged between 11 and 12 weeks. The rats were killed 8 weeks later and the tissue section was found to contain phagocytised titanium particles and numerous inflammatory cells. The authors concluded that the increase in inflammatory cells was probably due to the increased productions of superoxide anion and TNF-α production in the presence of titanium.

Kang et al. (2008) investigated the effects of fine (primary particle size 1,000 nm) and ultrafine (primary particle size 21 nm) TiO_2 particles on ROS generation and pro-inflammatory cellular cascades. Fine and ultrafine TiO_2 particles incubated with a mouse peritoneal macrophage cell line (RAW 264.7) for 24 h, at concentrations in the range of 0.5–200 μg/mL did not significantly affect cell viability, as measured by lactate dehydrogenase activity leakage. ROS generation was greater for ultrafine than fine TiO_2 particles at all concentrations tested in the range of 0.5–100 μg/mL at 4 h of incubation. At 24 h of incubation, ROS levels varied less with respect to particle size and were falling to control levels. Compared with controls, only ultrafine TiO_2 particles (0.5 μg/mL for 20 min) induced extracellular signal-regulated kinase-1/2 phosphorylation in a concentration-dependent manner in RAW 264.7 cells, whereas fine TiO_2 induced only minimal changes. Ultrafine TiO_2 (0.5–200 μg/mL) significantly increased TNF-α and macrophage inflammatory protein-2 (MIP-2) secretions in a concentration-dependent manner, compared with control, with peak responses at 200 μg/mL; 6.6-fold TNF-α and 5.8-fold MIP-2. The authors concluded that the effects of fine particles on increases in TNF-α and MIP-2 secretions were less pronounced at each concentration tested with peak responses at 200 μg/mL; 1.4-fold TNF-α and 3.1-fold MIP-2.

Morishige et al. (2010) investigated the effect of anatase and rutile TiO_2 particles of different sizes (anatase: 10 to < 50,000 nm; rutile: 40 to < 5,000 nm) on interleukin-1β (IL-1β) production in macrophage-like human THP-1 cells (acute monocytic leukaemia cell line). Differentiated cells were stimulated with 20, 100 or 500 μg TiO_2/mL for 24 h in the presence or absence of lipopolysaccharide as a THP-1 cell activator. At all concentrations, rutile TiO_2 induced greater IL-1β production than anatase TiO_2. Smaller anatase (compared with larger anatase particles) and larger rutile particles (compared with smaller rutile particles) provoked greater IL-1β production in differentiated THP-1 cells exposed for 6 h at all concentrations. At 20 and 100 μg/mL, spicula (needle-shaped) rutile particles also induced greater IL-1β production than similarly sized and structurally identical, but spherical rutile particles.

Becker et al. (2012) reported that following incubation with TiO_2 nanoparticles, macrophage-like cells readily take up TiO_2 after 6 h, and particles were also found intracellularly in intestinal cells. Incubation of cells with TiO_2 resulted in secretion of IL-1β and IL-8. According to the authors, this may aggravate inflammation in susceptible individuals.

Mice

Larsen et al. (2009) reported that nanosized TiO_2 may have an adjuvant effect after intraperitoneal injection into mice together with ovalbumin.

In mice receiving an intratracheal instillation of 0.5–50 mg/kg of TiO_2 nanoparticles, the levels of the proinflammatory cytokines, IL-1, TNF-α and IL-6, were significantly elevated in a dose-dependent manner 24 h after administration, and remained elevated for up to 14 days. Levels of the TH1 cytokines, IL-12 and interferon-gamma, and the TH2 cytokines, IL-4, IL-5 and IL-10, were also elevated dose dependently at day 1 and remain elevated for up to 14 days after instillation. Increased numbers of B lymphocytes were observed in both spleen and in blood, as well as increased immunoglobulin E production in BAL fluid and serum (Park et al., 2009).

In mice administered via intragastric gavage, TiO_2 nanoparticles caused congestion and proliferation of spleen tissue, with accompanying increases ROS in spleen tissue. The elevated ROS levels in spleens led to lipid peroxidation and upregulation of haem oxygenase expression, suggesting that TiO_2 nanoparticle accumulation in lymphoid organs may exert cytotoxic effects through the induction of oxidative stress (Wang et al., 2011).

Administration of 2.5, 5 or 10 mg/kg bw per day TiO_2 nanoparticles to mice via gavage for 6 months resulted in an accumulation of titanium in the liver and accompanying reductions in body weight, increases in liver damage indices, liver dysfunction, infiltration of inflammatory cells, and hepatocyte apoptosis and necrosis. Additionally, hepatic inflammation was increased, as measured by the upregulation of IL-4, IL-5, IL-12, interferon-gamma, GATA3, GATA4, T-bet, RORgt, STAT3, STAT6, eotaxin, MCP-1 and MIP-2. This indicated that prolonged exposure to TiO_2 nanoparticles may affect the cells and tissues of the lymphoid system, as well as peripheral organs including the liver, in which nanoparticle accumulation results in hepatic inflammation and toxicity (Hong et al., 2014).

Auttachoat et al. (2014) reported that after 28 days of oral gavage, TiO_2 nanoparticles (1.25–250 mg/kg in 0.5% methylcellulose) had no significant effects on innate, humoral or cell-mediated immune functions in female B6C3F1 mice. There were no effects on the weights of selected organs (spleen, thymus, liver, lung and kidneys). Following dermal exposure on the ears for 3 days, TiO_2 nanoparticles (2.5–10% w/v in 4:1 acetone/olive oil) did not affect auricular lymph node cell proliferation. Dermal sensitisation (2.5–10%) on the back and subsequent challenge (10%) on the right ear with TiO_2 nanoparticles produced no significant effects on percentage ear swelling in the mouse ear-swelling test. However, when TiO_2 nanoparticles were injected subcutaneously along the midline on top of the head at 125–250 mg/kg (in 0.5% methylcellulose), significant increases in auricular lymph node cell proliferation resulted. The authors concluded that immune effects of TiO_2 nanoparticle exposure are dependent on the route of exposure, and that hypersensitivity responses may occur following parenteral exposure or dermal administration of TiO_2 nanoparticles to compromised skin.

Rat studies

TiO_2 nanoparticles were shown to accumulate in the spleen of Sprague–Dawley rats after intravenous administration (5 mg/kg bw), with levels peaking at 24 h and decreasing slightly by days 14 and 28 (Fabian et al., 2008). The Panel noted that the dose injected was very high.

