Danish Ministry of the Environment

Occurrence and effects of nanosized anatase titanium dioxide in consumer products

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Occurrence and effects of nanosized anatase titanium dioxide in consumer products

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Foreword

The project “Prevalence and effects of nano-sized anatase titanium dioxide” has been carried out from September 2013 to December 2013.

The report describes the project results including a description of material characteristics, properties and toxicity, an overview of the (possible) prevalence of anatase nano-sized titanium dioxide according to literature, previous surveys, etc.; a mapping of selected products on the Danish market and a risk assessment of significant consumer products containing nano-sized titanium dioxide.

The results are based purely on available literature and for the selected products combined with information from the industry. No products have been subjected to any form of analysis.

The project has been carried out by Danish Technological Institute with COWI A/S as subcontractor and has been headed by project manager MSc, PMP, Gitte Sørensen.

Health and environmental assessments were headed by MSc Sonja Hagen Mikkelsen.

To assess the progress and results of the project, a steering committee was set up with the following members:

Flemming Ingerslev, the Danish EPA
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Sonja Hagen Mikkelsen, COWI A/S
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The project was financed by the Danish EPA as a part of the activities on the national action plan for chemicals 2010-13.

Summary

Titanium dioxide is one of the most widely used materials for a vast number of products, including consumer products. The major applications of titanium dioxide is as a white pigment in e.g. paints, coatings, plastic products, paper and printing inks, and a minor part is used as a colorant in foods, cosmetics, rubber, etc., as UV filter in sunscreen and as a filler.

A small part of the produced titanium dioxide that is used for specialist applications such as photocatalytic products, including paints, coatings, air cleaners, building and construction materials and other products with special requirements, where the deliberate use of nano-sized anatase titanium dioxide offers the optimal photocatalytic effects.

Titanium dioxide is of main industrial interest in the anatase and rutile crystal forms. The bulk materials are mainly produced by either a sulphate or chloride process from naturally occurring ilmenite mineral ores, producing a mix of anatase and rutile or pure rutile titanium dioxide, respectively. For direct titanium dioxide nanoparticle synthesis, sol-gel and spray pyrolysis syntheses are commonly used, while thin films or coatings may be obtained by applying titanium dioxide by chemical/ physical vapour deposition techniques (CVD/PVD) or sol-gel methods. The anatase and rutile crystal forms may be characterised and distinguished by X-ray diffraction or Raman/IR spectroscopy.

Titanium dioxide is inexpensive, inert, thermally stable, resistant to UV degradation and photoactive (in its uncoated form), and many of the properties possessed by titanium dioxide in bulk form are enhanced when the particle size is reduced to the nanosize due to the increased surface-to-volume ratio. Some material properties are therefore explicitly exploited by using particles with primary particle sizes in the nano range, namely the photocatalytic effects and the ability to absorb UV radiation at certain wavelengths. When used in practical applications, nanoparticles of titanium dioxide, though, tend to aggregate and agglomerate to particle sizes in the micro range. The increased surface-to-volume ratio is, however, still effective, thereby preserving the enhancement of desired properties.

The major use of anatase titanium dioxide may be grouped in three categories based on the function of the titanium dioxide, being pigment, photocatalyst and UV protection:

- Titanium dioxide is the most widely used white pigment, and both anatase and rutile titanium dioxide is used for pigmentation. It is used as a white pigment in paints, coatings, enamels, laquers, varnishes, plastic products, paper, printing inks, foods, cosmetics, rubber, fibre, ceramic, etc. Many titanium dioxide pigments have primary particle sizes larger than the nanoscale (0.2-0.3 µm) since this size range offers optimal opacity, and the pigments are coated with e.g. silica, alumina or organic coatings to improve properties such as particle dispersibility, dispersion stability, photostability, opacity and gloss.

- A minor use of titanium dioxide is as a photocatalyst in paints, coatings, air cleaners and construction materials such as roof tiles, concrete pavements and windows. Both anatase and rutile titanium dioxide exhibit a photocatalytic effect as they are able to generate radicals that cause degradation of organic compounds, thereby exhibiting e.g. self-cleaning, antibacterial or NOx-reducing properties. Smaller particle sizes strongly improve the photocatalytic effect, and most often nano-sized titanium dioxide is used to harvest an optimal effect. Studies report varying conclusions on how to obtain the best performance,

but it is generally accepted that the anatase crystal form exhibits a better photocatalytic effect, even though a mixture of anatase:rutile in the approximate ratio 75:25 in some cases has shown even better effects. The photocatalytic effect of both anatase and rutile titanium dioxide may be controlled by coating, which strongly reduces or hinders the effect and is not of interest to the products desiring this effect, or by doping with specific metals, which enhances the effect.

- Titanium dioxide is an effective UV filter, and it is used as both nano-sized as well as larger particles as a UV-A and UV-B filter, e.g. in sunscreen products and outdoor materials such as plastics and coatings. The nano-sized titanium dioxide particles scatter and transmit the UV-A and UV-B regions efficiently, and further, they are not visible to the eye. This allows e.g. sunscreen products with nano-sized titanium dioxide to offer an optimal UV protection while being transparent. Titanium dioxide particles used as a sun filter are coated by e.g. silanes or alumina coatings to reduce photocatalytic activity and prevent the formation of free radicals that may potentially harm the skin. Alternatively, trace amounts of specific metals are embedded within the particles to obtain the same effects. A number of studies have shown that both anatase and rutile titanium dioxide are used in sunscreen products, either as pure crystal forms or as a mixture of the two.

