# OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

# TITANIUM DIOXIDE

Colipa n° S75

adopted by the SCCNFP during the 14th plenary meeting
of 24 October 2000

# 1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. Request for inclusion of S75 in Annex VII, part 1 – List of UV filters which Cosmetic Products may contain – to Council Directive 76/768/EEC.

1.2. Request to the SCCNFP

The SCCNFP is requested to answer the following questions:

* Does the SCCNFP consider the safety profile of titanium dioxide to be sufficient to permit the listing of this material in part I of Annex VII?

* Does the SCCNFP concur with the proposed concentration limit of up to 25% or is a different figure recommended?

* Does the SCCNFP recommend additional requirements for its use in cosmetic products?

# 2. Toxicological Evaluation and Characterisation

## 2.1. General

In the following summary, the reports of the various experiments are preceded by letters indicating the nature of the material tested. M = micro-crystalline; C = coated; NM = not micro-crystalline; UC = uncoated. These letters have sometimes had to be assigned on the basis of probabilities, if the report is insufficiently detailed.

The preparations given the Colipa code S75 seem to consist of either (a) a micro-crystalline preparation of titanium dioxide, the particles of which are covered with an outer layer of various substances, or (b) a preparation of coarse particles ("pigmentary"). The coatings are primarily intended to improve the colour of the preparations and to assist in the incorporation of the titanium dioxide in the cosmetic. The coatings also reduce the photo-catalysis characteristic of titanium dioxide. It is proposed for use in concentrations up to 25%. This opinion concerns crystalline titanium dioxide, whether or not subjected to various treatments (coating, doping, etc.), irrespective of particle size, provided only that such treatments do not compromise the safety of the product.

### 2.1.1. Primary name

Titanium (IV) oxide

### 2.1.2. Chemical name

Titanium (IV) oxide

### 2.1.3. Trade names and abbreviations used in the toxicological studies submitted.

The following trade names (TN) and compositions of the various preparations are given in this order : trade name; crystal type; coating(s) and concentration(s) of coating material(s); average crystal size; manufacturer.

Mirasun TiW60; anatase; alumina 3-7%, silica 12-18% (also uncoated, same TN) 60 nm; Rhodia.

T805. rutile/anatase; silicone dioxide <2.5%; 21 nm. Degussa. (This preparation has been allotted the CAS number 100209-12-9)

T817: as previous with doping with diiron trioxide.

MT100TV; rutile; alumina 1-15%, aluminium stearate 1-13%; 15 nm; Mitsu-bishi/Tayca

MT100Z: as previous but alumina 6-10%, stearic acid 10-16%.

UV-Titan X200: rutile; uncoated; 20 nm; Kemira.

Eusolex T-2000 alumina 8-11%, simethicone 1-3%; 14 nm; Merck.

Kemira AFDC anatase; uncoated; 200 μ; Kemira.

UV-Titan M262: rutile; alumina 5-6.5% dimethicone 1-4%; 20 nm; Rhodia (Kemira also given).

UV-Titan M160 rutile; alumina 5.5-7.5% stearic acid 10%; 17-20 nm; Kemira.

UV-Titan X200 rutile uncoated 20 nm; Kemira.

MT-100TV; rutile; alumina 3-8% (1-15% also given) stearic acid 5-11% (aluminium stearate 1-15% and 1-13% also given); 15 nm; Mitsubishi/Tayca

Solaveil fine particle powder; rutile; alumina 10.5-12.5% (5-15% also given), silica 3.5-5.5%; 11-28 nm; Uniquema. With the same TN (and manufacturer), composition of coating also given is alumina 5-15%, aluminium stearate 5-15%; 10-28 nm.

UV-Titan M212;; rutile; alumina 5-6.5%, glycerol 1%, 20 nm, Kemira.
UV-Titan X161; rutile; alumina 8.5-11.5%, stearic acid 10%; 15 nm, Kemira.

MT-100 T-L-1; rutile; alumina 3.3-7.3%, stearic acid 5-11%; 15 nm; Mitsu-bishi/ Tayca.

MT-100SA; rutile; alumina 4-7.5%, silica 2-4%; 15 nm; Mitsubishi/Tayca.

MT-500SA; rutile; alumina 1-2.5%, silica 4-7%; 35 nm; Mitsubishi/Tayca.

MT-100 AR. crystal type?; alumina 4-8%, silica 7-10%; 15 nm; Mitsubishi/Tayca

TTO 51C. rutile; alumina 11%, silica 1-7%, stearic acid 3-7%, 35 nm; Merck.

TTO 51A. rutile; alumina 11%, silica 1-7%; 35 nm; Merck.

MT-100Z. rutile; alumina 6-10%, stearic acid 10-16%; Mitsubishi/Tayca.

MT100 AQ; rutile; alumina 4-8%, silica 7-11%; 15 nm; Mitsubishi/Tayca.

It is known that other preparations of types similar to the above are available, or may become available, which are not in the above list.

### 2.1.4. CAS no.

The CAS number of titanium dioxide is 13463-67-7.

### 2.1.5 Structural formula

TiO2 (anatase / rutile crystal type)

### 2.1.6. Empirical formula

Emp. Formula : $TiO_2$.
Mol weight : 79.9

### 2.1.7. Purity, composition and substance codes

The purity of titanium dioxide as used in the above proprietary preparations (section 2.1.3) varies somewhat; in the majority of cases, it is stated to be > 99.5%; in one case it is stated to be > 96.5% and in another about 95%.

### 2.1.8. Physical properties

(Remarks refer to titanium dioxide).

Appearance : Fine white powder.
Melting point : Between 1820$^o$ and 1850$^o$
Boiling point : Not applicable.
Density : 100 to 500
Specific gravity : 3.5 to 4.2
Rel. vap. dens. : Not applicable
Vapour Press. : Not applicable.

Log $P_{ow}$ : Not applicable (insoluble in water. Some preparations can be made hydrophilic by appropriate surface treatment).

The primary size of micro-crystals lies mainly in the area of 15 to 25 nm; one preparation has a crystal size of 60 nm. Most micro-crystalline preparations form agglomerates up to about 100nm diameter. Pigmentary titanium dioxide also forms agglomerates.

M/UC. A review of the photo-catalytic activity of titanium dioxide is presented. Much of this work has been carried out by the paint industry, and physical chemists are also interested in the semiconductor state which can be produced by titanium dioxide (among other metal oxides). The main point in relation to the use of titanium dioxide in sunscreens is that photo-catalytic activity is produced at the surface of particles of titanium dioxide, and that this activity can be greatly reduced by coating the surface with various compounds. For example, the photo-catalytic activity of titanium dioxide when coated with silicon dioxide and alumina to the extent of 3.5% of the weight of titanium dioxide will reduce photo-catalysis to 1% of that found in the uncoated titanium dioxide
Microcrystalline titanium dioxide (< 50 nm) absorbs ultraviolet radiation as well as reflecting it. Most of the coating materials are already used in cosmetic formulations, and have not given adverse effects. The chief reason for using coatings is to make the material easier to use in formulations.

(C/?M) The stability of the coating is estimated by subjecting a suspension of the coated material, in methanol/water 80/20, to various stresses - e.g. - shear stress, by high speed emulsifying procedures; changes in pH, changes in temperature, etc. After a stated period, the sample is subjected to centrifugation, and the centrifugate is then analysed for the percentage of the concentration of the coating in relation to the concentration of the titanium dioxide. Results are given for three coatings, viz., carbon, aluminium oxide, and silicon dioxide, from four companies.
The stresses used were shear stress, temperature of $80^{o}$ for one hour, and pH changes from pH 5 to pH 9. The results show the coatings in these cases are highly stable. The trade names of the compounds tested are not, however, given.

A number of preparations has been tested for stability of the various coatings. The coatings tested ($Al_2O_3$, $Al_2O_3$ : $SiO_2$ in different concentrations) have been found to be stable.

(M/C) An investigation was carried out into the nature of a coated titanium dioxide preparation; UV-Titan M160® (Kemira) The crystals were stated to have a diameter of 15 to 20 nm. This appears to have been nominal, since the crystals are acicular and in the investigated sample were much larger than stated. The process of coating for this product is to apply an aluminium coating chemically and then treat the particles by immersion in an emulsion containing stearic acid. After this the product is re-milled; it is not known how this second milling affects the coating layer. The investigator states that the stearate layer would so position itself on the particles that the stearate would be at the surface, and the formation of micelles and agglomerates would be expected. The product as tested had particles of 200 to 300 nm, thought to be due to agglomerates forming between the micelles. It was thought likely that a coating of (say) silicone would not form agglomerates of this kind, However, in the specimen under test, all the particles,

of whatever size, were found to be covered with stearate, so that photo-catalysis would be less likely to occur than in uncoated material.

Titanium Dioxide (T 817®, T 805®) was investigated on its properties and abilities of a possible influence on the photo-stability of UV-filter cpds used in cosmetic products. Such changes could be of interest as producing dermal incompatibilities by photo-reaction products and also reducing efficiency in UV-protection.

In "in vitro" systems using appropriate conditions - solar simulator (SOL 500), measuring UVA 320-400 nm → 1.7 mW/cm$^2$ and UVB 280-320 nm → 17 µW/cm$^2$ with a radiometer (IL 1700), resulting in about 2.5 MED/h. test samples of otherwise known photo-stable UV-filter/products were treated together with the test substance (purity > 97 % $TiO_2$ + 2 ± 1 % $Fe_2O_3$).

The results confirmed that Methylbenzylidene Camphor, Octyl Triazone and Octocrylene are photo-stable in the presence of Titanium Dioxide under these test conditions (> 95 % recovery of unchanged parent compounds after 10 MED with simulated sunlight).

Butyl-Methoxydibenzoylmethane is photo-stable under these test conditions (> 90 % when stabilized with 4 % Methylbenzylidene Camphor / 2 % Octyl Triazone, > 95 % when stabilised with 10 % Octocrylene). Without stabilisation the proportion of unchanged Butyl-Methoxydibenzoylmethane drops to 40 % after irradiation (reference).