In the study by Olmedo et al. (2008), male Wistar rats were injected intraperitoneally with a suspension of TiO_2 rutile powder at the dose of 1.60 g/100 g bw. After 6 months, the presence of titanium was assessed in serum, blood cells, liver, spleen and lung. Titanium was found in phagocytic mononuclear cells, serum and in the parenchyma of all the organs tested. According to the authors, TiO_2-rutile generated an increase in the percentage of reactive cells, which was smaller than that previously reported with TiO_2-anatase, suggesting that TiO_2-rutile is less reactive than TiO_2-anatase. The Panel noted that both the very high dose injected and the route of injection were not representative of the use of TiO_2 as a food additive.

As reported by Liu et al. (2010), 42 rats were instilled intratracheally with 0.5, 5 or 50 mg/kg bw of nano- (NP-1) and microsized (F-1) TiO_2 particles with a median size of 5 and 200 nm, respectively. Exposure to NP-1 TiO_2 decreased the chemotactic ability of the macrophages and the expression of Fc receptors and major histocompatibility complex class II on their surface. According to the authors, the mechanism responsible for these changes was mediated via altering nitric oxide (NO) and TNF-α expression by the porcine alveolar macrophages (PAMs). The amount of nitric oxide and TNF-α secreted by macrophages gradually increased as the dose of TiO_2 nanoparticles increased. Contrary to the 200 nm TiO_2 particles, 50 nm TiO_2 nanoparticles elicited strong nitric oxide and TNF-α production.

Sprague–Dawley rats were instilled intratracheally with TiO_2 nanoparticles (21 nm) at doses of 0.5, 4 and 32 mg/kg bw, or 32 mg/kg bw TiO_2 microparticles (1–2 μm) twice a week, for four consecutive weeks. Immune function response was characterised by increased proliferation of T cells and B cells following mitogen stimulation and enhanced natural killer cell killing activity in spleen, accompanying by an increased number of B cells in blood. No significant changes of Th1-type cytokines (IL-2 and interferon-gamma) and Th2-type cytokines (TNF-α and IL-6) were observed (Fu et al., 2014).

The Panel noted that in most of these studies, the administered doses used were very high.

3.2.6.2. Hypersensitivity

Humans

The SCCNFP (2000) evaluation reported that five sunscreen formulations were tested in 76 human volunteers (males and females), three forms containing 40% TiO_2 and two forms containing 10% TiO_2. The Shelanski repeated insult patch test method was used. The formulations were applied for 24 h on 2 × 2 cm patches on the lateral surface of the upper arm. Each subject had the same material applied to the same site throughout. Patches were applied 3 days a week for the first 3 weeks. Fourteen days later, challenge patches were applied to both arms, on one side to the original sites, and on the other to previously untreated sites. Scoring was at 48 and 96 h. Some mild erythematous reactions during the induction phase of the trial were recorded. There were no reactions to the challenge and the materials tested were judged not to cause sensitisation.

The SCCNFP (2000) evaluation also reported that a 5% preparation of TiO_2 in petrolatum was used to test 918 patients with various skin diseases (the occluded contact time was 48 h), including a group of 290 dermatitis patients (BIBRA, 1990). TiO_2 was reported not to cause any reaction. The same researchers also reported testing TiO_2 in 50 healthy volunteers and no reaction was observed (no further information) (SCCNFP, 2000).

Overall, the Panel noted that most of the published studies reporting effects of TiO_2 on the immune system have been carried out using nanosized TiO_2 and high doses of administration. However, an adequate characterisation of the size and the nature (rutile or anatase) were rarely provided and it was not clear to what extent the material used was representative of the food grade TiO_2. Finally, the route of administration (intratracheal or intraperitoneal) was often not representative of the use of TiO_2 as a food additive.

- *In vitro*, TiO_2 nanoparticles were readily internalised by immune system cells and might influence multiple manifestations of immune cell activity including cytokine production, proliferation, inflammation, ROS production and adhesion molecule expression, among others.
- *In vivo*, administration of TiO_2 nanoparticles has been reported to have multiple immunomodulatory effects, characterised by nanoparticle accumulation in local (Peyer's patches) and peripheral lymphoid organs, alterations in immune cell number, viability and function. In a few studies, microsized TiO_2 also induced some effects but only at high doses. Although ambiguity remains surrounding the specific immunomodulatory and inflammatory effects resulting from *in vivo* TiO_2 nanoparticle exposure, it seems clear that whereas TiO_2 nanoparticles have such a potential, TiO_2 particles with a larger size, over 100 nm, that is closer to food grade, are less active.

3.2.6.3. Other studies

The greatest number of studies on TiO_2 addressed the consequences of the exposure via inhalation and, in particular, the impact of particle size on the observed effects. The studies performed on pulmonary exposure to TiO_2 showed that toxicity was primarily dictated by particle size and crystal structure, whereby decreasing particle size and anatase as the crystalline form of TiO_2 enhanced particle toxicity (Ferin et al., 1992; Wang et al., 2008a,b).

Although the results of such studies cannot simply be used as basis for the safety evaluation of TiO_2 when taken orally, the studies give an indication on potential biological effects resulting from particles size when exposed by inhalation.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that had become available since then and the data available following

public calls for data. The Panel noted that not all original studies on which previous evaluations were based were available.

TiO_2 is a food colour authorised as a food additive in the EU. It was previously evaluated by the SCF in 1975 and 1977, by JECFA in 1969 (JECFA, 1970) and by EFSA in 2004. It has also been reviewed by TemaNord in 2002. In 1969, JECFA allocated an ADI 'not limited except for good manufacturing practice'. In 1975, the SCF did not establish an ADI for TiO_2, whereas in 1977, the SCF included TiO_2 in the category 'colours for which an ADI was not established but which could be used in food'. In 2002, TemaNord concluded that 'the inertness of the substance and the lack of absorption and tissue storage does not warrant further testing or a re-evaluation of the safety in use of this compound'. In 2004, the EFSA AFC Panel assessed the safety of platelet forms of rutile TiO_2 as an alternative to the permitted anatase form, and concluded that 'the bioavailability of these forms was essentially the same. The toxicological database would, therefore, be applicable to either form and that the platelet forms of rutile TiO_2 could be used to replace anatase TiO_2 in any of its current applications'.

The Panel is aware that the ECHA is carrying out an evaluation for a proposal for CLH on TiO_2, for which ANSES is the Rapporteur on behalf of the French Member State Competent Authority. ANSES prepared a report in which concluded that TiO_2 should be considered as being potentially carcinogenic to humans when inhaled and thus be classified Carc. Cat 1B – H350i. However, it also concluded that there was no carcinogenic concern after oral or dermal administration. A public consultation on this report is currently underway.[13]

In nature, TiO_2 exists in different crystalline forms, anatase and rutile being the two most important natural forms. The food additive TiO_2 (E 171) is a white to slightly coloured powder and it is insoluble in water and organic solvents (Commission Regulation (EU) No 231/2012).