Mapping of nano-sized anatase titanium dioxide in photocatalytic paints and sunscreens

On the Danish market, photocatalytic paints and sunscreen products were mapped to determine the prevalence of nano-sized anatase titanium dioxide.

Five companies producing and/or importing photocatalytic paints were initially identified on the Danish market, and all were approached with a questionnaire. The responses from three large international companies producing a wide range of paints and coatings show that they have all withdrawn the photocatalytic paints from the Danish market, and two of the companies stress that the products have been withdrawn because of limited sales. However, one of the companies is planning the introduction of improved photocatalytic products, implying a faith in the future of the products. This is confirmed by the remaining two companies, which have specialised in and market several niche products, including photocatalytic paints. They have experienced increased sales in photocatalytic paints and expect this trend to continue. The size of the titanium dioxide is by one company stated to be nano-sized, while one other company states the titanium dioxide to be in the micrometer range (0.2-2 μm). Whether the measures account to aggregates and agglomerates or the primary particle size is unknown. The photocatalytic paints contain 5-10 % photocatalytically active titanium dioxide, either as a mixture of rutile and anatase or as pure anatase titanium dioxide.

An extensive number of companies producing/importing sunscreen products in Denmark are present and more than 30 brands are marketed, covering both large international and more narrowly targeted brands. The mapping obtained most information from Danish producers, and the findings are to be considered with care since these are not necessarily representative for the general sunscreen market. The Danish companies have a strong focus on the Nordic Ecolabel 'Svanen' which does not allow the content of nanosized titanium dioxide in sunscreens. 11 companies as well as an expert were approached.

The sunscreen mapping indicates a general decrease in the use of titanium dioxide and a trend of substituting titanium dioxide as a sun filter by chemical sun filters, primarily based on Danish-produced products. The only company in the mapping offering sunscreen products containing titanium dioxide on the Danish market was a large international company; however, it is anticipated that more international brands may contain titanium dioxide. The information obtained on the characteristics of titanium dioxide is very limited. The expert has tested seven sunscreens containing titanium dioxide that are available on the Danish market and found that two used anatase titanium dioxide as the primary form, while it was pure rutile in the rest. The sunscreens based primarily on the anatase crystal form showed a photocatalytic activity superior to the rutile-based products.

Toxicity of anatase titanium dioxide

Absorption, distribution, metabolism, and excretion (ADME) of titanium dioxide nanoparticles may be influenced by factors such as exposure routes and particle size, particle agglomeration, aggregation and surface coating. Absorption into the body can occur through the lung and gastro-intestinal tract, but no specific information is available regarding the magnitude of the absorption and transport for the purpose of systemic risk assessment. Rutile titanium dioxide seems to be better absorbed orally than the anatase form, although absorption is generally low. Titanium dioxide nanoparticles do not seem penetrate into the viable epidermis or dermis cells of healthy skin, psoriatic skin or skin exposed to UV-B radiation. There is some evidence that titanium can be found in vacant hair follicles. For dermal and inhalation exposure, available evidence does not allow identifying any possible difference in absorption between the anatase and rutile forms, coated or uncoated.

The irritation and sensitisation potential of anatase and rutile nanosized titanium dioxide is low.

Titanium dioxide nanoparticles injected intravenously or intraperitoneally have been found to distribute to different organs, such as liver, spleen, kidneys, lung, lymph nodes and brain.

Mixtures of anatase and rutile titanium dioxide (85 % anatase and 15 % rutile) coated with trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane show low acute oral toxicity. When inhaled the anatase form has been shown to be more toxic than the rutile form. The pulmonary response includes inflammation, epithelial damage, increased permeability of the lung epithelium, oxidative stress and cytotoxicity as well as morphological alteration within the lung. Results from studies in rats show that they are particularly susceptible to high lung burdens causing inflammation and histopathological lesions.

Nano-crystalline titanium dioxide generates reactive species (ROS) quite efficiently, particularly under ultraviolet (UV) illumination and is has been shown that anatase titanium dioxide was 100 times more toxic than an equivalent sample of rutile titanium dioxide and that the toxic response of nano-sized titanium dioxide increased substantially with UV illumination. Oxidant reactivity (generation of ROS) exhibited by titanium dioxide particles with similar size but different crystal structures has been demonstrated to be highest for amorphous samples, followed by pure anatase, lower for anatase/rutile mixtures and lowest for pure rutile.

IARC has evaluated and categorised titanium dioxide (even the microform – if exposure is high enough) to be a Class 2B carcinogen (possibly carcinogenic to humans). It is however, generally considered a result of lung overload in the rat at high exposures, and of less relevance for humans.

Not much information is available to conclude regarding the differences in toxicity between coated and uncoated anatase titanium dioxide.

Consumer exposure and risk

Based on the results of the mapping of specific products on the Danish market, direct exposure of consumers (apart from intake via food) is expected to occur primarily from use of cosmetics (sunscreen products) and from application of paints and coatings. In addition, exposure to anatase titanium dioxide bound in matrices is possible through contact with paper, ceramics, rubber and fibres containing titanium dioxide as pigment.

Exposure and risk related to worst case scenarios involving dermal and inhalation exposure have been considered. Due to lack of systemic absorption by dermal exposure the dermal scenario has been calculated to estimate at which level of human dermal absorption a risk might be foreseen if it is compared to effects seen after oral absorption in mice. Carrying out this risk evaluation based on very precautionary assumptions and using results from an oral study for putting dermal risk into perspective, shows that for the worst case dermal summer scenario, an unacceptable risk is not likely to be expected.