No destabilisation of the investigated UV-filters can be recognised under these test conditions after the admixture of the commercial Titanium Dioxide types T 805® respectively T 817® to photo-stable UV-filter systems. In both cases the coating of the Titanium Dioxide is effective.

### 2.1.9. Solubility

Insoluble in water and other solvents. Some preparations can be made hydrophilic by suitable surface treatment.

### 2.2. Function and uses

The crystals of titanium dioxide are covered with various coating materials, which may be inorganic or organic; in this form they are proposed for use in sunscreen formulations.

## TOXICOLOGICAL CHARACTERISATION

### 2.3.1. Acute oral toxicity

(M/C) Titanium dioxide T805® was investigated according to GLP and in accordance with Council Directive 92/32/EEC. The material, suspended in arachid oil, was given by gavage as a single dose of 2150 mg/kg bw to 10 rats (strain Hsd/Win:WU), 5 males and 5 females. There were no toxic effects, and no deaths. Observation was for 14 days. There was no effect on body weights. Gross necropsy revealed no abnormalities. The $LD_{50}$ was > 2150 mg/kg bw.

Ref. : 5

(NM/UC) Titanium dioxide (code name H20762, duPont) in deionised water was administered as single doses, ranging from 670 to 11 000 mg/kg bw by gavage in the rat. Observation was for 14 days. There were no deaths and no signs of toxicity. The lethal dose was found to be > 11 000 mg/kg bw.

Ref. : 1

(NM/UC) Albino inbred mouse. A single dose of titanium dioxide, (10 000 mg/kg bw) suspended in water, was given by gavage to 10 mice. This was the highest dose that could be given because of the viscosity of higher concentrations. No toxic effects were produced. The $LD_{50}$ was estimated to be >10 000 mg/kg bw.

Ref. : 2

(NM/UC). Titanium dioxide in the form of P25 as a single dose of 10 000 mg/kg bw in the rat had no toxic effects. No further details are given. The $LD_{50}$ was estimated to be > 10 000 mg/kg bw.

Ref. : 3

(NM/UC) Titanium dioxide is said to have an $LD_{50}$ of greater than 25 000 as a single oral dose. No further details are given.

Ref. : 4

Titanium Dioxide T 817® was investigated according to GLP and in accordance with Council Directive 92/69/EEC following OECD No 401.
HsdCpb : WU-rats (5/5 per dose) received under “Limit Test”-conditions a (single) oral dose of 2.150 mg/kg bw (by gavage in two equal parts, time interval 3 hours) of the test substance, a fluffy powder (purity > 97 % $TiO_2$ + 2 ± 1 % $Fe_2O_3$) in olive oil. Observation period 14 days. The only side effect was a diarrhoea in some animals, obviously due to the used olive oil as a vehicle for the test substance. No other symptoms were noted.

As the result it was stated that, under the experimental conditions used, the $LD_{50}$ of the test substance is above 2.150 mg/kg for both sexes.

Ref. : 104

### 2.3.2. Acute dermal toxicity

(NM/UC) Rabbit: Approximate lethal dose > 10 000 mg/kg bw. No details about the experiment are given.

Ref. : 4

(M/?C: status doubtful) Ultra-fine titanium dioxide 40% in a mixture of mineral oil and glycerine, with dispersant (NP 88/296), was tested according to OECD 402 in 5 male and 5 female Sprague Dawley rats.
A single dose of 2000 mg/kg bw was applied once under occlusion for 24 hours. Clinical signs were confined to the day after dosing; the animals showed hypo-kinesia, ataxia, and chromodacryorrhoea, and were hot to the touch.
All animals were normal 2 days after dosing. There were no deaths. Body weight gains were as would be expected in this strain. Gross necropsy showed no abnormalities. The dermal $LD_{50}$ was estimated to be > 2 000 mg/kg bw.

Ref. : 6

### 2.3.3. Sub-chronic oral toxicity

(NM/UC) Titanium dioxide (anatase) was administered in the diet to groups of 10 male and 10 female F334 rats and to 10 male and 10 female B6C3FI mice for 13 weeks. The dose levels were 0, 6 250, 12 500, 25 000, 50 000, and 100 000 ppm. There were no deaths; body weights were not affected, and no gross or microscopic pathological changes were found which could be attributed to the test compound. This investigation was the basis for a chronic feeding study.

Ref. : 22

### 2.3.4. Chronic toxicity

(NM/UC) Rats were exposed to 10, 50 and 250 mg/$m^3$ of titanium dioxide dust for 24 months. In the top dose animals squamous cell carcinomas appeared.

Ref. : 4

## 2.4. Irritation & corrosivity

### 2.4.1. Irritation (skin)

(M/C) The commercial preparation T805 was tested according to GLP and 92/32/EEC in 3 Russian white rabbits. Two areas of the skin of the back were clipped the day before the experiment. One area was for the test, the other served as a control. A dose of 0.5 gram of the test substance was moistened with 0.64 ml of paraffin oil, and applied to the skin on the left side of the vertebral column and covered with an occlusive dressing for 4 hours. The site on the opposite side was treated identically except that no active ingredient was applied. Reading was at 24, 48 and 72 hours. There was slight erythema (grade 1) at 24 hours in 2 animals at 24 hours, absent at 48 hours. Slight oedema (grade 1 at 24 hours) was noted in one animal, absent at 48 hours. There were no systemic toxic effects. The primary irritation index was 0.3/8, so the material was judged to be non-irritant.

Ref. : 13

(NM/UC) Titanium dioxide was applied to intact and abraded skin in 4 male albino rabbits for 24 hours. The application seems not to have been occluded. The amount was 100 mg, applied once. Reading was at 48 hours. The substance was not irritating.

Ref. : 2

(NM/UC) A dose of 0.5 gm of titanium dioxide (code H-20762, du Pont) was placed on a gauze pad, and moistened with distilled water to the consistency of a paste. This preparation was applied to the shaved intact skin of 6 NZW rabbits (3 male and 3 female), covered with a semi-occlusive dressing and left in place for 4 hours. The sites were scored on a Draize scale 1, 24, 48 and 72 hours after removal of the dressings. Three rabbits showed no irritation. At 1 hour one animal showed mild erythema and two animals showed slight erythema. At 24 hours, slight erythema was observed in three rabbits. Slight erythema was observed in one animal only at 48 and 72 hours. The substance was judged to be slightly irritant under the conditions of the study.

Ref. : 10

(NM/UC). Titanium dioxide powder was tested in 6 albino rabbits. The animals were clipped free of hair and 0.5 grams of powder was moistened with physiological saline. It was applied on patches of 2.25 square inches and covered under semi-occlusive conditions for 24 hours. Observation was at 24 and 48 hours. No irritation was found.

Ref.: 11

(NM/UC) Titanium dioxide (99.5% pure) was tested in 12 male albino guinea pigs. The material was applied as a powder (0.5 gram) or as a 28% paste (0.1 gram) to the shaved intact skin and occluded for 24 hours; observation was at 24 and 48 hours. No irritation was found.

Ref.: 12

(M/UC) Two proprietary dispersions of titanium dioxide were tested according to GLP in the rabbit. The materials tested were micro-crystalline titanium dioxide in mineral oil/triglyceride with dispersing agent (NP89/98) and the same in octyl palmitate with dispersing agent (NP89/97), as supplied by the manufacturer. Control applications were not made. The hair was clipped from the dorsum of the animals 24 hours before the experiment, with care to avoid damage to the skin. Four sites, 2 on either side of the vertebral column, were delineated as test sites. The sites on the right side were abraded. Applications of 0.5 ml of the test materials were made, on a patch of filter paper 2.5 X 2.5 cm, to the intact and abraded skin of two of the sites; the other two sites served as vehicle controls. The sites were then occluded for 23 hours, at which time the dressings were removed and the test areas scored according to a conventional scheme. These applications were repeated (to the same sites) a further 4 times.
NP89/97: Slight well defined erythema was found at both intact and abraded sites. Very slight oedema was found at abraded sites only. Mean irritation score: 1.58.
NP89/98: Slight to moderate erythema was noted at both intact and abraded sites. Very slight oedema was noted at abraded sites only. Mean irritation score 1.92.
One rabbit died on day 4; this was not considered related to the treatment.

Ref. : 14

(M/UC) A study identical to the above (ref. 14) was carried out on a dispersion of ultra-fine titanium dioxide, 40% in mineral oil/triglyceride + dispersant (manufacturer's code NP 88/296). was tested in 3 NZW rabbits, except that two dilutions were used, viz., 40% and 10%. The undiluted preparation and a 10% dilution in mineral oil/triglyceride were tested. One rabbit showed no reactions; the other two showed only slight and non-persistent oedema. The mean irritation score for both applications was 0.13.

Ref. : 15

(NM/UC) In an attempt to improve the sensitivity of skin testing, and to shorten the time required for experimental procedures, a technique was developed which involved the production of a grid of abrasions on human skin, and the application of the test substance in an aluminium chamber sealed to the skin. The test material was applied daily for 3 days, apparently for 24 hours each time. Readings were made at each removal of the chamber, but the final reading, after 3 applications, was regarded as the most important. The subjects were 5 male and female volunteers. A very large number of substances was tested: one of these was titanium dioxide as a powder, which was found to have a very low irritancy.

Ref. : 16

Titanium Dioxide T 817® was investigated according to GLP and in accordance with OECD Guideline No 404 and EC Guideline 92/69 EEC; The Gilman et al-irritation index was used.

White Russian albino rabbits were treated on a dorsal clipped skin area with 0,5 g of the test substance (purity > 97 % + 2 ± 1 % $Fe_2O_3$) moistened with 1,0 g peanut oil under patch (6.25 $cm^2$) conditions for 4 hours. Observation time 1, 24, 48 and 72 hours after removal of the patches.

As the result there were no changes registered and it was stated that the test substance, under the conditions used, was classified : non-irritant.