Interested parties provided analytical data on the particle size characteristics of TiO_2 (E 171; anatase or rutile) used as a food/feed additive and additional information was available from public literature. The Panel noted that determination of the fraction of TiO_2 nanoparticles in the food additive (E 171) is method dependent. The Panel also noted that, according to the data provided by industries and from the literature, TiO_2 (E 171) as a food additive would not be considered as a nanomaterial according to the EU Recommendation on the definition of a nanomaterial.[8]

The Panel noted that there are no set limits for the particle size of TiO_2 in the EU specifications, and therefore characterisation of the particle size in the food additive E 171 should be included among the specifications.

The Panel noted that the manufacturing process for powdered or particulate food additives resulted in material with a range of sizes. Although the median size of the particles is generally significantly greater than 100 nm, a small fraction will always be, and has been, with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nano fraction. The test requirements stipulated in current EFSA guidance documents and European Commission guidelines for the intended use in the food/feed area apply in principle to unintended nano forms, as well as to engineered nanomaterials. Therefore, the Panel considers that, in principle, for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive, including its nanoparticulate fraction. The Panel considers that for the re-evaluation of food additives, this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014 (EFSA, 2015). In addition, the Panel noted analytical data provided by interested parties on the particle size distribution of food-grade TiO_2, which confirmed the small percentage in the nanoscale (< 100 nm), but that actual values depended on the method used. From this information, a percentage value of 3.2% of nanoparticles by mass, was considered by the Panel to be reasonable to address in a conservative way a preliminary content estimate in the food additive TiO_2 (E 171).

The Panel was provided with the unpublished results of a number of RIVM studies on TiO_2 nanoparticles. These studies were evaluated along with the published literature and they did not affect the Panel's conclusions drawn from the whole dataset. The Panel recommends that, once publicly available, further information on the RIVM studies should be published as an addendum to this Opinion.

In absorption, distribution and excretion studies in animals (rat and mice), differences in the observed results appear to be dependent on study design and duration.

The Panel concluded that:

- the absorption of orally administered TiO_2 is extremely low,
- the bioavailability of TiO_2 (measured either as particles or as titanium) is low,
- the bioavailability measured as titanium appeared to be independent of particle size,
- the vast majority of an oral dose of TiO_2 is eliminated unchanged in faeces,
- a small amount (maximum of 0.1%) of orally ingested TiO_2 was absorbed by the GALT and subsequently distributed to various organs and elimination rates from these organs were variable,
- there were significant and highly variable background (basal) levels of titanium in animals and humans, which presented challenges in the analysis at the low levels of titanium uptake reported and could complicate interpretation of the reported findings in some studies.

The acute oral toxicity of TiO_2 is very low, with oral LD_{50} values > 10 g/kg bw per day for mice and > 25 g/kg bw per day for rats.

Overall, the Panel noted that there was rather limited information available on the short-term and subchronic toxicity of the food additive TiO_2 (E 171). In a well-performed 28-day gavage study in rats with non-coated pigment-grade TiO_2 (rutile form; d_{50} 173 nm) at a dose of 24,000 mg TiO_2/kg bw, no treatment-related adverse effects were observed. Occurrence of particles in intestinal lymphoid tissue was not regarded as adverse. The NOAEL for the study was 24,000 mg/kg bw per day. Although the study was not performed using the food additive TiO_2 (E 171), the Panel considered the results useful as supporting evidence in the assessment of the use of TiO_2 as a food colour. In a 90-day study, doses up to 16,900 mg TiO_2/kg bw per day for male mice and up to 8,100 mg TiO_2/kg bw per day for male rats did not result in differences in body weight or in relevant gross or microscopic pathology as compared with the control. However, no haematological parameters and no biochemical parameters in urine and blood were measured.

The Panel concluded that the available mixed results provided some evidence of *in vitro* genotoxicity for TiO_2 micro- and nanoparticles. The Panel noted that most positive results have been reported under experimental conditions associated with the induction of oxidative stress, and that the genotoxic effects observed mainly concern indicator assays, which in some studies were shown not to be associated with permanent chromosome damage.

In vivo, overall negative results were obtained in genotoxicity studies with microsized TiO_2 pigment. Limited evidence of genotoxicity, if any, was provided by studies with orally administered TiO_2 nanoparticles. Limited or no indication of genotoxicity of TiO_2 nanoparticles was also provided by studies using the intravenous route of administration, which allowed maximum exposure of target tissues.

The Panel concluded that the use of TiO_2 as a food additive does not raise a concern with respect to genotoxicity.

Two carcinogenicity studies, performed with TiO_2 administered to mice and rats via the oral route were available and the outcome of these studies was reported to be negative for both mice and rats. Based on these data, and on earlier data reported in the JECFA (1970) evaluation, the Panel concluded that TiO_2 is not carcinogenic after oral administration. This is in line with the recent assessment performed by ANSES for the ECHA evaluation in which it is concluded that there was no carcinogenic concern after oral or dermal administration. The Panel identified a NOAEL of 2,250 mg TiO_2/kg bw per day, the highest dose tested, from a chronic toxicity and carcinogenicity study in rats.

No reproductive (one- or two-generation toxicity) studies with TiO_2 (as the food additive, micro- or nanosized) performed according to the OECD guidelines were available for evaluation. However, the Panel noted that in the NCI (1979) chronic toxicity and carcinogenicity study, no histopathological changes in the male and female reproductive organs were reported at the highest doses tested of 6,500 and 8,350 mg/kg bw per day for male and female mice, respectively, and at the highest doses tested of 2,250 and 2,900 mg/kg bw per day for male and female rats, respectively.

Overall, the Panel noted that prenatal developmental studies with three pigment-grade (pg-1, pg-2, pg-3) and three ultrafine (uf-1, uf-2, uf-3)/nanoscale (anatase and/or rutile) TiO_2 particulates performed according to the OECD guidelines (TG 414) did not give concern for maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg bw per day). However, the Panel noted that reproductive toxicity studies performed according to the OECD guidelines using TiO_2, meeting the food additive specifications were not available. Furthermore, the Panel noted that results from other reproductive and developmental studies with TiO_2 nanoparticles (Jia et al., 2014; Tassinari et al., 2014) indicating effects on the reproductive system, showed contradictory results in the change

in hormone levels. Because of deficiencies in the study designs and inadequate data reporting, the Panel considered that the relevance of these findings is currently uncertain for the risk assessment of TiO_2 as a food additive.