Calculations have also been carried out for a scenario involving indoor spray application of a photocatalytic paint containing anatase titanium dioxide. The risk evaluation is however, not fully developed due to the deficiencies of literature data to precisely express DNEL and exposure in the calculation. The risk characterisation ratio (RCR) was 1.13 using this very conservative approach where the DNEL (REL) is derived from an average exposure of up to 10 hours per day during a 40-hour work week over a working lifetime and considering lung cancer as the endpoint, and an exposure estimation based on results from an experimental study with a consumer product containing nanosized titanium dioxide. When adjusting from the obvious uncertainties, e.g. that the worker exposure is 125 times the estimated realistic worst case consumer exposure, the RCR was considerably below 1, indicating that no unacceptable risk is expected.

Dansk sammenfatning

Titandioxid er blandt de mest anvendte materialer og er at finde i en lang række af forskellige produkter, deriblandt forbrugerprodukter. Den mest væsentlige anvendelse af titandioxid er som pigment i fx malinger, coatinger, plastprodukter og printerblæk, mens en mindre del anvendes som farvestof i fødevarer, kosmetik, gummi osv., som UV-filter i solcreme samt som fyldstof.

En lille del af den mængde af titandioxid, der produceres, er at finde i specialprodukter med fx en fotokatalytisk effekt, bl.a. malinger, coatinger, luftrensere, byggematerialer og andre produkter, hvor den specielle fotokatalytiske effekt af netop anatase titandioxid i nanostørrelse tilfører den fotokatalyiske effekt bedst muligt.

Den primære industrielle interesse i titandioxid gælder de to krystalformer anatase og rutil. Bulkmaterialerne produceres hovedsageligt vha. enten en sulfat- eller en chloridproces baseret på det naturligt forekommende mineral ilmenit. Processerne giver hhv. en blanding af de to krystalformer eller den rene form af rutil titandioxid. Titandioxidpartikler i nanostørrelse fremstilles oftest direkte ved enten en sol-gel-proces eller spray-pyrolyse, mens tynde film eller coatinger af titandioxidpartikler i nanostørrelse kan fremstilles vha. *chemical/ physical vapour deposition*-teknikker (CVD/PVD) eller sol-gel-metoder. Det er muligt at karakterisere og skelne mellem de to krystalformer anaase og rutil ved anvendelse af røntgendiffraktion eller Raman-/IR-spektroskopi.

Titandioxid er billigt, inert, termisk stabilt, modstandsdygtigt over for UV-nedbrydning og er fotoreaktivt, og mange af de egenskaber, som bulk-kemikaliet titandioxid besidder, er forbedrede eller forstærkede, når partikelstørrelsen mindskes, da forholdet mellem overflade og volumen af partiklerne øges. Nogle materialeegenskaber udnyttes derfor specifikt ved at anvende partikler med en primær partikelstørrelse i nanoområdet, og dette gør sig især gældende for de fotokatalytiske egenskaber samt muligheden for at absorbere bestemte bølgelængder i UV-spektret. På trods af at titandioxid-nanopartikler har en tendens til at aggregere eller agglomerere til partikler i mikrostørrelse bevares det øgede forhold mellem overflade og volumen, hvorfor de forstærkede egenskaber bevares.

Den primære brug af anatase titandioxid kan opdeles i følgende tre kategorier baseret på materialets funktion i produktet: Pigment, fotokatalysator og UV-beskyttelse:

- Titandioxid er det mest bredt anvendte hvide pigment, og både anatase og rutil titandioxid anvendes til pigmentering. Det anvendes bredt som hvidt pigment i malinger, coatinger, lakker, emaljer, fernis, plastprodukter, papir, priterblæk, fødevarer, kosmetik, gummi, fibre, keramik osv. Mange titandioxidpigmenter har en primær partikelstørrelse, som ligger over nanoområdet (0,2-0,3 µm), da titandioxidpartikler i denne størrelse giver en optimal dækkeevne. Desuden er pigmenterne coatet med fx en silika-, aluminiums- eller organisk coating, som kan forbedre fx dispersibiliteten, stabiliteten af dispersioner, fotostabilitet, dækkeevnen og glansen af farven.
- Titandioxid anvendes i mindre grad som fotokatalysator i specialmalinger og -coatinger, luftrensere og byggematerialer som fx tagsten, fortovsfliser og vinduer. Både anatase og rutil titandioxid kan virke fotokatalytisk ved at generere radikaler, som kan nedbryde organiske forbindelser, vha. af UV-belysning, og overfladen med titandioxid kan dermed

virke fx selvrensende, antibakteriel eller NOx-reducerende. Mindre partikelstørrelse forbedrer denne fotokatalytiske effekt væsentligt, og oftest anvendes titandioxidpartikler i nanostørrelse til netop at opnå en optimal effekt. Litteraturen rapporterer varierende konklusioner på, hvordan man opnår de bedste resultater, men det er overordnet set generelt accepteret, at anatase-krystalformen giver en bedre fotokatalytisk effekt, selvom enkelte studier viser, at en blanding af anatase og rutil i et forhold på ca. 75:25 giver en endnu bedre effekt. Den fotokatalytiske effekt af både anatase og rutil titandioxid kan kontrolleres ved at coate partiklerne, så effekten reduceres kraftigt eller helt forsvinder, eller partiklerne kan dopes med bestemte metaller, som forbedrer effekten yderligere.