Ref. : 108

In 21 healthy male and female human volunteers (30 - 76 y) an investigation on irritative effects on the skin of different preparations has been carried out, amongst which one preparation (Code K: PHYWIO 90 Micropigment Lotion LF 25/100/BDF contained micronised $TiO_2$. The product was administered in the pure undiluted state in a quantity of 0.75 µl using an occlusive epicutaneous adhesive patch testing method (Finn Chamber® with Scanpore®) on the back of the volunteers every day unless a skin reaction was noted (score registration).

Results: Only after 3 days consecutive applications very slight/doubtful reactions in 1 proband on day 3, moderate reactions in 2 probands on days 4 and 5 were registered.

In conclusion the author stated that the test product (Code K) under the conditions of the test was classified as “non irritant”.

Ref. : 131

### 2.4.2. Irritation (mucous membranes)

(NM/UC) Titanium dioxide with 2% or 3.7% antimony oxide (the presence of the two additives is stated in the Colipa summary, but does not seem to be mentioned in the original paper provided) was tested. It was placed in the rabbit eye. The material was judged to be slightly irritant due to physical rather than chemical properties. No other information is offered.

Ref. : 4

(NM/UC) Titanium dioxide in a dose of 100 mg was placed in the lower conjunctival sac of the right eye of a rabbit. The eye was rinsed with distilled water after 5 minutes. Reading was at 12, 24. 48 and 72 hours. The material was non-irritant.

Ref. : 2

(NM/UC) Titanium dioxide (manufacturer’s code number H-20762) of 100% purity was placed in the right lower conjunctival sac of 2 female NZW rabbits .The amount used was 100 mg. The left eye of each animal served as a control. Both eyes of one rabbit were rinsed after 20 seconds; the eyes of the other animals were not. Observations were 48 and 72 hours.
Moderate conjunctival redness and slight chemosis were found in both the rinsed and non-rinsed test eyes. Those eyes which were rinsed were normal after 1 day; those which were not were normal after 3 days. Scoring according to Draize gave a total score of 10 in the unwashed eye and 5 in the washed eye (total possible score 80). The substance was judged to be a moderate eye irritant.

Ref. : 7

(M/UC) Micro-crystalline titanium dioxide, (manufacturer’s code number NP/88/296) 40% in mineral oil/triglyceride + dispersant, was tested in 2 male and 3 female NZW rabbits according

to OECD 405 and GLP. A dose of 0.1 ml of the material was placed in the conjunctival sac. Observations were at 1, 24, 48 and 72 hours. Slight conjunctival redness (score 1) was noted at 1 hour and 24 hours in all animals. At 48 hours there was slight redness in the eye of one animal only. No abnormality was noted at 72 hours. The material was considered to be slightly irritant.

Ref. : 8

(M/C) A formulation of titanium dioxide, T805 was tested according to 92/32/EEC and OECD 405, and according to GLP, in 3 male white Russian rabbits. A volume of 0.1 ml was used. Rinsing was not carried out; the untreated eye in each animal served as a control. Observation was at 1, 24, 48 and 72 hours. No irritation was found.

Ref. : 9

Titanium Dioxide T 817® was investigated for irritant effects on the eye of White Russian albino rabbits under GLP conditions in accordance with OECD # 405 and EC Guideline 92/69 EEC. 11.5 mg to 16.8 mg (representing a volume about 0.1 ml of the test substance) (purity > 97 % $TiO_2$ + 2 ± 1 % $Fe_2O_3$) were applied into the conjunctival sac of the right eye of three rabbits and not rinsed. Observation time 1, 24, 48 and 72 hours after application.
As the result the corneae and the iris of all animals were without any findings in all animals. In the conjunctivae of two rabbits a slight injection was recorded only for one hour after application. Irritation index 0.3. Classification “non irritant” in the used test system.

Ref. : 106

## 2.5. Sensitisation

(M/C) A maximisation study was carried out in the guinea pig according to GLP and 84/449/EEC. Twelve male and ten female animals of the Pirbright White strain were used. Two control groups consisted of 3 males and 3 females each.. The test group was composed of 6 males and 4 females. The active ingredient was T805. It was suspended in light paraffin oil at a concentration of 0.5%. This had been determined to be the maximum non-irritating concentration (when administered intradermally) in a preliminary experiment.
An area 6 X 8 cm in the scapular area was prepared by clipping. Three pairs of intradermal injections each of 0.1 ml, were made: (1) Freund’s complete adjuvant (FCA) in physiological saline 1/1 (treatment and control animals); (2) active ingredient (T) or vehicle (C); (3) active ingredient + FCA 1/1 (T) or FCA + vehicle 1/1 (C).
On day 7, the scapular area was again clipped and a 10% aqueous solution of sodium lauryl sulphate was applied to the skin. On day 8, the area was exposed to a 30% solution of the test substance or the vehicle as appropriate and covered with an occlusive patch for 48 hours.
On day 21, areas of about 5 cm X 5 cm were clipped free of hair on both flanks of the animals. The left flanks of the test animals and the control group 1 animals were treated with 0.2 ml of the test substance at 5%, and the right flanks with 0.2 ml of the vehicle. These sites were covered occlusively for 24 hours. This procedure was repeated once. Observation was at 24 and 48 hours and scored on a Draize scale.

Results : There were no reactions of any kind following the epidermal challenge. There was no evidence of sensitisation. There were no signs of systemic toxicity; body weight gain was as would be expected

Ref. : 19

(NM/UC) A test was carried out in guinea pigs. The article describes a method involving 10 intradermal injections of a 0.1% solution of an active ingredient over three weeks; in the second and third weeks Freund's complete adjuvant is incorporated. After a 14 day rest, occlusive epidermal applications are made.

Ref. : 18

(M/UC) A Magnusson-Kligman maximisation test was carried out in guinea pigs according to GLP and in conformity with EEC and OECD guidelines. Thirty-six female guinea pigs were used, 20 test and 16 control. The active ingredient was a micro-crystalline titanium dioxide (Tioveil MOTG®). It was suspended in mineral oil/triglyceride + dispersant, the latter having been supplied by the manufacturer. The maximal non-irritant concentration of the active ingredient had already been determined in previous experiments with another batch of the same material.
Induction. The test material was a 10% suspension of a.i. Sites about 4 X 6 cm were prepared in the scapular area. The test animals were given 6 intradermal injections, 3 on each side, of (a) 0.1 ml of Freund's complete adjuvant (FCA); (b) 0.1 ml of test material; (c) 0.05 ml of test material emulsified with 0.05 ml of FCA. The control animals were similarly treated but with the vehicle in place of the test material. The sites were scored 1 and 24 hours after the injections. Six days later, the scapular site was again prepared and 10% sodium lauryl sulphate was applied. Twenty-four hours later, a 2 x 4 cm patch of filter paper, saturated with the test material at 100%, was applied and maintained under occlusion for 48 hours. The control animals were treated with vehicle only. The sites were scored at 1 and 24 hours.
Challenge. Two weeks later both test and control animals were challenged with concentrations of 100% and 50% of the a.i. applied to the prepared left flanks of the animals and occluded for 24 hours. Observation was at 24 and 48 hours after removal of the patches.
Results. The induction sites showed grade 1 changes at 24 hours, and grade 2 at 48 hours, with both test and control solutions The challenge sites showed no reaction of any sort.
There was no contemporaneous positive control, but animals of this strain are regularly tested with 2,4-dinitrochlorobenzene; at the most recent such test, 100% of the animals showed sensitisation.
It was concluded that the active ingredient was not a sensitiser.

Ref. : 20

(?M/UC) Five sunscreen formulations were tested. They were: NP 89/350 Tioveil MOTG®; NP89/351 Tioveil OP®; NP89/352, Tioveil AQ®; NP89/407 and NP89/466. The first three preparations contain 40% titanium dioxide with dispersant in carrier; the carriers are, respectively, mineral oil/triglyceride, octyl palmitate and water. The fourth and fifth are sunscreen formulations containing 10% titanium dioxide.
The subjects were 76 human volunteers who completed the test, out of an original number recruited of 83 (21 males and 62 females). No subject dropped out because of reactions to the test material. The method of testing was the Shelanski repeated insult patch test. The test materials, as supplied by the manufacturer, were applied on 2 x 2 cm patches on the lateral surface of the upper arm. Exposure was for 24 hours. The patches were initially applied in a random sequence, but after that each subject had the same material applied to the same site throughout. Patches were applied on Mondays, Wednesdays and Fridays for the first three 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 hours.
There were some mild erythematous reactions during the induction phase of the trial. There were no reactions to the challenge. The materials tested were judged not to cause sensitisation under the circumstances of the experiment.

Ref. : 21

(NM/UC) A study in man of 29 potential allergens commonly found in cosmetics was carried out. Among those tested was titanium dioxide. Among other materials, a 5% preparation of titanium dioxide in petrolatum was used to test 918 patients suffering from various diseases of the skin. Patches of filter paper were applied to the skin of the patients and occluded; the contact time was 48 hours. Titanium dioxide did not cause any reaction. The same investigators tested a similar range of allergens in 50 healthy volunteers, and again found no reactions to titanium dioxide.

Ref. : 17

Titanium Dioxide T 817® was investigated according to GLP in the Buehler test in order to determine its sensitising properties.. The method used was in accordance with OECD No 406 and EC Guideline 96/54 EEC.
HsdPCC: DH Guinea pigs (20Е/20Г) were used, dived in two vehicle (paraffin oil), control groups (5/5) and 1 test substance (purity > 97 % + 2 ± 1 % $Fe_2O_3$) group (10/10).

Following a proper epidermal challenge neither treated nor control animals showed any changes at the exposed skin. It is concluded that, under the test conditions used, Titanium Dioxide T 817® has no sensitising properties.

Ref. : 110

A test for primary or cumulative irritation and skin sensitisation in human volunteers was performed with test material 0685115 (Repeated Insult Patch Test).
Due to the lack of specification of the test material included in the study, SCCNFP was of the advice to consider this study inadequate.