For the safety assessment of TiO_2 used as a food additive, based on information reported in the examined literature and information supplied following calls for data taking into account the following considerations:

- the food additive E 171 mainly consists of microsized TiO_2 particles, with a nanosized (< 100 nm) fraction less than 3.2% by mass;
- the absorption of orally administered TiO_2 particles (micro- and nanosized) in the gastrointestinal tract is negligible, estimated at most as 0.02–0.1% of the administered dose;
- no difference is observed in the absorption, distribution, and excretion of orally administered microsized and nanosized TiO_2 particles;
- no adverse effect resulting from the eventual accumulation of the absorbed particles is expected based on the results of long-term studies which did not highlight any toxicity up to the highest administered dose;
- the uncertainties in the toxicological database arising from limitations in the available reproductive toxicity studies;

the Panel considered that an ADI should not be established, and that a margin of safety (MoS) approach would be appropriate (EFSA ANS Panel, 2012).

As regards hypersensitivity, the Panel noted that the available studies on the effects of TiO_2 (nano)particles on the immune systems pointed to different outcomes. However, they indicated that the reported effects were dependent on the core composition, size and concentration of the particles, and on the duration and route of exposure. The Panel considered that, given the absence of clear characterisation of the material used, the difference in effects observed following various routes of administration and the diversity in the effects reported, a conclusion on the possible immunotoxic effects of the food additive TiO_2 cannot be reached. However, the Panel noted that the larger the TiO_2 particles, the lower their potential to induce effects, and that from animal data it appeared that the route of injection influences the response, TiO_2 particles being less reactive after oral administration.

To assess the dietary exposure to TiO_2 (E 171) from its use as a food additive, the exposure was calculated based on: maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and reported use levels (defined as the *refined exposure assessment scenario*) as provided by industry and Member States.

Based on the available dataset, the Panel calculated two refined exposure estimates based on different assumptions: a *brand-loyal consumer scenario*, in which it is assumed that the population is exposed over a long period of time to the food additive present at the maximum reported use/analytical levels for one food category and to a mean reported use/analytical level for the remaining food categories; and a *non-brand-loyal scenario*, in which it is assumed that the population is exposed over a long period of time to the food additive present at the mean reported use/analytical levels in all relevant food categories.

The Panel considered that the refined exposure assessment approach was a more realistic scenario, because it was based on the range of usage and analytical data, assumed that the processed foods and beverages contain the additive at the mean concentration level for all products (non-brand-loyal consumer scenario) and considers one product containing TiO_2 at the maximum concentration level (brand-loyal consumer scenario). However, the Panel noted that due to the low amount of data provided to EFSA (reported use levels or analytical data), only 14 food categories were taken into account, representing between 60% and 80% of food (by weight) authorised to contain TiO_2 according to annex II.

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of TiO_2.

For the *maximum level exposure assessment scenario*, at the mean, the exposure estimates ranged from 0.4 mg/kg bw per day for infants and the elderly to 10.4 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 1.2 mg/kg bw per day for the elderly to 32.4 mg/kg bw per day for children.

For the *refined estimated exposure scenario*, in the *brand-loyal scenario*, the exposure estimates ranged, at the mean, from 0.4 mg/kg bw per day for infants and the elderly to 8.8 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 1.1 mg/kg bw per day for the elderly to 30.2 mg/kg bw per day for children. In the *non-brand-loyal scenario*, the exposure estimates

ranged, at the mean, from 0.2 mg/kg bw per day for infants and the elderly to 5.5 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.5 mg/kg bw per day for the elderly to 14.8 mg/kg bw per day for children.

In the case of TiO_2, the Panel did not identify brand loyalty to a specific food category and therefore the Panel considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to food additives present at the mean reported use/analytical levels in processed food.

Based on a NOAEL of 2,250 mg TiO_2/kg bw per day and the exposure data for the non-brand loyal scenario, the Panel calculated the MoS values for the different population groups (Table 16).

Table 16: MoS values calculated based on the exposure estimated through the non-brand loyal scenario estimates as presented in Table 8, in six population groups (min–max across the dietary surveys)

Population groups	MoS calculation based on exposure to the non-brand loyal scenario	
	Mean	p95
Infants	2,800 11,000	600 3,200
Toddlers	500 3,800	350 1,200
Children	400 2,500	150 950
Adolescents	550 5,700	200 1,800
Adults	550 7,500	250 2,100
The elderly	800 11,000	300 4,500

MoS: margin of safety.

The Panel noted that the lowest MoS calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the available toxicological data and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) considered in this opinion is above 100. In the Guidance for submission of food additives (EFSA ANS Panel, 2012), the Panel considered that, for non-genotoxic and non-carcinogenic compounds "a MoS of 100 or more between a NOAEL or BMDL and the anticipated exposure would be sufficient to account for uncertainty factors for extrapolating between individuals and species". Consequently, the Panel considered that the reported use/analytical levels of TiO_2 (E 171) considered in this opinion would not be of safety concern.

The Panel considered that once definitive and reliable data on the reproductive toxicity of E 171 were available, the full dataset would enable the Panel to establish a health-based guidance value (ADI).

For the purpose of providing an indicative estimate of exposure to nanoparticles of titanium dioxide from the use of TiO_2 as a food additive, the Panel considered that the highest reported weight percentage value of 3.2% of nanoparticles by mass could reasonably be used in a conservative way to address this issue.

Based on this maximum reported level of 3.2% of nanoparticles by mass in all foods categories considered in the exposure assessment from the use of E 171 as a food additive, the Panel noted that indicative estimates of exposure to nanoparticles of titanium dioxide coming from TiO_2 (E 171) ranged for the maximum level exposure assessment scenario, at the mean, from 0.01 mg/kg bw per day for infants and the elderly to 0.33 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.04 mg/kg bw per day for infants and the elderly to 1.04 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the brand-loyal scenario, the exposure estimates ranged at the mean from 0.01 mg/kg bw per day for infants and the elderly to 0.28 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.03 mg/kg bw per day for the elderly to 0.97 mg/kg bw per day for children.

For the refined estimated exposure scenario, in the non-brand-loyal scenario, the exposure estimates ranged at the mean from 0.01 mg/kg bw per day for infants, adolescents, adults and the elderly to 0.18 mg/kg bw per day for children. At the 95th percentile, exposure estimates ranged from 0.02 mg/kg bw per day for infants and the elderly to 0.47 mg/kg bw per day for children.

The Panel noted that from its indicative estimates of exposure to nanoparticles that could be present in the food additive TiO_2, the uncertainties identified could result in an overestimation if all

food categories considered in its exposure assessment had nanoparticles present at the maximum reported percentage value by mass (3.2%).

Conclusions

From the available data on absorption, distribution and excretion, the Panel concluded that:

- the absorption of orally administered TiO_2 is extremely low;
- the bioavailability of TiO_2 (measured either as particles or as titanium) is low;
- the bioavailability measured as titanium appeared to be independent of particle size;
- the vast majority of an oral dose of TiO_2 is eliminated unchanged in the faeces;
- a small amount (maximum of 0.1%) of orally ingested TiO_2 was absorbed by the gut-associated lymphoid tissue (GALT) and subsequently distributed to various organs and elimination rates from these organs were variable.