- Titandioxid er et effektivt UV-filter, og det bruges både i nanostørrelse og som større partikler som UV-A- og UV-B-filter i bl.a. solcremer og udendørs materialer som fx plastprodukter og coatinger. Titandioxidpartikler i nanostørrelse spreder og reflekterer effektivt lys i UV-A- og UV-B-regionen og kan ikke ses med det blotte øje, hvorfor fx solcreme med titandioxidpartikler i nanostørrelse giver både god UV-beskyttelse og en usynlig creme. Titandioxidpartikler, der anvendes som solfilter, er coatet med fx silaner, silika eller aluminiumsforbindelser for at reducere den fotokatalytiske aktivitet og dannelsen af frie radikaler, som potentielt kan skade huden. Alternativt er partiklerne dopede med specifikke metaller for at opnå samme effekt. Flere studier har vist, at både anatase og rutil titandioxid anvendes i solcremer, og der kan både anvendes de rene krystalformer eller en blanding.

Kortlægning af nanostørrelse anatase titandioxid i fotokatalytiske maling og solcreme
Fotokatalytiske malinger samt solcremer på det danske marked er kortlagt for at bestemme forekomsten af anatase titandioxid i nanostørrelse.

Fem virksomheder, som producerer og/eller importerer fotokatalytiske malinger, blev identificeret på det danske marked, og de blev alle interviewet. Svarene fra tre store, internationale virksomheder, som producerer en bred vifte af malinger og coatinger, viser, at de alle har trukket fotokatalytisk aktive malinger tilbage fra det danske marked, og to af disse virksomheder understreger, at tilbagetrækningen er sket pga. et begrænset salg. Én af de tre store virksomheder planlægger at reintroducere en forbedret fotokatalytisk maling, hvilket viser en tro på deres berettigelse fremadrettet. Denne holdning deles af de to mindre virksomheder, som har specialiseret sig inden for nicheprodukter, deriblandt fotokatalytiske malinger. Disse virksomheder har oplevet et stigende salg af fotokatalytiske malinger og forventer, at stigningen vil fortsætte. Størrelsen af titandioxidpartiklerne oplyses af en virksomhed til at være i nanostørrelse, mens en anden virksomhed oplyser en partikelstørrelse på mikrometerskala (0,2-2 µm). Om dette er baseret på størrelsen af aggregater eller den primære partikelstørrelse er dog uvist. Fotokatalytisk maling indeholder 5-10 % fotokatalytisk aktivt titandioxid, enten som et mix af anatase og rutil eller som ren anatase titandioxid.

Der er mange virksomheder, som producerer og/eller importerer solcreme i Danmark, og der markedsføres mere end 30 mærker, som dækker over både store internationale brands og smalt fokuserede brands. I kortlægningen er overvejende opnået information fra danske producenter, og resultaterne skal derfor tolkes med forsigtighed, da svarene ikke nødvendigvis repræsenterer det generelle marked for solcremer, som købes i Danmark. De danske producenter har et væsentligt fokus på mærkning med ”Svanen”, som ikke tillader anvendelse af titandioxid i nanostørrelse i sine solcreme. Der er i kortlægningen af solcreme kontaktet 11 virksomheder samt en ekspert.

Kortlægningen af solcreme indikerer et generelt fald i anvendelsen af titandioxid og en tendens til at erstatte det fysiske titandioxid-solfilter med kemiske solfiltre i danskproducerede produkter. Den eneste virksomhed i kortlægningen, som tilbyder solcreme med titandioxid på det danske marked er en stor international virksomhed; det forventes dog, at flere internationale brands kan indeholde titandioxid. Der er opnået en meget begrænset mængde oplysninger omkring den anvendte titandioxids karakteristika. Eksperten, som er kontaktet i forbindelse med kortlægningen, har testet syv solcremer med titandioxid på det danske marked og fundet, at to af solcremerne primært indeholder den anatase form af titandioxid, mens de fem øvrige anvender ren rutil titandioxid som solfilter. Solcremerne, som anvender anatase som den primære form, viste en overlegen fotokatalytisk effekt sammenlignet med de øvrige.

Toksicitet af anatase titandioxid
Absorption, distribution, metabolisme og udskillelse (ADME) af titandioxid nanopartikler kan være påvirket af faktorer såsom eksponeringsvej og partikelstørrelse, partikel agglomerering samt overfladebehandling (coating). Systemisk absorption kan ske gennem lungerne og mave-tarmkanalen, men der er ikke fundet specifikke oplysninger vedrørende omfanget af absorption og distribution til forskellige organer med henblik på vurdering af den systemiske risiko. Rutil titandioxid ser ud at at absorberes oralt i større udstrækning end anatase, selv om absorptionen generelt er lav. Titandioxid nanopartikler synes ikke trænge ned i de levende lag af huden i hverken sund hud, hud påvirket af psoriasis eller solbrændt (forbrændt) hud, der har været udsat for UV -B-stråling. I en enkelt test er titanium er fundet i tomme hårsække. Det er ikke muligt at identificere en eventuel forskel i absorptionen mellem anatase og rutil titandioxid (med eller uden overfladebehandling), ved hverken inhalation eller dermal eksponering.

Potentialet for irritation og sensibilisering er vist at være lavt for både anatase og rutil nanotitandioxid.

Titandioxid nanopartikler indgivet intravenøst eller intraperitonealt transporteres med blodet og er påvist i forskellige organer, såsom lever, milt, nyrer, lunger, lymfeknuder og hjernen.

Blandinger af anatase og rutil-titandioxid (85% anatase og 15% rutil), behandlet med trimethoxy - caprylylsilane eller trimethoxy-n-octyl-silan udviser lav akut oral toksicitet. Ved inhalation har anataseformen har vist sig at være mere toksiske end rutilformen. Reaktionern i lungerne omfatter inflammation, beskadigelse af lungeepitelet, forøget permeabilitet af lungeepitelet, oxidativt stress og cytotoksicitet, samt morfologiske ændringer i lungen. Resultater fra studier med rotter har vist, at de er særligt følsomme over for høj partikelbelastning af lungerne, som forårsager inflammation og histopatologiske skader.