Ref. : 129

## 2.6. Reproductive toxicology

An in vitro test to investigate the release of DNA by $S_1$ nuclease with $TiO_2$ produced negative results. The scientific value of the test was considered of any relevance by SCCNFP.

Ref. : 36

## 2.7. Toxicokinetics (incl. percutaneous absorption)

(M/C) An investigation into the absorption of the active ingredient (a.i.) was carried out in pig skin *in vitro*, using Franz cells; the skin was thereafter stripped and the distribution of the a.i. studied by electron micrography.
For the first part of the investigation, the skin was placed in the cells, with an exposed area of approximately 5 $cm^2$. The temperature was maintained at $32^o$. The a.i. was formulated in an oil in water emulsion, containing 4% T805®. This is a coated preparation with a mean diameter of 20 nm, although electron micrography at a later stage of the experiment showed formation of agglomerates. The receptor fluid was saline with gentamicin and 1% bovine serum albumin. The application was at a rate of 4 mg of formulation per square centimetre. The cells were allowed to stand for 24 hours. Thereafter, the skin was cut into small areas by use of a punch and prepared for electron microscopy (both transmission and scanning); the identification of titanium in the sections was by energy dispersive X-ray analysis (EDXA) using a titanium-specific energy window.

Results. Surprisingly, no chemical investigation of the receptor fluid is reported. The authors studied the distribution of the a.i. by electron micrography. The a.i. was confined to the stratum corneum. It penetrated the outer portions of the hair follicles in minute amounts, but was not found in any of the cells lining the follicle. [No mention is made of sweat glands; this may be because of anatomical differences between pig and human skin]. Repeated stripping suggested that the a.i. was found initially on the ridges of the skin surface; as stripping continued, there was access to the material found in the furrows. The authors suggest that this gradually declining concentration of the a.i. with successive strippings is not to be interpreted as a penetration of the a.i. into the stratum corneum, but rather to a differential sampling of the skin, so that the early strippings, taking up the material on the ridges of the skin, show a higher amount of a.i. than the later strippings, which are derived from the furrows, and which contain a smaller amount of the a.i.
A study was also carried out *in vivo* on human forearm, which is stated to have confirmed the above findings, but this study is not reported in the present submission.

Ref. : 132

(?M/C) A proprietary preparation, Tioveil AQ-N, was spread on the forearm skin at a rate of 2 $mg/cm^2$. The formulation contained titanium dioxide coated with aluminium oxide and silica. The concentration of the titanium dioxide preparation is not given. Areas of skin had applications made 5 times on days 1, 2, and 3, and once on day 4. The sites were protected by fabric (apparently not occlusive). Estimation of the amounts of titanium dioxide were by X-ray fluorescence spectroscopy. Stripping was carried out 1, 24, and 96 hours after the last application. The amounts of titanium dioxide found on the various strippings ($\mu g/cm^2$ of tape) were, at one hour, 31 in the first stripping, becoming progressively less with each stripping, and as low as 0.08 in the deepest layer of the stratum corneum. After 24 hours, the figures were 2.8 to 0.04 (at about 50% of the depth of the stratum corneum); after 96 hours, the amounts ranged from 0.94 to 0.01 in the deepest layer of the stratum corneum. The author concludes that the material does not penetrate beyond the stratum corneum. The investigation, however, suggested that there were some titanium dioxide particles at the openings of the follicles.

Ref.: 63

(M/C) The experiments were performed according to GLP. The test substance was T805. The formulation contained 5% of the active ingredient. Skin samples were obtained post mortem from 3 female donors and one male. The ages of the donors ranged from 23 to 70 years. Fat was removed from the samples, and they were stored at $-20^o$ until required. Before the experiment, the skin was allowed to thaw, and the sample immersed in water at $60^o$ for a minute The epidermis was then removed with a forceps, and mounted in a cell; the formulation was applied at a rate of about 3.6 $mg/cm^2$. The area of the exposed skin was 0.32 $cm^2$. The receptor fluid was physiological saline with tetracycline hydrochloride 1 µg/ml, and was perfused at a rate of 1.5 $ml.h^{-1}$. The integrity of the membrane was checked by the use of tritiated water and calculation of the permeability coefficient for each skin. Skins with coefficients greater than $1.5 \times 10^{-3}$ cm. $h^{-1}$ were excluded. The diffusion was allowed to proceed for 8 hours. Following the diffusion process, the skins were fixed for electron microscopy.
The active ingredient was looked for in the receptor fluid using inductive coupled plasma mass spectrometry. This technique involves the introduction of the samples of receptor fluid as a spray into inductively coupled argon gas plasma, which converts (e.g.) titanium into an ion and allows quantitation according to the mass/charge quotient. The method gives a result linearly related to

the concentration of the analyte in the sample, and the limit of detection is 1.5 ng/ml in the original sample. The skin was also examined by transmission electron microscopy.
No titanium dioxide was found in the receptor fluid within the limits of detection. Using electron microscopy, the titanium dioxide was found to be confined to the outer layers of the stratum corneum. It was concluded that the active ingredient was not absorbed by human skin *ex vitro*.

Ref. : 24

(M/C). Commercial preparations (Eusolex TA® and Eusolex TC®) were tested using human abdominal epidermis in Franz cells. The active ingredient was 5% micro-crystalline titanium dioxide. The material is said to be coated, but the nature of the coating is not stated. Sixteen samples of skin (4 from each of 4 specimens of skin) were used in each of the tests of the formulations. The preparations were commercial formulations of the active ingredient in a sunscreen. The formulation was applied to the skin at a rate of 3.19 to 4.28 mg/cm$^2$. The receptor fluid was physiological saline. The receptor fluid was analysed for titanium by ICP-OES; the minimum detectable was 50 ng (no further details of the method are given). No titanium dioxide was found in the receptor fluid.
Microscopic examination of the skin samples showed no traces of titanium dioxide from Eusolex TA except for one skin which showed two particles located intracellularly in the stratum granulosum, but these may have been cellular detritus rather than titanium dioxide. In the case of Eusolex TC, titanium dioxide was found only on the outer surface of the stratum corneum.
It was concluded that the titanium dioxide did not penetrate the skin under the circumstances of the experiment

Ref. : 25

(M/C) Eusolex TC® was tested in the chorio-allantoic membrane of the hens egg. Three test groups, each of 3 eggs, and a control group of 2 eggs, were used. After 9 days of incubation, a 5% suspension of the material in water was applied to the egg membrane and to the chorio-allantoic membrane for one hour in each case; in addition, the test solution was applied to the chorio-allantoic membrane for 24 hours. The vehicle was applied to each of the membranes for 1 hour. The membranes were fixed and examined microscopically. There was no evidence of penetration of the test material. Photographs of the histological findings are mentioned, but do not appear in the submission. This alternative method has not been validated.

Ref. : 26

(M/C, but nature of coating not specified). Male and female volunteers with skin types I, II and III were used. Eusolex TA® and Eusolex TC® were applied at a rate of approximately 2 mg/cm$^2$ to lower forearm skin for 30 minutes. [The title of the submission states that abdominal skin was used, but this is nowhere found in the body of the submission]. The area was then stripped, and the strippings examined microscopically for evidence of deposition of titanium dioxide; in case of doubt, EDX (energy dispersive X-ray) analysis was also carried out. A 10% aqueous dispersion of titanium dioxide applied to the forearm was used as a positive control; stripping of this area showed titanium dioxide in the top two strippings. The vehicle was used in another subject as a negative control.
Titanium dioxide from the formulations penetrated only to the first few strippings (none beyond the fourth layer) and very few particles were found deeper than the second layer.
It was concluded that titanium dioxide from the formulations under test did not penetrate the skin.

Ref. : 27

(M/C, probably) The test material was Hombifine S35, an 8% formulation of titanium dioxide. Human and mouse skin, from a commercial source, were used in a Franz cell. The skins were

both stripped and unstripped; the former preparation was intended to mimic the condition of damaged skin. After diffusion for 24 hours, the skin and the receptor fluid were dried and fused with potassium bisulphate to solubilise the active ingredient. Estimation was by atomic absorption spectroscopy. The limit of detection was 2 µg/ml. The material was spread on the skin in a layer 400 microns thick and allowed to dry; the pH of the receptor fluid was adjusted to pH 7.4 and 5 in different experiments. In no case was any titanium dioxide found in the receptor fluid. The skin contained a varying amount of titanium dioxide (about 15%), but the greatest amount was recovered from the surface. There was no evidence of penetration of titanium dioxide through the skin.

Ref. : 28

(?M/?C) A test in 4 human volunteers was carried out using 3 preparations of titanium dioxide. (A). Tioveil AQG®. This was made up in an o/w lotion at 5% a.i. (B). Tioveil TG®. This was made up in a w/o cream at 7.5% a.i. (C). Tioveil OP®. This was made up as an o/w lotion at 7.5% a.i. A dose of 2 µl/cm$^2$ of each of the above preparations was applied to 4 cm$^2$ test areas on the forearm, and occluded for 8 hours. Thereafter, skin biopsies were taken from the treated areas by applying a microscope slide coated with a thin film of cyanoacrylate adhesive. This procedure was repeated 4 times. In addition, a sample was taken from an untreated area of skin. Each stripping removes a layer of stratum corneum about 2 µm thick. These samples were then analysed by X-ray microanalysis. In addition, the samples were subjected to X-ray fluorescence spectrophotometry and also digestion +solubilisation, followed by atomic absorption spectrometry. The last two procedures were carried out by the manufacturer.
No subject showed any titanium deeper than the first two strippings; in some samples titanium dioxide was found only in the first layer. The amounts were small: maximally about 0.6% of the amount applied.