The Panel further concluded that there were significant and highly variable background levels of titanium in animals and humans, which presented challenges in the analysis at the low levels of titanium uptake reported and could complicate interpretation of the reported findings.

The Panel concluded that, based on the available genotoxicity database and the Panel's evaluation of the data on absorption, distribution, and excretion of micro- and nanosized TiO_2 particles, orally ingested TiO_2 particles (micro- and nanosized) are unlikely to represent a genotoxic hazard *in vivo*.

The Panel noted that possible adverse effects in the reproductive system were identified in some studies conducted with material which was either non-food-grade or inadequately characterised nanomaterial (i.e. not E 171). There were no such indications in the available, albeit limited, database on reproductive endpoints for the food additive (E 171). The Panel was unable to reach a definitive conclusion on this endpoint due to the lack of an extended 90-day study as in the Guidance for submission of food additives (EFSA ANS Panel, 2012) or a multigeneration or extended-one generation reproduction toxicity study with the food additive (E 171). Therefore, the Panel did not establish an ADI.

From a carcinogenicity study with TiO_2 in mice and in rats, the Panel chose the lowest NOAEL reported which was 2,250 mg TiO_2/kg bw per day for males from the rat study, the highest dose tested in this species and sex.

The Panel considered that on the database currently available and the considerations on the absorption of TiO_2 the margins of safety calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the toxicological data available and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) considered in this opinion would not be of concern.

The Panel concluded that once definitive and reliable data on the reproductive toxicity of E 171 were available, the full dataset would enable the Panel to establish a health-based guidance value (ADI).

Recommendations

The Panel recommended that:

- In order to enable the Panel to establish a health-based guidance value (ADI) for the food additive TiO_2 (E 171), additional testing could be performed. An extended 90-day study or a multigeneration or extended-one generation reproduction toxicity study according to the current OECD guidelines could be considered. Such studies should be performed with TiO_2 (E 171) complying with the EU specifications and additionally including a characterisation of the particle size distribution of the test material. However, in deciding on actual testing, considerations of animal welfare need to be balanced against the improvement in the toxicological database within a tiered testing approach.
- The EU specifications for TiO_2 (E 171) should include a characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm) present in TiO_2 (E 171) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011).
- The maximum limits for the impurities of the toxic elements (arsenic, lead, mercury and cadmium) in the EU specification for TiO_2 (E 171) should be revised in order to ensure that TiO_2 (E 171) as a food additive will not be a significant source of exposure to those toxic elements in foods.

Documentation provided to EFSA

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Abbreviations

8-OH-dG	8-hydroxy 2′-deoxyguanosine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AECD	area equivalent circular diameter
AESGP	Association of the European Self-Medication Industry
AFC	EFSA Former Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AOM	azoxymethane
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
AUC	area under the curve
BIBRA	British Industrial Biological Research Association
bw	body weight
BMDL	benchmark dose modelling
CAC	colitis-associated cancer
CAS	Chemical Abstracts Service
CEFIC	European Chemical Industry Council
CHO	Chinese hamster ovary
C.I.	Colour Index
CIAA	Confederation of the Food and Drink Industries of the EU
CLH	harmonised classification and labelling
d_{50}	median particle size
DLS	dynamic light scattering
DSS	dextran sulfate sodium
ECHA	European Chemical Agency
EINECS	European Inventory of Existing Commercial Chemical Substances
FCS	Food Categorisation System
FDA	Food and Drug Administration
FDE	Food Drink Europe
FSANZ	Food Standard Agency of New Zealand
GALT	gut-associated lymphoid tissue
GNPD	Global New Products Database
HD	hydrodynamic diameter
hOGG	human 8-hydroxyguanine DNA-glycosylase
IACM	International Association of Colour Manufacturers
IARC	International Agency for Research in Cancer
ICGA	International Chewing Gum Association
ICRP	International Commission on Radiological Protection
IL	interleukin
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee of Food Additives
KEM	keratinocyte growth medium

LC	left-censored
LD_{50}	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
MIP-2	macrophage inflammatory protein-2
MoS	margin of safety
MPL	maximum permitted level
MS	Member State
MTT	methyl tetrazolium cytotoxicity
NCE	normochromatic erythrocytes
NCI	National Cancer Institute
N.F.	National Formulary
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observable adverse effect level
NSF	US National Sanitation Foundation
NTP	National Toxicological Programme
OECD TG	Organisation for Economic Co-operation and Development Testing Guidelines
PALS	phase analysis light scattering
PAMs	porcine alveolar macrophages
PCE	polychromatic erythrocytes
QS	quantum satis
RIVM	National Institute for Public Health and the Environment
ROS	reactive oxygen species
SCCNFP	Scientific Committee on Cosmetics and Non-Food Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
SEM	scanning electron microscopy
SHE	Syrian hamster embryo
STT	short-term test
TBIL	total bilirubin
TDMA	Titanium Dioxide Manufacturers Association
TEM	transmission electron microscopy
TK	thymidine kinase
TNF	tumour necrosis factor
UF	ultrafine
UV	ultraviolet
XSDC	X-ray disc centrifugation

Appendix A – Summary of reported use levels (mg/kg) of TiO_2 (E 171) provided by industry

Food category number	Food category name	MPL	Restriction/ exceptions	Total number of data	Reported use levels from industry			Information provided by	Comments
					Number of data	Typical mean (range)	Highest maximum level		
01.8	Dairy analogues, including beverage whiteners	QS		1	1	125	125	FDE	
03	Edible ices	QS		2	2	428	857	FDE	
05.2	Other confectionery, including breath-refreshening microsweets	QS		5	5	1,074	4,500	FDE	
05.3	Chewing gum	QS		2	1	3,400	3,800	FDE	
					1	2,829	16,000	ICGA	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS		13	13	1,296	20,000	FDE	
07.2	Fine bakery wares	QS		2	2	179	555	FDE	
08.2.3	Casings and coatings and decorations for meat	QS	Except edible external coating of pasturmas	2	2	18	35	FDE	
12.5	Soups and broths	QS		1	1	193	193	FDE	
12.6	Sauces	QS	Excluding tomato-based sauces	5	5	1,646	4,000	FDE	
12.7	Salads and savoury-based sandwich spreads	QS		1	1	2,500	3,000	FDE	
14.1.4	Flavoured drinks	QS	Excluding chocolate milk, malt products	6	6	28	70	FDE	
15.2	Processed nuts	QS		4	4	3,775	7,000	FDE	
16	Desserts, excluding products covered in category 1, 3 and 4	QS		1	1	140	200	FDE	
17.1	Food supplements supplied in a solid form, including capsules and tablets and similar forms, excluding chewable forms	QS		16	15	2,801	12,000	AESGP	
					1	2	4	Capsugel	Empty gelatin capsule

QS: quantum satis; FDE: FoodDrinkEurope; ICGA: International Chewing Gum Association; AESGP: Association of the European Self-Medication Industry.