Nano-krystallinsk titandioxid danner reaktive iltforbindelser (reactive oxygen species, ROS) ganske effektivt, især under ultraviolet (UV) belysning. Det er påvist, at anataseformen af titandioxid er 100 gange mere toksisk end rutil-formen, og at den toksiske effekt af nano-titandioxid vokser substantielt med UV-belysningen. Reaktiviteten (ROS-dannelsen) af partikler af titaniumdioxid med samme størrelse, men forskellig krystalstruktur har vist sig at være størst for amorf titandioxid, efterfulgt af rent anatase, lavere for anatase / rutil-blandinger og lavest for ren rutil.

IARC har vurderet og kategoriseret titandioxid (også mikroformen - hvis eksponeringen er høj nok) til at være kræftfremkaldende i klasse 2B (muligvis kræftfremkaldende for mennesker). Det antages dog at være en følge af overbelastning af rotternes lunger med partikler i store doser og dermed mindre relevant for mennesker, da rotten er særlig følsom på det område.

Der er ikke megen tilgængelig information, der gør det muligt at konkludere med hensyn til forskellene i toksicitet mellem overfladebehandlet og ubehandlet anatase titandioxid.

Forbrugereksponering og -risiko
Baseret på resultaterne af kortlægningen af specifikke produkter på det danske marked, forventes direkte eksponering af forbrugerne (bortset fra indtagelse via fødevarer) at forekomme primært fra brugen af kosmetik (solprodukter) og fra anvendelse af maling og lak. Desuden er udsættelse for

anatase titandioxid bundet i matricer mulig ved kontakt med papir, keramik, gummi og fibre, der indeholder titandioxid som pigment. Eksponering og risiko er derfor evalueret ved hjælp af to worst case scenarier, der involverer eksponering via hud og ved indånding.

Da der ikke er set systemisk absorption af anatase titandioxid igennem huden, er scenariet for hudeksponering vurderet ved at estimere hvor meget der skal absorberes dermalt for at udgøre en risiko, hvis det antages, at man kan sammenligne med effekter der ses efter oral absorption i mus. En sådan risikoevaluering sat i perspektiv ift til data fra et oralt studie, er baseret på meget forsigtige antagelser. Evalueringen viser, at en uacceptabel risiko ikke er sandsynlig, selv ved sommer-scenariet hvor en høj mængde solcreme er blevet anvendt.

Scenariet for inhalation omfatter anvendelse af indendørs spraybemaling med fotokatalytisk maling med anatase titandioksid. Scenariet indgår i en risikoevaluering, der er begrænset på grund af at data i litteraturen er for mangelfulde til at man kan udtrykke DNEL og eksponering med tilstrækkelig præcision i beregningerne. Ved at anvende meget konservative antagelser beregnedes risikokarakteriseringsratioen (RCR) til 1,13. I antagelserne indgik en gennemsnitlig livstidseksponering svarende til 10 timer pr dag i en 40 timers arbejdsuge og lungecancer blev vurderet som den mulige kritiske effekt. Det blev antaget af eksponeringen kunne estimeres ud fra eksperimentelle data for et forbrugerprodukt med nano titandioksid. Hvis denne vurdering justeredes for åbenlyse overestimeringer (fx at en forbrugers eksponering antages at være 125 gange mindre end en arbejders eksponering), blev RCR betydeligt lavere end 1 hvilket indikerer at der ikke kan forventes en risiko.

1. Introduction to the survey

This report describes titanium dioxide and the use thereof with specific attention to nano-sized anatase titanium dioxide. This description includes production, characteristics, properties and methods of characterisation as well as the toxicity of the substance. Further, the report gives an overview of products and product groups containing nano-sized anatase titanium dioxide, describing prevalence, use and users as well as exposure to nano-sized anatase titanium dioxide from the products.

Two significant products with regard to prevalence and exposure that contain nano-sized anatase titanium dioxide –being sunscreen and photocatalytic paints – have been mapped in more detail by contact to relevant Danish companies. The overall contents are illustrated in image 1 below.