Ref. : 29

(M/C) An oil in water cream containing 5% Titan M160® was tested according to GLP, using human abdominal skin in a flow through cell. Seventeen samples of epidermis taken from 3 specimens of abdominal skin were used for the test procedure; 4 samples were used as controls The methods used in this part of the experiment were the same as those described in reference 46 (above). The test material was applied to the epidermis at a rate of 2 mg/ml, and allowed to diffuse for 8 hours. The limit of detection was 1 µg/l. There was no evidence of absorption of the active ingredient. The analyses of the receptor fluid were carried out by inductively coupled plasma atomic absorption spectrometry (ICP-AES) by the manufacturer.
The skin samples were fixed at the end of the experiment and embedded in epoxy resin. The results of examination of these specimens are not reported

Ref. : 30

(NM/UC) Human abdominal skin, both stripped and unstripped, was used in a diffusion cell. The surface area of the skin was 1.13 cm$^2$. The receptor fluid was physiological saline containing 0.1% $NaN_3$. An emulsion containing 10% titanium dioxide was applied in a dose of 10 mg, and the receptor fluid sampled at 4 and 8 hours. After 24 hours, the skin was washed and dried, and the washings and the skin analysed for titanium dioxide. The epidermis was then separated from the skin to enable the determination of the amounts in each. The skin was also subjected to electron microscopy. The method of estimation of titanium dioxide is stated to be inductively coupled plasma atomic absorption spectrometry, with a detection limit of 30 ppb.
Electron microscopy showed that the titanium dioxide was confined to the surface of the skin; there was no penetration of the stratum corneum or the epidermis, whether the skin had been

stripped or not. No titanium dioxide could be detected in the receptor fluid, or in the separated dermis, at 24 hours.

Ref. : 31

M/C An oil-in-water emulsion containing UV-Titan-M160® was tested. The concentration of active ingredient is not stated. The preparation was applied to the left forearm of human volunteers in a dosage of 2 mg/cm$^2$. The areas treated were demarcated in each case and measured 160 cm$^2$. The applications were made 5 times on the first, second and third day, and once on the fourth day. The opposite forearm had the formulation without titanium dioxide similarly applied as a control. One hour after the completion of the application(s) on each day, the area was stripped with adhesive film, and the stripping repeated until the strips were free of corneocytes. Biopsies of the treated area were taken following stripping, to avoid any contamination of the biopsies by titanium dioxide remaining on the more superficial layers of the stratum corneum. It is not clear how many biopsies were taken. The number of corneocytes on each stripping was measured by UV/VIS spectroscopy. The authors state that this method enabled each stripping to be referred to a specific layer of the stratum corneum. The amount of titanium dioxide on each tape was extracted into chloro-nitrous acid and determined by quantitative X-ray fluoroscopy. The distribution of titanium dioxide on the tapes was determined by space-resolved Raman spectroscopy. The titanium dioxide particles on the tape were determined by laser fluorescence and reflection: by these means the contribution of the coating could be distinguished from that of the active ingredient. The biopsies consisted of dermis only; the amounts of titanium dioxide were determined by X-ray fluoroscopy using an electron microscope. The presence of a follicle in an area where titanium dioxide occurred could be determined by the electron microscope.
Results. The strippings following repeated applications of the formulation showed that most of the titanium dioxide occurred in the superficial part of the stratum corneum. Nevertheless, there was some titanium dioxide to be found in the deepest strippings. Electron microscopy showed that the titanium dioxide in the deepest layers was associated with the follicles only. The authors point out that the follicles also are surrounded by stratum corneum, so that there was no penetration of the active ingredient into the dermis proper.
The technical aspects of this paper are unusual, and in some cases of recent development, so that a critical analysis is difficult. It would seem, however, that the methods used allowed a confident statement that titanium dioxide does not penetrate to the dermis, and so is not absorbed by the body.

Ref.: 62, 70

In “in vitro”-studies a 5 % cream preparation of micronised Titanium Dioxide T 805® and human skin samples in diffusion cells (0.32 cm$^2$) - estimated applied dose of formulation was ~3.55 mg. cm$^2$ - were used for an estimation of the percutaneous absorption rate of the test substance ($TiO_2$). The used methodology follows acknowledged rules and is described appropriately in detail. A total of 14 samples of epidermis from 4 different donor abdominal epidermis specimen were used. Chemical analyses of Ti-cpds were carried out by IPCMS.

In addition to normal methodology, after the exposure described, Transmission Electron Microscopy (TEM) of the skin samples was carried out.
As a result of all procedures applied, it could be shown that no statistical relevant differences of titanium contents between samples and blank solutions were detected in the receptor fluids. The result of the TEM revealed that $TiO_2$ was only present in the outer layers of the stratum corneum.

In conclusion it can be stated that, under the used experimental conditions, $TiO_2$ (T 805®) did not penetrate through human epidermis in vitro.

Ref. : 112

In 25(29) healthy male and female human volunteers (18 - 60 y) the photoallergic potential of the test material ($TiO_2$, 0685115) was determined by repetitive epidermal contact. The conditions of the test: inclusion and exclusion criteria as well as the used methodologies are appropriately described.

The application of the test substance was made on the lower back, between scapulae and beltline.

A Xenon Arc Solar Simulator (150w) was used as the source for the ultraviolet light (290 - 400 nanometers [nm]) for 3 min each exposure. During the challenge phase a Schott WG 345 filter was used to block the range 290 - 320 nm. Individual sensitivity was obtained by a determination of a progressive sequence of timed UV light exposures.

The induction phase was carried out with approx. 0.2 ml of the test material to a 1"x1" gauze portion of an adhesive dressing (semi-occluded patch) two times a week for 3 weeks (6 inductions). The challenge phase, approximately two weeks following the last induction evaluation was carried out in a similar way. All of the challenge sites were evaluated at 24h, 48h and 72h following irradiation.

In an evaluation of all readings and the conditions of this study, the test material did not induce a response indicative of a photo-allergic reaction.

Ref. : 130

## 2.8. Mutagenicity

(NM/UC) A study was carried out in CHOK1 cells, to determine whether titanium dioxide could produce sister chromatid exchanges or micronuclei. The titanium dioxide used was obtained from Merck; it is not stated to have been coated, and probably was not a coated preparation. It was suspended in DMSO at a concentration of 1 or 2 mM. Up to 50 μM of this suspension was added to the culture medium, and the final DMSO concentration adjusted to 0.5%. Under these circumstances, no precipitate could be seen using a phase contrast microscope. The concentrations of titanium dioxide in the culture medium were 0, 2, 5 and 10 micromolar. The culture medium contained rapidly growing cells, and was allowed to incubate for 24 hours. Following centrifugation, repeated washing and overnight digestion with nitric acid, the amount of titanium dioxide in the cells was determined by atomic absorption spectroscopy. It was found that there was a dose related accumulation of titanium dioxide in the cells (up to 4.5 ng/$10^6$ cells).
A test for cytotoxicity was carried out using the concentrations of titanium dioxide used above, and incubating the cells for 24 hours; 200 cells per dish were then replated. Titanium dioxide was non-toxic at 10 micromolar, and even at 20 micromolar the survival of the cells was 93%. Sister chromatid exchanges were looked for at concentrations of titanium dioxide of 0 to 5 micromolar. Significantly increased SCE frequency was found at all concentrations of titanium dioxide, reaching $p < 0.01$ at 5 micromolar.
Micronucleus formation was studied using 0 to 20 micromolar titanium dioxide. Cytochalasin B was used to induce cytokinesis block. There was a highly significant increase in micronuclei ($p < 0.001$) at all levels of titanium dioxide. This may be explained by possible strain differences in

the cells used. The mechanisms underlying the present findings may be due to the release of reactive oxygen radicals.

Ref. : 65, 66

P-25, anatase type Titanium dioxide particles (Nippon-Aerosil, Tokyo, Japan), WA Anatase type 255 nm particles size (WACO, Tokyo, Japan), TP-3, rutile type, 420 nm particle size (Fuji Titan, Kanagawa, Japan) and WR, rutile type, 255 nm particle size (WACO, Tokyo, Japan) have been tested for the evaluation of their photo-genotoxic potential in the *Salmonella*/microsome assay, on TA100, TA98 and TA102 strains, in the mammalian *in vitro* cell gene mutation assay on L5178Y $tk^{+/-}$ cell line (mouse lymphona), in the SCG (Single Cell Electrophoresis) method on L5178Y $tk^{+/-}$ mouse lymphona cell line and in the *in vitro* chromosome aberration assay on CHL/IV cells under UV light produced by a sunlight simulator (Sol 500 with filter, with 50% transmission at 333 nm UVA : UVB = 25:1) at doses of 1-5 J/$cm^2$.

The results of these tests were : negative evidence on *Salmonella*/microsome assay and *in vitro* mammalian gene mutation; positive evidence in the SCG (Comet assay) and in the *in vitro* chromosome aberration assay.

Ref. : 134

(NM/UC) Titanium dioxide, a commercial product of 98.5% purity, was used in an Ames test, using strains TA98, TA100, TA1535,TA1537 and TA1538, with and without activation. This test was carried out as part of a much larger programme to study the reproducibility of microbial assays for mutagenicity. The assays were carried out in 4 laboratories. The chemicals tested were those which had already been studied for carcinogenic activity in NCI/NTP programmes. Three sources of S9 were also tested: those deriving from rat, mouse, and hamster liver. There was no evidence of mutagenesis with titanium dioxide.

As part of the same investigation, titanium dioxide was tested in *E. coli* WP2 uvrA. There was no evidence of mutagenic activity.

Ref. : 32

(NM/UC) A programme of testing a large range of chemicals for mutagenic activity was carried out. These had previously been tested for carcinogenicity in rodent assays. The following tests for *in vitro* gene mutation were carried out: an Ames test using 4 - 5 strains of *Salmonella typhimurium*; a test in the mouse L5178Y lymphoma $TK^{+/-}$; a test for the production of chromosomal aberrations in CHO cells; and an assay for sister chromatid exchange in CHO cells.

These tests were carried out with and without activation. Among the chemicals tested was titanium dioxide. No mutagenic effect was found with the compound.

Ref. : 33

(NM/UC) In the course of testing of a large number of compounds for mutagenic activity, titanium dioxide was tested in a mouse lymphoma cell (L5178Y) mutation assay, with and without activation. A suspension of the material in water was added to the cultures to give a dose range of 1.56 to 50 µg/ml. The results were negative, and so the experiment was repeated using activation. The results were negative.