Appendix B – Summary of analytical results (mg/kg) of TiO_2 (E 171) provided by Members States

Food category number	Food category name	MPL	N	No LC	Range		All data				Positive values				
					LOD	LOQ	Min	Median	Mean	Max	Number of positive values	Min	Median	Mean	Max
13	Foods intended for particular nutritional uses as defined by Directive 2009/39/EC	QS	2	0	2,000	5,000	16,950	18,150	18,150	19,350	2	16,950	18,150	18,150	19,350
17	Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council, excluding food supplements for infants and young children	QS	26	4	2,000	5,000	1,000	15,150	14,438	26,950	22	6,000	16,900	16,745	26,950

LC: left-censored data; LOD: limit of detection; LOQ: limit of quantification; Max: maximum; Min: minimum; MPL: maximum permitted level; N: number of analytical results.

Appendix C – Number and percentage of food products labelled with TiO_2 (E 171) out of the total number of food products present in Mintel GNPD per food subcategory between 2011 and 2015

Mintel sub-category[a]	Total number of products	Products labelled with TiO_2 (E 171)	
		Number	%
Gum	1,262	642	50.9
Sticks, Liquids & Sprays	88	22	25.0
Mixed Assortments	271	56	20.7
Pastilles, Gums, Jellies & Chews	3,346	345	10.3
Lollipops	341	34	10.0
Liquorice	690	54	7.8
Other Sugar Confectionery	950	66	6.9
Yeast Extracts	15	1	6.7
Non-Individually Wrapped Chocolate Pieces	4,687	312	6.7
Standard & Power Mints	787	44	5.6
Creamers	182	10	5.5
Other Frozen Desserts	1,396	76	5.4
Seasonal Chocolate	4,962	219	4.4
Boiled Sweets	858	35	4.1
Beverage Mixes	767	26	3.4
Marshmallows	431	14	3.2
Cakes, Pastries & Sweet Goods	11,877	385	3.2
Baking Ingredients & Mixes	8,031	234	2.9
Mayonnaise	802	21	2.6
Dairy-Based Frozen Products	7,001	174	2.5
Dessert Toppings	573	12	2.1
Toffees, Caramels & Nougat	1,738	30	1.7
Medicated Confectionery	891	14	1.6
Other Chocolate Confectionery	263	4	1.5
Beverage Concentrates	2,097	23	1.1
Sweet Biscuits/Cookies	15,483	162	1.0
Chilled Desserts	5,583	54	1.0
Chocolate Spreads	979	9	0.9
Dressings & Vinegar	3,035	27	0.9
Chocolate Tablets	7,344	64	0.9
Instant Rice	120	1	0.8
Shelf-Stable Desserts	2,945	21	0.7
Individually Wrapped Chocolate Pieces	2,296	14	0.6
Spoonable Yoghurt	8,752	49	0.6
Processed Cheese	1,875	10	0.5
Nuts	4,018	21	0.5
Instant Noodles	995	5	0.5
Sandwiches/Wraps	2,406	12	0.5
Snack Mixes	1,273	6	0.5
Eggs & Egg Products	1,298	6	0.5
Chocolate Countlines	2,059	9	0.4
Caramel & Cream Spreads	243	1	0.4
Nectars	3,581	12	0.3
Table Sauces	5,376	17	0.3

Mintel sub-category[a]	Total number of products	Products labelled with TiO_2 (E 171)	
		Number	%
Meat Substitutes	1,908	6	0.3
Soft Cheese & Semi-Soft Cheese	4,995	15	0.3
Salads	2,337	7	0.3
Meat Pastes & Pates	2,776	8	0.3
Water-Based Frozen Desserts	1,072	3	0.3
Meal Kits	1,809	5	0.3
Snack/Cereal/Energy Bars	4,232	11	0.3
Fish Products	10,920	26	0.2
Soft Cheese Desserts	1,364	3	0.2
Noodles	482	1	0.2
Sucrose	975	2	0.2
Meal Replacements & Other Drinks	990	2	0.2
Instant Pasta	549	1	0.2
Cooking Sauces	4,446	7	0.2
Prepared Meals	9,894	14	0.1
Hors d'oeuvres/Canapes	3,631	5	0.1
Energy Drinks	1,484	2	0.1
Poultry Products	5,483	7	0.1
Fresh Cheese & Cream Cheese	2,457	3	0.1
Flavoured Alcoholic Beverages	1,800	2	0.1
Sandwich Fillers/Spreads	901	1	0.1
Malt & Other Hot Beverages	921	1	0.1
Popcorn	981	1	0.1
Dips	1,282	1	0.1
Potato Snacks	4,388	3	0.1
Rice	2,932	2	0.1
Liqueur	1,467	1	0.1
Hard Cheese & Semi-Hard Cheese	5,903	4	0.1
Wheat & Other Grain-Based Snacks	1,689	1	0.1
Corn-Based Snacks	1,955	1	0.1
Pasta	8,874	4	0.0
Fruit/Flavoured Still Drinks	2,590	1	0.0
Meat Products	13,984	4	0.0
Seasonings	8,423	2	0.0
Savoury Biscuits/Crackers	4,214	1	0.0
Vegetables	9,283	2	0.0
Cold Cereals	5,472	1	0.0
Juice	6,949	1	0.0
Bread & Bread Products	8,926	1	0.0
Total sample	278,705	3,516	1.3

(a): According to Mintel food categorisation.