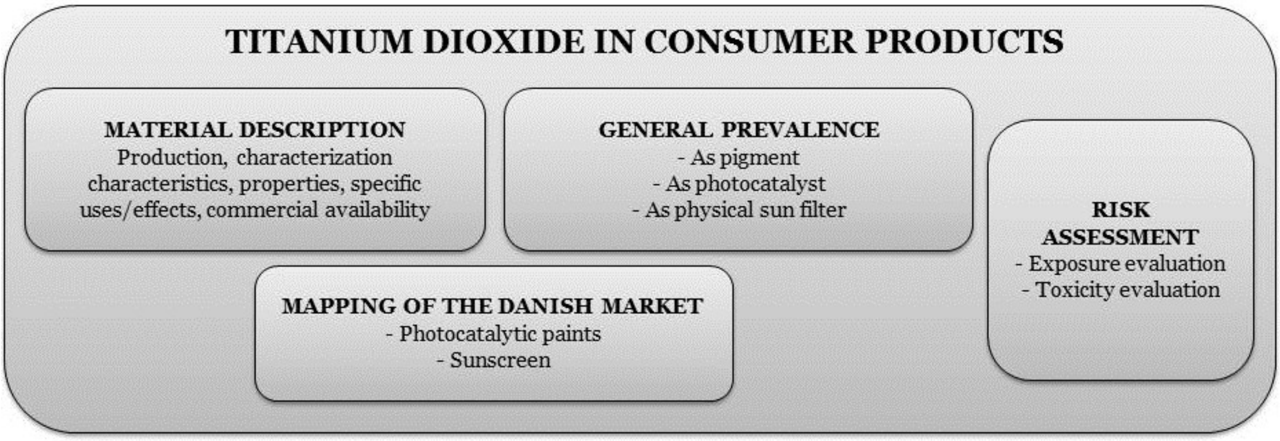

IMAGE 1
STRUCTURAL OVERVIEW OF THE REPORT. EACH CHAPTER IS DESCRRIBED IN FURTHER DETIALS IN SECTION 1.3,

Titanium dioxide is one of the most widely used materials for a vast number of products, including consumer products. According to the Titanium Dioxide Manufacturers Association (TDMA), the global production of titanium dioxide was estimated to €6.6 billion in 2006, and the demand is expected to increase in the next years according to a recent market forecast (Pira International).

The main part of the titanium dioxide produced is used as a white pigment in e.g. paints, coatings, plastic products, paper and printing inks, which constitute the major applications. A minor part of pigmentary titanium dioxide is used in foods, cosmetics, sunscreen, pharmaceuticals, rubber, etc. Most titanium dioxide will often contain a range of particles, including a fraction with nanoscale dimensions.

A limited part of the produced titanium dioxide is used for specialist applications, of which many are relevant to the present report since they often base their function on the nano-sized anatase titanium dioxide that is the subject of this survey. The applications include e.g. sunscreen and photocatalytic products such as self-cleaning paints and roof tiles.

Specifically the anatase form of titanium dioxide has been debated in recent years, since it forms potentially harmful radicals when exerting a photocatalytic effect, which is desired in a number of the specialist applications. Further, nano-sized particles offer enhanced effects regarding photoactivity as well as other properties due to the increased surface area per volume. Both the radical formation and the use of nano-sized particles are being heavily debated with regard to safety, since there are concerns about the health effects and toxicity thereof.

Because of the infinite prevalence of titanium dioxide in consumer products combined with the safety and health concerns of one specific form of titanium dioxide, it is important to gain knowledge on the possible exposure to nano-sized anatase titanium dioxide. To establish this knowledge, an overview of the applications of specifically nano-sized anatase titanium dioxide is established along with a review of available safety data combined with exposure estimates based on a mapping of the prevalence of the relevant products.

1.1 Objective of the survey

The objective of the survey is to identify consumer products containing nano-sized anatase titanium dioxide and to assess if applications of nano-sized anatase titanium dioxide in consumer products may pose a health risk.

1.2 Survey methods

A preliminary screening based on previous surveys of titanium dioxide, recent scientific literature/reviews on titanium dioxide and present and future use of nanomaterials as well as relevant databases (including the “Substances in Preparations In the Nordic countries” (SPIN) database, the Danish product register and online nanodatabases such as BUND and the Woodrow Wilson database) has been carried out to identify relevant products. The identified products have been catalogued, and the products have been described with regard to overall prevalence, the form of titanium dioxide used and the function of titanium dioxide in the products.

Based on the results of the preliminary product screening, the steering group assessed the knowledge gained on material properties, prevalence, use and users combined with initial exposure and toxicity evaluations to select relevant products for further mapping on the Danish market as well as risk assessment.

To map the prevalence of sunscreens and photocatalytic paints containing nano-sized anatase titanium dioxide on the Danish market, relevant Danish producers, importers, retailers and trade organisations have been questioned. The respondents have generally answered the survey based on current knowledge of the titanium dioxide raw materials used/contained as well as the properties of the titanium dioxide.

1.2.1 Industry contact

Companies relevant to the selected product groups were contacted, asking them to complete a short questionnaire (see Appendix 1). The companies that did not respond to the first enquiry were contacted again with a request to complete the questionnaire, and the contact was supplemented by an interview to clarify further, when relevant.

A limited number of five producers/importers of photocatalytic paints in Denmark were initially identified, covering both specialised companies only focusing narrowly on niche paints with specific properties as well as large paint companies offering a wide range of paint and coating products. All five companies have contributed to the mapping by completing the questionnaire, supplying information by e-mail and/or being interviewed.

For sunscreen products, a vast amount of Danish producers and importers were identified. 11 companies were contacted, representing both small and medium-size producers and importers/retailers of major and minor brands. Six of the companies contributed to the mapping by completing the questionnaire, supplying information by e-mail and/or being interviewed.

1.3 Structure of the report

The report reflects the main contents as illustrated in image 1 above and the delimitation process throughout the project. This means that the chapters have been structured as described below:

- What is titanium dioxide? (Chapter 2): a description of production, characterization, characteristics, properties and commercial availability; all with primary focus on the nano-sized anatase titanium dioxide.
- Anatase titanium dioxide in consumer products (Chapter 3): a preliminary screening outlining the primary use of nano-sized anatase titanium dioxide.
- Mapping (Chapter 4) of the selected product groups sunscreen and photocatalytic paints containing nano-sized anatase titanium dioxide.
- Toxicity of and exposure to nano-sized anatase titanium dioxide (Chapter 5): risk evaluation of nano-sized anatase titanium dioxide by evaluation of exposure and toxicity evaluations with focus on the selected product groups sunscreen and photocatalytic paints.