Ref. : 34

(NM/UC) In a programme to try to correlate rodent carcinogenicity studies with tests for mutagenesis in a very large number of chemicals, a commercial preparation of titanium dioxide (98.5% pure) was tested for ability to induce chromosomal aberrations and sister chromatid exchange in CHO cells, with and without activation. Tests were carried out according to a conventional protocol, and both positive and negative controls were used. The tests were carried out with and without activation. The tests were negative.

Ref. : 35

(NM/UC) In the course of an investigation of the effects of various types of asbestos fibre on the incorporation of tritiated thymidine into cultured embryonic lung fibroblasts, several other substances, not suspected of being carcinogenic, were studied. Among these latter was titanium dioxide. There was no evidence of increased DNA synthesis.

Ref. : 37

(NM/UC) In the course of a series of investigations into possible *in vitro* tests for carcinogenic metals, titanium dioxide was tested. The test system was Syrian hamster embryo cells infected with the simian adenovirus, SA7. The active ingredient was dissolved in acetone/water 1/1 and incorporated into cultures of the cells in concentrations up to 12.5 mM. Those cells showing enhancement of virus transformation were distinguished by viral foci in the cell. Titanium dioxide had no such effect.

Ref. : 38

(NM/UC) The effect of various types of mineral fibres on the morphological transformation of cultures of Syrian hamster cells was studied. In addition to the fibres, titanium dioxide was studied. There was no evidence of transformation with the latter.

Ref. : 39

(NM/UC) In the course of an investigation into the use of cultured embryonic Syrian hamster cells to evaluate various carcinogenic metals, titanium dioxide was included. Morphological changes in the colonies were used as the end point. Titanium dioxide showed no such activity.

Ref. : 40

(One preparation M/C; the other NM/UC). In the light of evidence that a preparation of titanium dioxide (Degussa P25) can cause lung tumours in rats following inhalation, and that titanium dioxide can potentiate the carcinogenic action of asbestos and benzo(a)pyrene, two preparations of titanium dioxide, UV-Titan M160 and Degussa P25 were tested *in vitro*. The experimental method was the production of micronuclei in cultured rat liver epithelial cells. Pigmentary titanium dioxide and mitomycin-C were also tested. The Titan M160 was washed in ethanol to remove the stearic acid, in order to produce satisfactory suspensions, so its coating status is uncertain. The tests were for capacity to produce micronuclei and a single cell electrophoresis assay using rat liver epithelial cells. Cytotoxicity was tested for by the use of cytochalasin B to determine the number of cells with one nucleus only. On the basis of this test, doses of 0, 5, 10 and 20 $\mu g/cm^2$ were used in the main test. No chromosomal or DNA damage was produced. In an additional experiment, the cultures were exposed to ultraviolet light at 365 nm. The intensity is not specified. No mutagenic effect was produced by this procedure.

Ref. : 41, 42

(NM/UC) Commercial titanium dioxide was used in a somatic mutation assay (wing spot test) in *Drosophila melanogaster*. No evidence of genotoxicity was found.

Ref. : 44

### 2.8.1 Mutagenicity, photo-mutagenicity and photo-toxicity *in vitro*.

Different types of $TiO_2$ particles have been tested for mutagenicity, photo-mutagenicity and photo-toxicity by different validated *in vitro* methods. The results are reported in the following tables.

**Table 1**

| | **Rutile/Anatase** | |
|---|---|---|
| | **PSMA 1** | |
| *Trade Name* | T805 DEGUSSA20/80 RU/AN | T817 DEGUSSA79/12/2 RU/AN/Fe |
| *Crystal size* | 21 | 21 |
| *Coating* | SILICON DIOXIDE <2.5 % | SILIC. DIOX. <2.5 % DIIRON TRIOX 2 % |
| *Photo-mut.* | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) |
| *Mutagen.* | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) |
| *Photo-tox.* | NRU (neg.) | NRU (neg.) |

PSMA 1 to 6 : code names referring to the crystal types and to the various coatings
BAC : bacterial assay
CA : Chromosome aberration
NRU : Neutral Red Uptake Photo-toxicity test

Ref. : 67, 113-116, 121-124, 126, 127

**Table 2A**

| | **Rutile** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PSMA 2** | **PSMA 4** | | **PMSA 5** | | | | | |
| *Trade Name* | EUSOLEX 2000 MERCK | UV-TITAN M262 KEMIRA | UV-TITAN M212 KEMIRA | UV-TITAN M160 KEMIRA | UV-TITAN X161 KEMIRA | MT-100TV MITS/TAY | MT-100Z MITS/TA | MT-100T-L-1 MITS/TA | SOLAVEIL FINE PART. POWDER UNIGEMA |
| *Crystal size* | 14 | 20 | 20 | 17/20 | 15 | 15 | 15 | 15 | 10-28 |
| *Coating* | ALUM. 8-11 % SOMETHICONE 1-3 % | ALUM. 5-6.5 % DIME-THICONE 1-4 % | ALUM. 5-6.5 % GLYC. 1 % | ALUM. 5.5-7.5 % STEAR. ACID. 10 % | ALUM. 8.5-11.5 % STEAR. ACID. 10 % | ALUM. 1-15 % ALL. STEAR. 1-13 % | ALUM. 6-10 % STEAR. ACID. 10-16 % | ALUM. 3.3-7.3 % STEAR. ACID. 5-11 % | ALUM. 5-15 % ALL. STEAR. 5-15 % |
| *Photo-mut.* | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) | BAC (neg.) No data | BAC (neg.) No data | No data No data | BAC (neg.) CA (neg.) | No data No data | No data No data | No data No data |
| *Mutagen.* | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data |
| *Photo-tox.* | No data | No data | No data | No data | No data | NRU (neg.) | No data | No data | No data |

**Table 2B**

| | **Rutile** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **PSMA 5** | | **PSMA 6** | | | | | |
| *Trade Name* | MT-100TV MITS. TAYCA | UV-TITAN x-200 KEMIRA | SOLAVEIL FINE PART. POWDER UNIGEMA | MT-100SA MITS/TA | MT-500SA MITS/TA | MT-100AQ MITS/TA | TTO 51C MERCK | TTO 51A MERCK |
| *Crystal size* | 15 | 20 | 11-28 | 15 | 35 | 15 | 35 | 35 |
| *Coating* | / | / | ALUM. 10.5–12.5% SILICA 3.5 – 5.5 % | ALUM. 4 – 7.5 % SILICA 2 – 4 % | ALUM. 1– 2.5 % SILICA 4 – 7 % | ALUM. 4 – 8 % SILICA 7 – 11 % | ALUM. 11 % SILICA 1 – 7 % ST.AC. 3 – 7% | ALUM. 11 % SILICA 1 7 % |
| *Photo-mut.* | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) | No data CA (neg.) | No data No data | No data No data | No data No data | BAC (neg.) No data | BAC (neg.) No data |
| *Mutagen.* | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data | No data No data |
| *Photo-tox.* | NRU (neg.) | NRU (neg.) | No data | No data | No data | No data | No data | No data |

**Table 2C**

| | **Anatase** | | |
|---|---|---|---|
| | **PSMA 3** | | |
| *Trade Name* | MIRASUN TiW60 RHODIA | AFDC KEMIRA | MIRASUN TIWGO RHODIA |
| *Crystal size* | 60 | 200 µ | 60 |
| *Coating* | ALUM. 3 – 7 % SILICA 12 – 18 % | / | / |
| *Photo-mut.* | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) | BAC (neg.) CA (neg.) |
| *Mutagen.* | No data No data | No data No data | No data No data |
| *Photo-tox.* | No data | NRU (neg.) | No data |

Ref. : 50-52, 69, 73-83, 86-91, 97-102

## 2.9. Carcinogenicity

(NM/UC; used as a coating on mica particles) Fischer 344 rats were treated with titanium dioxide coated mica consisting of 28% titanium dioxide and 72% mica. The material was administered in the feed. The doses used were 0, 750, 1 500 and 3 700 mg/kg bw/day. The number of animals used was 480 - 60/sex/dose. Feeding was carried out for 130 weeks.
The results showed no important differences between the groups. Body weights and body weight gains were unaffected. There was an increased mortality in female animals at the low dose, but this only occurred in the last two weeks of the trial. Haematological and clinical chemistry showed no important changes. There was a slightly increased incidence of adrenal medullary hyperplasia in high dose males only, without evidence of progression to phaeochromocytoma. There was also an increased incidence of cataract in male rats only; this was attributed to viral infection. It was concluded that there was no evidence that the material under test was carcinogenic when administered in the diet under the circumstances of the experiment.

Ref. : 23

(NM/UC) Fisher 344 rats were subjected to a 103 week feeding study using titanium dioxide (anatase). The number of animals was 50/sex/dose. The doses used were (ppm) 0, 25000 and 50000, equivalent to 0, 1875 and 3750 mg/kg bw/day. The end of the feeding study was followed by a one week observation period. All animals were subjected to autopsy, with the exception of a few which died during the procedure, and were cannibalised or too autolysed for satisfactory autopsy. A large number of tissues was fixed for histo-pathological examination.

There was an increased "hunched" appearance in dosed animals from week 87. White faeces were noted in the dosed animals. There were no differences in body weights between the groups. The number of surviving animals did not show any statistical differences between the groups. In female rats, C-cell adenomas and carcinomas of the thyroid occurred at a dose related incidence, but were not significantly increased over control animals. (Control 1/48, low dose 0/47, high dose 6/44. Similarly, in the females, the incidence of stromal endometrial polyps was higher in female rats in the dosed groups (control, 7/50, low dose 15/50, high dose 10/50), but the differences were not statistically significant. Other tumours found were randomly distributed, and were known to be common in this strain of rat.
There was no evidence of carcinogenesis.