Appendix D – Concentration levels of TiO_2 (E 171) used in the refined exposure scenarios (mg/kg)

FCS category no.	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Max	
01.4	Flavoured fermented milk products, including heat-treated products	QS			Not taken into account (no concentration data)
01.5	Dehydrated milk as defined by Directive 2001/114/EC	QS			Not taken into account (no concentration data)
01.6.3	Other creams	QS			Not taken into account (no concentration data)
01.7.1	Unripened cheese, excluding products falling in category 16 (except mozzarella and unflavoured live fermented unripened cheese)	QS			Not taken into account (no concentration data)
01.7.3	Edible cheese rind	QS			Not taken into account (no corresponding FoodEx code/ no concentration data)
01.7.4	Whey cheese	QS			Not taken into account (no concentration data)
01.7.5	Processed cheese	QS			Not taken into account (no concentration data)
01.7.6	Cheese products	QS			Not taken into account (no concentration data)
01.8	Dairy analogues, including beverage whiteners	QS	125	125	Reported use levels
03	Edible ices	QS	429	857	Reported use levels
04.2.4.1	Fruit and vegetable preparations excluding compote only mostarda di frutta	QS			Not taken into account (no corresponding FoodEx code/ no concentration data)
04.2.4.1	Fruit and vegetable preparations excluding compote only seaweed-based fish analogues	QS			Not taken into account (no corresponding FoodEx code/ no concentration data)
04.2.5.3	Other similar fruit or vegetable spreads, except crème de pruneaux	QS			Not taken into account (no concentration data)
05.2	Other confectionery, including breath-refreshening microsweets	QS	1,074	4,500	Reported use levels
05.3	Chewing gum	QS	3,115	16,000	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS			Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	QS			Not taken into account (no concentration data)
06.5	Noodles	QS			Not taken into account (no concentration data)
06.6	Batters	QS			Not taken into account (no corresponding FoodEx code/ no concentration data)
06.7	Pre-cooked or processed cereals	QS			Not taken into account (no corresponding FoodEx code/ no concentration data)

FCS category no.	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Max	
07.2	Fine bakery wares	QS	160	318	Reported use levels
08.2.3	Casings and coatings and decorations for meat	QS			Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products, including molluscs and crustaceans only surimi and similar products and salmon substitutes	QS			Not taken into account (no concentration data)
09.2	Processed fish and fishery products, including molluscs and crustaceans only fish paste and crustacean paste	QS			Not taken into account (no concentration data)
09.2	Processed fish and fishery products, including molluscs and crustaceans only precooked crustacean	QS			Not taken into account (no concentration data)
09.2	Processed fish and fishery products, including molluscs and crustaceans only smoked fish	QS			Not taken into account (no concentration data)
09.3	Fish roe only processed fish roe	QS			Not taken into account (no concentration data)
12.2.2	Seasonings and condiments	QS			Not taken into account (no concentration data)
12.4	Mustard	QS			Not taken into account (no concentration data)
12.5	Soups and broths	QS	193	193	Reported use levels
12.6	Sauces	QS	1,433	4,000	Reported use levels
12.7	Salads and savoury-based sandwich spreads	QS	2,500	3,000	Reported use levels
12.9	Protein products, excluding products covered in category 1.8	QS			Not taken into account (no concentration data)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC	QS			Not taken into account (no concentration data)
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal	QS			Not taken into account (no concentration data)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation	QS			Not taken into account (no concentration data)
14.1.4	Flavoured drinks	QS	39	70	Reported use levels
14.2.3	Cider and perry	QS			Not taken into account (no concentration data)
14.2.4	Fruit wine and made wine	QS			Not taken into account (no corresponding FoodEx code/ no data provided)
14.2.5	Mead	QS			Not taken into account (no corresponding FoodEx code/ no data provided)

FCS category no.	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Max	
14.2.6	Spirit drinks as defined in Regulation (except whisky or whiskey)	QS			Not taken into account (no concentration data)
14.2.7.1	Aromatised wines	QS			Not taken into account (no concentration data)
14.2.7.2	Aromatised wine-based drinks	QS			Not taken into account (no concentration data)
14.2.7.3	Aromatised wine-product cocktails	QS			Not taken into account (no concentration data)
14.2.8	Other alcoholic drinks, including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol and	QS			Not taken into account (no concentration data)
15.1	Potato-, cereal-, flour- or starch-based snacks	QS			Not taken into account (no concentration data)
15.2	Processed nuts	QS	3,775	7,000	Reported use levels
16	Desserts, excluding products covered in category 1, 3 and 4	QS	140	200	Reported use levels
17.1	Food supplements supplied in a solid form, including capsules and tablets and similar forms, excluding chewable forms	QS	14,438	26,950	Analytical data
17.2	Food supplements supplied in a liquid form	QS			
17.3	Food supplements supplied in a syrup-type or chewable form	QS			

FCS: Food Categorisation System; MPL: maximum permitted level; QS: quantum satis.

Appendix E – Summary of total estimated exposure of TiO_2 (E 171) from its use as a food additive for maximum scenario and refined exposure scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

	Number of subjects	Maximum scenario		Brand-loyal scenario		Non brand-loyal scenario	
		Mean	p95	Mean	p95	Mean	p95
Infants							
Bulgaria (NUTRICHILD)	659	0.4	1.8	0.4	1.8	0.2	0.9
Germany (VELS)	159	1.4	6.6	1.3	5.3	0.6	2.8
Denmark (IAT 2006_07)	826	0.5	2.3	0.4	1.9	0.2	1.1
Finland (DIPP 2001 2009)	500	0.6	1.4	0.6	1.2	0.3	0.7
United Kingdom (DNSIYC 2011)	1,366	1.9	9.6	1.8	9.2	0.8	3.9
Italy (INRAN_SCAI_2005_06)	12	1.0		0.9		0.7	
Toddlers							
Belgium (Regional Flanders)	36	9.2		7.6		4.6	
Bulgaria (NUTRICHILD)	428	2.3	7.5	2.1	6.7	1.0	2.9
Germany (VELS)	348	7.0	15.0	5.3	12.5	2.8	6.4
Denmark (IAT 2006 07)	917	3.7	10.1	2.9	7.8	1.4	3.6
Spain (enKid)	17	2.3		1.9		1.1	
Finland (DIPP 2001 2009)	500	1.2	4.0	1.1	3.6	0.6	2.0
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	5.9	17.3	5.0	14.2	2.6	6.8
United Kingdom (DNSIYC 2011)	1,314	4.3	14.0	3.8	12.9	1.9	5.9
Italy (INRAN SCAI 2005 06)	36	1.8		1.6		0.9	
Netherlands (VCP kids)	322	7.1	19.3	5.7	14.7	2.9	6.8
Children							
Austria (ASNS Children)	128	4.7	12.2	3.6	10.8	2.4	7.5
Belgium (Regional Flanders)	625	7.3	15.3	6.0	12.7	3.5	7.1
Bulgaria (NUTRICHILD)	433	3.3	9.8	2.8	8.3	1.5	3.9
Czech Republic (SISP04)	389	5.7	18.8	4.7	15.3	2.2	6.4
Germany (EsKiMo)	835	4.3	12.0	3.4	10.0	1.7	4.6
Germany (VELS)	293	8.0	16.7	5.8	12.4	3.1	6.1
Denmark (DANSDA 2005-08)	298	5.5	13.5	3.9	9.8	1.9	4.6
Spain (enKid)	156	4.5	12.5	3.7	10.3	1.8	5.6
Spain (NUT INK05)	399	5.1	14.1	4.4	12.8	2.2	5.9
Finland (DIPP 2001 2009)	750	10.4	32.4	8.8	30.2	3.2	9.2
France (INCA2)	482	4.6	9.5	3.5	7.1	2.0	4.2
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	6.4	15.5	5.1	13.3	2.7	6.2
Greece (Regional Crete)	838	4.4	13.7	3.9	12.7	2.9	10.5
Italy (INRAN SCAI 2005 06)	193	1.8	4.9	1.5	4.1	0.9	2.4
Latvia (EFSA TEST)	187	9.1	23.1	8.0	19.9	5.5	14.8
Netherlands (VCP kids)	957	7.3	16.5	5.6	12.7	2.9	6.3
Netherlands (VCPBasis AVL2007 2010)	447	8.6	17.7	6.2	13.7	3.5	7.1
Sweden (NFA)	1,473	10.4	22.1	8.0	17.0	4.4	9.0