1.4 The nanomaterial definition

In the literature, industry and legislation, several different definitions of a nanomaterial are in use, exemplified by the definitions suggested by e.g. the International Organization for Standardization (ISO), by the European Food Safety Authority (EFSA) and by Organisation for Economic Co-operation and development (OECD) as recommended by the European Commission. This heterogeneity severely complicates a common understanding and, thereby, also discussions on prevalence and effects of nanomaterials and their use.

In this survey, a nanomaterial is defined according to the Recommendation of the EU Commission on the definition of nanomaterials (2011/696/EU).

Recommendation from the EU Commission on the definition of nanomaterials (2011/696/EU)

"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 nm - 100 nm.

"Particle", "agglomerate" and "aggregate" are defined as follows:
(a) "Particle" means a minute piece of matter with defined physical boundaries;
(b) "Agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;
(c) "Aggregate" means a particle comprising strongly bound or fused particles."

2. What is titanium dioxide?

Titanium dioxide is also known as titanium(IV) oxide or titania. It is the naturally occurring oxide of titanium with the molecular formula TiO_2, and it belongs to the family of transition metal oxides. Due to high refractive index and light scattering, titanium dioxide has been used widely as a white pigment, and the annual production of titanium dioxide is estimated to 4.5 million metric tons (United States EPA 2009). When used as a pigment, it is commonly referred to as Titanium White and Pigment White, and when used as a food colorant it has the E number E 171. Almost 90 % of the total production is used for paints, coatings, plastic, rubber and paper. Minor end-use sectors are textiles, food, leather, pharmaceutical and cosmetics. In addition, titanium dioxide can be used as a catalyst in photocatalytic processes to generate electricity or to degrade specific organic compounds.

Titanium dioxide is found in four different polymorphs in nature: "anatase", "brookite", "rutile", and "TiO_2 (B)". Additionally, several high-pressure forms have been synthesized (Carp *et al.* 2004) as well as amorphous titanium dioxide. However, only rutile titanium dioxide and anatase titanium dioxide are reported in commercial products (IARC 2010), and will therefore only be the subject of this report. In the subsequent sections, a general description of titanium dioxide is presented with particular emphasis on differences between the anatase and rutile crystal forms.

Rutile titanium dioxide and anatase titanium dioxide are assigned with CAS Registry numbers 1317–70–0 (EC No.: 215-280-1) and 1317–80–2 (EC No.: 1317-80-2), respectively. In addition, the CAS Registry number 13463–67–7 (EC No.: 236-675-5) is used for titanium dioxide as collective term (both anatase and rutile). Titanium dioxide (CAS Registry No.: 13463–67–7) is registered in REACH, and the majority of registrations derive from a lead dossier of a REACH registration joint submission.

2.1 Difference in crystalline forms

The difference in anatase and rutile form lies in the arrangement of the titanium and oxygen atoms in the unit cell. In all four crystal forms, the titanium atoms are coordinated octahedrally by oxygen. Rutile and anatase titanium dioxide have tetragonal unit cells, while brookite is orthorhombic and titanium dioxide (B) is monoclinic in regard to the unit cells. Comparing anatase and rutile, anatase has a relatively larger unit cell than rutile and a less dense structure, which leads to it having a density of 3.79-3.97 $g \cdot cm^{-3}$, whereas rutile has a density of 4.23 $g \cdot cm^{-3}$ (Anthony 1990). Rutile is the thermodynamically stable form of titanium dioxide; the other polymorphs and amorphous state are metastable and eventually converts into rutile upon heating (Rayner-Canham 2003).

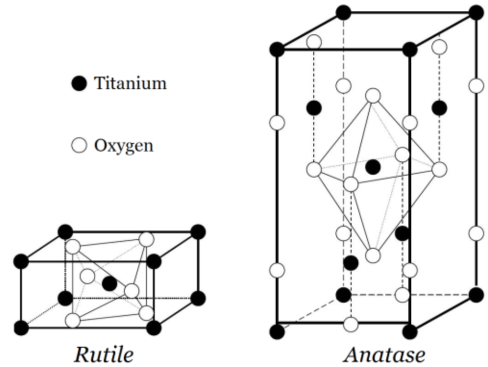

IMAGE 2
CRYSTAL LATTICE OF RUTILE AND ANATASE FORMS OF TITANIUM DIOXIDE.

2.2 Characterization

Polymorph structures of titanium dioxide may be characterised and distinguished in several ways. The most common technique is X-ray diffraction, where the X-rays are scattered by the atoms in the crystal lattices. To a lesser extent, Raman/IR spectroscopy is used where the vibration energy of the bonds present in the crystal are measured. These techniques can be used both for titanium dioxide powders and for coatings (Birkholz 2006; Shapovalov 2010).

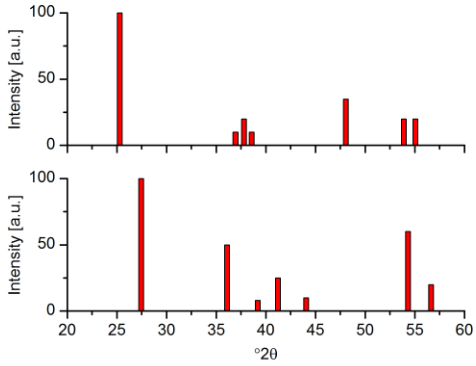

IMAGE 3
X-RAY DIFFRACTION PATTERNS OF RUTILE (TOP) AND ANATASE (BOTTOM) BASED ON POWDER X-RAY ANALYSIS.

X-ray diffraction is used not only to distinguish and quantify the various amorphous and crystalline phases of titanium dioxide, but also reveals information about grain sizes in the nanometer range, crystallite orientation and lattice defects. The ratio of anatase to rutile in pigments may be determined according to the ASTM standard test method ASTM D3720 - 90(2011).