Ref. : 22

(NM/UC) A similar test to the preceding (22) was carried out in the same laboratory at the same time in B63CF1 mice. The main difference was that the dose levels were (mg/kg bw/day) 0, 3 750 and 7 500. Again, white faeces were observed in dosed animals. There were no effects on body weight, but the mortality in female mice was significantly higher than in controls (Survivals: males: control, 32/50; low dose, 40/50; high dose, 40/50; females: 45/50, 39/50; 33/50). There was no significant increase in tumours in the test compared with the control groups.
There was no evidence of carcinogenesis.

Ref. : 22

The above experiments (references 22 and 23) were carried out using pigmentary titanium dioxide. Colipa has obtained the values for the distribution of particle size of the titanium dioxide used in the these experiments, and reports that about 10% of the material had a small crystal diameter. Thus the NOAEL found in these experiments could be calculated to give a value of one-tenth if present day small crystal material had been used - about 375 and 750 mg/kg bw/day in rats and mice respectively.

## 2.10. Special investigations

(M/C) A test for photo-irritation was carried out using the preparation T805. The test animals were 3 female NZW rabbits. Single applications of the material in ethanol in concentrations of 3%, 10% and 30% were made to the skin of both flanks for 100 minutes, without occlusion; one flank was irradiated with UVA, 310 to 420 nm, for 50 minutes, to administer 10 J/cm$^2$. The areas were inspected at 30 minutes, and 24, 48 and 72 hours. There were no signs of photo-irritation.

Ref. : 45

(M probably/UC) A preparation of micro-crystalline titanium dioxide in mineral oil/triglyceride + dispersant (code NP89/367; code for vehicle NP 89/310) was tested in 20 female Dunkin Hartley albino guinea pigs (10 test and 10 control). The material was applied to areas of 2 cm$^2$ on the skin of the back in concentrations of 2%, 5%, 10% and 25%. UVA, 320-400 nm was applied in a dose of 20 J/cm$^2$. Observation was at 24, 48 and 72 hours after irradiation. There was no evidence of photo-irritation. As a positive control 8-methoxypsoralen was also applied. The response to 8-methoxy-psoralen is not stated.

Ref. : 46

(M/C) A 10% dispersion of titanium dioxide, of which the particles were coated with silica, alumina and cerium, was tested in male Dunkin Hartley guinea pigs (4 test, 3 control). The

crystals were anatase, 40 to 50 nm in diameter. The material was applied to a shaved area of the skin of the back, and the test area then irradiated with a sub-erythemogenic dose: UVA 9 J/cm$^2$ and UVB 0.1 J/cm$^2$. The wavelengths were 365 and 312 nm respectively. Observation was at 24 and 48 hours after the challenge. No evidence of any effect was found, with or without irradiation. The substance did not cause photo-irritation.

Ref. : 47

(M/C) A test for photo-irritation was carried out using the preparation T817 (highly dispersed hydrophobic titanium dioxide and iron oxide). The test animals were SPF New Zealand white albino rabbits.
Three male rabbits were treated with T.O. + UV irradiation; 8-MOP was used as positive control. Evaluations were made ½, 24, 48 and 72h after treatment on the back flanks of the animals, removed from the hair.
0.05 ml of a 20 % dilution of the T817 in 96 % ethanol were used for the treatment.
The dose of UVA was ca. 10 J/cm$^2$, by employing a UVA fluorescent lamp (Philips TLD 36 W/08), at wavelength peak at 365 nm).
The test substance did not induce skin reactions; the positive control, 8-MOP, induced photo-irritation.

Ref. : 118

(M/C) A test for photo-sensitisation was carried out using the preparation T817 (highly dispersed hydrophobic titanium dioxide and iron oxide).
The test animals were SPF bred albino guinea pigs, males (10 treated and 5 control animals).
The induction was made by topical treatment of dorsal neck region, which was closely clipped with electric clippers; the interested region was depiled.
A 20 % dilution of the test substance was used for the topical application, preceded by 4 injections of 0.1 ml. Freund's Complete Adjuvant on the next day.
The treated animals and the controls were irradiated with UVA-light by means of an irradiation device containing four UVA fluorescent lamps (Philips TLD 36 W/08, wavelength peak at 365 nm), until a dose of ca. 10 Joules/cm$^2$ was received.
Skin readings were made immediately after irradiation; the procedure was repeated five times within a 2-week period. The positive control was represented by musk ambrette.
No positive signs of photo-sensitisation were observed on the T817 treated animals, whereas musk ambrette treated animals presented clear signs of photo-sensitisation.

Ref. : 120

(M/C) A test for photo-sensitisation was carried out in 15 SPF guinea pigs, 8 males and 7 females. The test group comprised 5 males and 5 females; the control group 3 males and 2 females.. The test substance was T805®. The induction was in the nuchal region, in which 4 injections of Freund's complete adjuvant were made at the corners of an area which was then treated with 0.2 ml of a 30% suspension of the active ingredient in ethanol 5 times in two weeks.

The negative control was vehicle only. UVA, 310-420 nm, was applied for about 50 minutes after each application, to give a dose of 10 J/cm$^2$. Twelve days after the last induction procedure, the same areas were irradiated with the same dose of UVA. Observation was at 24 and 48 hours later. No skin effects were observed, with or without irradiation. In the laboratory, there had been regular tests to the same protocol using musk ambrette as a positive control.

Ref. : 48

(M probably/UC) A test for photo-sensitisation according to GLP was carried out using a micro-crystalline titanium dioxide preparation (code NP89/367) in mineral oil/triglyceride + dispersant (code NP89/310). Thirty-five female Dunkin Hartley guinea pigs were used, 10 test, and 2 control groups, each of 10 animals. Following a range finding test in 5 animals, a concentration of 10% was decided upon for the experiment, this being a dose which was non-irritant and sufficiently dilute to allow the action of irradiation on the skin.
The hair was clipped from an area 6.25 $cm^2$ in the scapular region. Four injections of 0.1 ml of Freund's complete adjuvant were injected at each corner of the prepared area. A volume of 0.1 ml of the 10% dilution of the preparation was applied to the skin area 5 times over 10 days. Thirty minutes after each application, UVA 320-400 nm, was applied to the treated area to give a dose of 10.2 $J/cm^2$. Animals of the first control group (irritation control) were treated with vehicle only, and of the second group (sensitisation control) with active ingredient without irradiation. These sites were assessed for irritation 24 hours afterwards. This procedure was repeated a further 4 times, thus giving a total of 5 induction procedures over 10 days.
Challenge was made 21days after the final induction application. An area of 6.25 $cm^2$ was prepared on either flank. The active ingredient (10%) in a dose of 0.1 ml was applied to both flanks of the test animals and the animals of control group 1, and the right side covered with light proof material. Animals of control group 2 had the test material applied to one side and the vehicle to the other. Thirty minutes later, animals of the test and control 1 groups were irradiated in the same manner as for the induction; animals of control group 2 were not irradiated. Observation was at 24, 48 and 72 hours.
After 4 applications of the active ingredient + radiation in the test group during the induction, there was slight irritation, more marked after the fifth application. The control groups showed no such reaction. Following the challenge + UVR, 3 animals of the test group showed reactions in the areas which had been irradiated; there were no reactions in the areas protected from radiation. There were, however, reactions in 5 of the animals in control group 1 (which had been induced using vehicle only) in the areas exposed to radiation. The reactions found were therefore attributed to "hyper-reactivity due to induction pre-treatment". In the view of the authors, there was no evidence of photo-sensitisation.

Ref. : 49

(M/UC) Fine particle titanium dioxide MT100® (Teikoku Kako, Osaka) with a mean particle size of 25 nm and a maximum of 70 nm was tested for its ability to prevent photo-toxicity in hairless mice. The concentrations of titanium dioxide tested were (%) 1, 5 and 10. In addition to a control group, the sunscreens 4-aminobenzoic acid and urocanic acid (both at 1%) were also tested. Damage was tested for by measuring the incorporation of tritiated thymidine in the epidermal DNA. Radiation was from lamps emitting from 280 to 370 nm, with a peak at 305 nm. Radiation intensity was 293 $mJ/cm^2$/minute. Groups of animals were irradiated with ($mJ/cm^2$) 440, 880 and 1760. They were injected with the thymidine at 1, 24 and 48 hours after exposure, and were then sacrificed one hour later. The number of animals in each group is not given.

In control animals, DNA synthesis was much inhibited 1 hour after the irradiation, but increased about 5 fold 48 hours later. The titanium dioxide preparations at 1% and 5% showed much greater protection than the other two sunscreens; at 10% it was able to inhibit the changes produced even by the highest dose of radiation.

Ref. : 133

(M/C) Five groups, each of 30 hairless mice, were formed. Group 1 had irradiation followed by croton oil promotion; group 2, the same irradiation and promotion + titanium dioxide sunscreen formulation; group 3, irradiation + sunscreen; group 4, sunscreen alone + promotion; group 5,

promotion alone. It appears that a commercial preparation containing micro-crystalline titanium dioxide coated with aluminium stearate with a SPF value of 20 was used; the concentration of the active ingredient is not given. The irradiation was by simulated solar light, with the intensities ($W/cm^{2)}$) UVB 2.7 X $10^{-4}$; UVA 5.2 X $10^{-3}$. The duration of exposure was so adjusted as to give a minimal erythema dose. Exposure was for 12 weeks.
The results show that all of the mice which had received ultraviolet light and promotion developed tumours within 52 weeks. Where the sunscreen was used without promotion, the incidence was 3.7%. When the sunscreen was used with promotion, the incidence was about 15%. This indicates photo-proctective activity.