	Number of subjects	Maximum scenario		Brand-loyal scenario		Non brand-loyal scenario	
		Mean	p95	Mean	p95	Mean	p95
Adolescents							
Austria (ASNS Children)	237	2.7	7.7	2.3	7.1	1.6	5.8
Belgium (Diet National 2004)	576	4.6	12.4	3.9	10.5	2.0	5.2
Cyprus (Childhealth)	303	0.8	3.3	0.7	2.7	0.4	1.3
Czech Republic (SISP04)	298	3.7	12.2	3.1	10.7	1.5	4.9
Germany (National Nutrition Survey II)	1,011	4.5	13.4	3.9	11.6	1.8	5.1
Germany (EsKiMo)	393	3.3	9.5	2.6	7.6	1.3	3.6
Denmark (DANSDA 2005-08)	377	3.1	7.6	2.3	5.5	1.1	2.4
Spain (AESAN FIAB)	86	2.3	7.4	2.1	6.5	0.8	2.8
Spain (enKid)	209	3.8	10.4	3.1	8.3	1.5	4.0
Spain (NUT INK05)	651	3.4	8.6	2.9	7.0	1.4	3.5
Finland (NWSSP07 08)	306	6.7	23.5	5.9	21.2	1.9	6.2
France (INCA2)	973	2.7	6.3	2.1	4.8	1.1	2.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	3.7	9.4	3.1	8.0	1.5	3.7
Italy (INRAN SCAI 2005 06)	247	1.1	3.1	0.9	2.5	0.6	1.5
Latvia (EFSA TEST)	453	6.5	18.0	5.6	15.0	4.1	10.8
Netherlands (VCPBasis AVL2007 2010)	1,142	5.6	13.9	4.3	10.6	2.3	5.2
Sweden (NFA)	1,018	6.2	14.8	4.9	11.6	2.6	6.0
Adults							
Austria (ASNS Adults)	308	4.5	12.7	3.9	11.0	2.6	7.2
Belgium (Diet National 2004)	1,292	3.3	9.7	2.9	8.7	1.5	4.1
Czech Republic (SISP04)	1,666	1.7	6.1	1.5	5.2	0.9	3.7
Germany (National Nutrition Survey II)	10,419	3.6	10.4	3.2	9.0	1.5	4.0
Denmark (DANSDA 2005-08)	1,739	1.9	5.2	1.4	4.1	0.7	1.8
Spain (AESAN)	410	1.3	4.7	1.2	3.7	0.7	2.1
Spain (AESAN FIAB)	981	1.7	4.4	1.5	3.7	0.7	2.0
Finland (FINDIET2012)	1,295	4.2	15.0	3.6	13.6	1.6	5.2
France (INCA2)	2,276	1.8	4.5	1.5	3.7	0.8	1.9
United Kingdom (NDNS-RollingProgrammeYears1-3)	1266	2.9	7.8	2.5	6.4	1.3	3.4
Hungary (National Repr Surv)	1,074	0.8	3.4	0.7	3.3	0.4	1.8
Ireland (NANS 2012)	1,274	3.2	9.0	2.7	7.8	1.3	3.8
Italy (INRAN SCAI 2005 06)	2,313	0.7	2.4	0.6	2.1	0.4	1.4
Latvia (EFSA TEST)	1,271	4.7	13.0	4.3	11.7	3.2	9.2
Netherlands (VCPBasis AVL2007 2010)	2,057	3.7	9.0	3.0	7.5	1.6	3.9
Romania (Dieta Pilot Adults)	1,254	0.6	2.2	0.5	1.9	0.3	1.1
Sweden (Riksmaten 2010)	1,430	6.8	14.8	5.7	12.4	4.0	9.7
The elderly							
Austria (ASNS Adults)	92	3.6	9.4	3.1	7.4	2.4	6.3
Belgium (Diet National 2004)	1,215	2.2	6.4	2.0	6.0	1.2	2.9
Germany (National Nutrition Survey II)	2,496	1.9	5.7	1.7	5.2	0.9	2.4
Denmark (DANSDA 2005-08)	286	1.0	3.1	0.8	2.5	0.4	1.3
Finland (FINDIET2012)	413	2.4	7.9	2.0	7.2	1.0	3.5

	Number of subjects	Maximum scenario		Brand-loyal scenario		Non brand-loyal scenario	
		Mean	p95	Mean	p95	Mean	p95
France (INCA2)	348	1.4	3.3	1.2	2.8	0.6	1.5
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	2.5	6.5	2.1	5.8	1.2	2.9
Hungary (National Repr Surv)	286	0.6	2.3	0.6	2.2	0.3	1.2
Ireland (NANS 2012)	226	2.3	6.5	2.0	6.2	1.1	2.9
Italy (INRAN SCAI 2005 06)	518	0.5	2.1	0.5	1.9	0.3	1.1
Netherlands (VCPBasis AVL2007 2010)	173	2.7	6.8	2.2	5.4	1.3	3.1
Netherlands (VCP-Elderly)	739	2.9	6.8	2.4	5.9	1.4	3.4
Romania (Dieta Pilot Adults)	128	0.4	1.2	0.4	1.1	0.2	0.5
Sweden (Riksmaten 2010)	367	4.5	10.7	3.9	9.2	2.8	7.0

Appendix F – Toxicological studies with coated TiO_2 nanoparticles considered by the Panel

Brun E, Jugan M-L, Herlin-Boime N, Jaillard D, Fayard B, Flank A-M, Mabondzo A and Carriere M, 2011. Investigation of TiO_2 nanoparticles translocation through a Caco-2 monolayer. Journal of Physics: Conference Series, 304, 012048.

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Warheit DB, Brown SC and Donner EM, 2015b. Acute and subchronic oral toxicity studies in rats with nanoscale and pigment grade titanium dioxide particles. Food and Chemical Toxicology, 84, 208 224.