Ref. : 59

(M/UC) A mouse model was used to study the ability of various sunscreens to prevent the development of squamous cell cancer of the skin. The cancers were induced by a preliminary application of a subcarcinogenic dose of 7,12-dimethylbenzanthra-cene; this was followed by 32 weeks of solar UV irradiation at a low dose, insufficient to cause oedema. Each group consisted of 15 animals.
One group of animals was protected with titanium dioxide and another with 2-ethyl-hexyl-4-methoxy-cinnamate. Probably uncoated titanium dioxide was used. A third group of animals was unprotected by sunscreen. Other groups were also studied, but they are judged not to be relevant in the present context. After 48 weeks, 87% of the animals treated with DMBA and ultraviolet radiation had tumours. The two sunscreens mentioned completely protected the animals. It is noted that there is evidence that titanium dioxide had been shown to be much more active in the prevention of immunosuppression produced by ultraviolet radiation than the cinnamate; yet both agents protected against the production of tumours under the conditions of the experiment. It would appear from this experiment that titanium dioxide (probably uncoated) did not induce tumours following treatment with DMBA and ultraviolet light, but rather protected against such tumours.

Ref. : 58

(M/UC) A culture of HeLa cells was used to test for a possible anti-cancer effect of irradiated titanium dioxide. It is known that photo-excited titanium dioxide in aqueous solution can give rise to OH, $O_2^-$, and $H_2O_2$. The titanium dioxide used was p25 (Nippon-Aerosil, Tokyo), average diameter of particles 30 nm. The material was suspended in the culture medium in doses (µg/ml) of 0, 50, 100 and 150. In the absence of ultraviolet radiation, no toxic effect on the cultured cells was found. The irradiation was carried out using a 500 W high pressure mercury lamp; filters were used to obtain ultraviolet radiation between 300 and 400 nm, and the dose was 7 - 10 $J/cm^2$. The cells were killed completely at 100 µg/ml, and at 50 µg/ml if the dose of radiation were doubled. Titanium dioxide had no effect on cell survival if radiation had not been carried out.
In an extension to the experiment, tumour cells were injected subcutaneously into nude mice. When the tumours had grown, groups of mice received one of the following treatments: (a) injection of 0.4 mg titanium dioxide (into the tumour); (b) the same, with ultraviolet irradiation for an hour; (c) irradiation only; (d) the same as (b) with a second similar treatment 13 days later. Only the combination of irradiation and injection of titanium dioxide caused marked inhibition of tumour growth; this inhibition was even more marked if a second treatment (group d) was given.

Ref. : 60

(M/UC) Human T-24 bladder cancer cells were cultured in the dark with titanium dioxide (uncoated anatase, average diameter 30 nm., agglomerates 30 to 100 nm). The doses seem to

have been 0, 10, 100 and 300 μg/ml. Some of the cultures were then irradiated at 300 to 400 nm, at 7 to 10 J/cm$^2$. The culture was then continued for a further 10 days. Compared with control cultures, radiation alone produced little cytotoxicity, but this was markedly increased if titanium dioxide had been present during the irradiation. The use of catalase and L-cysteine, known to scavenge hydrogen peroxide and hydroxy radicals, partially protected the colonies against the effects of irradiation + titanium dioxide.
In an extension of this work, cultured T24 cells were injected into the backs of 16 nude mice. When the tumours had grown, titanium dioxide was injected into the tumours in 2 groups of 4 animals. After 3 days, the tumours were exposed surgically. One of the group of animals which had had injections of titanium, and one which had not, had the tumours exposed to ultraviolet radiation. Another group of animals formed an untreated control group. The further tumour growth was much inhibited in those animals which had had both titanium dioxide and ultraviolet treatment. Titanium dioxide by itself, or radiation by itself, showed no differences from the controls.

Ref. : 61

(?M/?C) A test for photo-stability was carried out by studying the oxidation of propan-2-ol to propanone under the influence of photo-excited titanium dioxide. The authors state that for maximum photo-catalytic activity in this system, the ultraviolet emission from a medium pressure mercury arc (at 365 nm) is ideal. The exposure time in these experiments was 60 seconds. Determination of the propanone was by GLC. The material tested was micro-crystalline titanium dioxide coated with silica 5% and alumina 12% (Degussa p25. Thus in the submission, but there may be some confusion, as p25 is said to be uncoated and not micro-crystalline) The manufacturer also provided other (pigmentary) powders for comparison (titanium dioxide and zinc oxide based; presumably not micro-crystalline). It was found that the photo-activity of these powders was much less than that of micro-crystalline titanium dioxide. [The summary states "The photo-activity of the powder (micro-crystalline coated titanium dioxide) is less than that of commercial grades of powders used for food additives and cosmetic colorants. The inorganic coating technology is used to give substantial reductions in photo-activity."] The photo-activity of the various compounds in these experiments is expressed as the number of moles of propanone produced per gram of titanium dioxide per hour (in the presence of ultraviolet radiation). The figure for the active ingredient is about 200; the figures for the other comparator products were considerably lower (range: undetectable to 28). This seems to be at odds with the Colipa statement above, but the photo-protection afforded by the titanium dioxide to the test complex may complicate analysis. It is difficult to know what conclusions may be drawn form this submission.

Ref. : 53

(M/UC) A test for photo-stability was carried out on micro-crystalline titanium dioxide compared with anatase of pigment grade for cosmetic use. The test used was the oxidation of glycerol by photo-activated titanium dioxide. This in turn leads to the reduction and blackening of lead carbonate, which can be estimated by reflectance. The photo-activity of the micro-crystalline titanium dioxide is said to be not greater than that of the pigmentary grade material. Figures are not given. [Part of this paper is missing from the submission, so that the comparison between the photo-catalytic activity of micronised titanium dioxide and pigmentary titanium dioxide has been obtained from the summary. Thus, full reliance cannot be placed on this submission].

Ref. : 54(1)

(M/C) In a paper similar to the above (ref. 54(1)) the author uses a similar method to study the photo-catalytic activity of titanium dioxide - in this case a compound coated with aluminium oxide and silicon dioxide. The main burden of the paper is the chemical explanation of the reactions observed.

Ref. : 54(2)

(M/UC) Photo-stability was tested by studying the oxidation of propan-2-ol to propanone under the influence of photo-activated micro-crystalline titanium dioxide. The method of estimation was by IR-spectroscopy. The photo-activity was decreased by inorganic and organic coating of the crystals of titanium dioxide.

Ref. : 85

(M/C) The summary of this paper suggests that tests were carried out on the commercial preparations UV-Titan M210® and UV-Titan M262®. This does not appear from the paper submitted. It consists of an investigation into the photo-stability of various preparations of titanium dioxide. In general, the authors found that rutile crystals, heavily coated with aluminium hydroxide followed by a final treatment with dimethicone gave the best results, both from the point of view of stability and also of favourable physical properties for use in cosmetic formulations. Rutile crystals were much less satisfactory.

Ref. : 56

(NM/UC) The photo-catalytic activity of uncoated titanium dioxide was found to be reduced by the addition of hydroxypropylcellulose to sunscreen formulations. This is thought to be absorbed on the crystal surface and thus deactivates the catalytic centres.

Ref. : 57

A test with $TiO_2$, considered as a non-carcinogenic material, to evaluate the influence on five biochemical parameters, demonstrated that it did not induce hepatic DNA damage.
The scientific value of the study for the risk assessment of consumers' exposure to $TiO_2$ was considered not relevant by SCCNFP.

Ref. : 43

## 2.11. Conclusions

Both titanium dioxide itself (in micro-crystalline form in most experiments), and various coated and doped preparations of micro-crystalline titanium dioxide, have been used in the experiments summarised above.
Titanium dioxide is photo-catalytic in ultraviolet light, but this activity is found to be much less in coated material.
Acute oral toxicity is very low, both in coated and uncoated material; acute dermal toxicity is also low, but in this case uncoated material was used. Sub-chronic oral toxicity is low (uncoated). Long term feeding studies in rat and mouse with uncoated pigmentary material showed no evidence of carcinogenesis. Inhalation studies in rats, and epidemiological evidence in man, using uncoated finely divided material, suggest that it causes an increase in the incidence of lung tumours. This, however, probably reflects the actions of irritating dusts generally.
Irritation of the skin is low or absent, both in animals and humans subjects, using both coated and uncoated material. Irritation of mucous membranes is low or absent, both with coated and uncoated material; in one experiment in the rabbit, the uncoated material was judged to be

moderately irritant. Sensitisation in animals and man was not found, using either coated or uncoated material. Titanium dioxide did not show photo-toxic activity in studies *in vivo* or *in vitro*, and no photo-sensitisation or photo-irritation was observed. Titanium dioxide is photo-catalytic in ultraviolet light, but the relevance of this is doubtful in the absence of dermal penetration, as well as the fact that the coated preparations show much less photo-catalytic activity than the uncoated material.
Extensive tests for percutaneous absorption, mostly *in vitro*, indicate that absorption does not occur, either with coated or uncoated material; one experiment found some evidence that a little of the material could be found in the openings of the follicles.
Numerous tests for mutagenicity and clastogenicity have been carried out, and consistently show negative results.
The toxicological profile of this material does not give rise to concern in human use, since the substance is not absorbed through the skin. In view, also, of the lack of percutaneous absorption, a calculation of the margin of safety has not been carried out.
Many of the coating substances already used as ingredients in cosmetics, and if they are acceptable in this role they should be acceptable as coatings for titanium dioxide.
It is also suggested that if a coating material were proposed for use which is not at present approved as a cosmetic ingredient, it would have to be submitted for approval in the same way as any other new ingredient.

**Classification :** 1 at maximum concentration of 25% for the intended use.

## 2.12. Safety evaluation

### CALCULATION OF THE MARGIN OF SAFETY

Not calculated as there appears to be no percutaneous absorption of the products tested.

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## 3. Opinion of the SCCNFP

The SCCNFP is of the opinion that titanium dioxide is safe for use in cosmetic products at a maximum concentration of 25% in order to protect the skin from certain harmful effects of UV radiation.

This opinion concerns crystalline (anatase and/or rutile) titanium dioxide, whether or not subjected to various treatments (coating, doping, etc.), irrespective of particle size, provided only that such treatments do not compromise the safety of the product.

The SCCNFP proposes no further restrictions or conditions for its use in cosmetic products.

## 4. Other considerations

Not applicable

## 5. Minority opinions

Not applicable