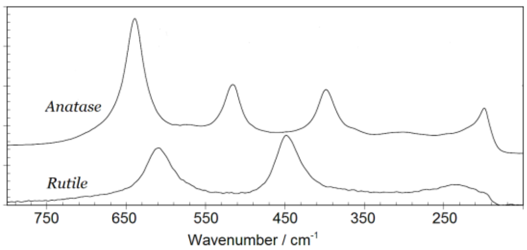

IMAGE 4
RAMAN SPECTRA OF ANATASE AND RUTILE TITANIUM DIOXIDE.

Raman spectroscopy uses monochromatic light, usually from a laser in the visible or near-infrared/ultraviolet range, as the exciting light source to probe the vibrational modes of the crystalline lattice. The vibrational information is specific to the chemical bonds and symmetry of the crystalline lattice, and thereby the vibrational pattern reveals valuable structural information. Infrared spectroscopy offers similar, but complementary, structural information. (West 1999; Shapovalov 2010)

2.3 Sources and production

Since there is no natural resource/occurrence containing only titanium dioxide, different refinement procedures are applied to obtain the pure titanium dioxide from naturally occurring mineral ores. Various routes are available with the main ones being the sulphate process and the chloride process, both illustrated in image 5. In the sulphate process, titanium dioxide is refined from the mineral ilmenite ($FeTiO_3$) by treating it with sulphuric acid (H_2SO_4). This causes the titanium to be dissolved as titanyl sulphate ($TiOSO_4$), which can be isolated and subsequently hydrolysed at elevated temperature and dried to produce titanium dioxide. The chloride process is a refinement of different polymorphs of titanium dioxide and titanium slag. Impure titanium dioxide is converted to liquid titanium tetrachloride ($TiCl_4$), which is separated and subsequently oxidized at elevated temperature (calcination) to form pure rutile titanium dioxide. It has been estimated that approx. 65 % of the global production of titanium dioxide is based on the chloride process.[1]

[1] According to the reference library *The Essential Chemical Industry – online*, 2013.

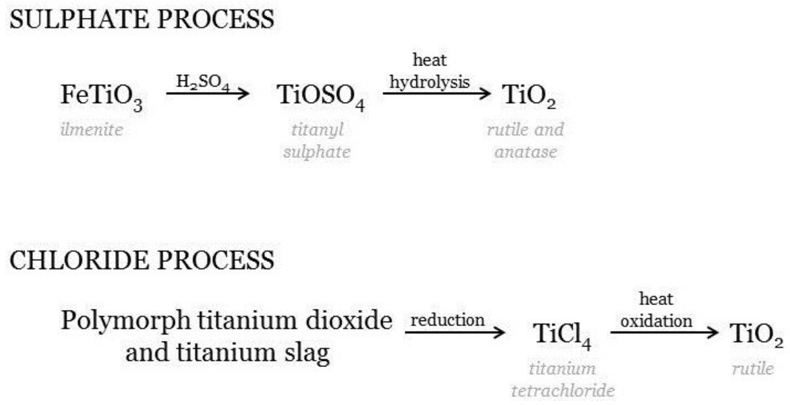

IMAGE 5
SCHEME OF PRODUCTION OF TITNIUM DIOXIDE VIA THE SULPHATE AND CHLORIDE PROCESS, RESPECTIVELY.

Both the anatase and rutile forms of titanium dioxide can be produced by the sulphate process. At elevated temperatures (>600 °C), titanium dioxide converts to the more stable rutile form. Consequently, only rutile titanium dioxide can be synthesized using the chloride process due to the elevated temperature in the calcination step. In finishing steps, the crude form of titanium dioxide is milled to produce a specific distribution of particle size.

Titanium dioxide nanoparticles can be synthesized using various methods, including the sulphate and chloride processes, by applying additional post-manufacturing processes of sufficient energy to break apart the aggregates/agglomerates. Surfactants or solvents can be used to help keep the smaller particles from re-aggregating after separation (United States EPA 2009).

A vast number of methods have been specifically developed to prepare titanium dioxide nanoparticles, including solvothermal methods, chemical/physical vapour deposition, spray pyrolysis precipitation, microemulsion and sol-gel methods (Carp *et al.* 2004; Chen *et al.* 2007), as these syntheses provide a higher degree of control. Many of the syntheses apply the intermediate titanium tetrachloride from the chloride process as a precursor. The sol-gel and the spray pyrolysis syntheses are commonly used to synthesise titanium dioxide nanoparticles. In the sol-gel synthesis the particle size and morphology are controlled by the pH of the gel (IARC 2010).

2.3.1 Thin-film synthesis

Titanium dioxide thin films and coatings is today commercially used to create functional surfaces for e.g. self-cleaning windows and construction parts, low-emission and solar control films on large area glass, anti-fogging mirrors and car windscreens as well as anti-reflective coatings for optics. Furthermore, extensive research in how to apply and benefit from titanium dioxide antibacterial coatings is carried out. Several methods for synthesizing and depositing titanium dioxide thin films and coatings are available, the most common being chemical/ physical vapour deposition techniques (CVD/PVD) or sol-gel methods (Bumjoon *et al.* 2002).

The CVD-based techniques use chemical vapours and gasses as reactants in a reaction chamber to form a solid coating of titanium dioxide. PVD-based methods usually employ a solid target as the evaporation source in an oxygen-containing atmosphere. These techniques enable excellent means to accurately tailor the properties of the deposited titanium dioxide coatings such as coatings thicknesses from a few nanometers to several micrometers, controlled grain size and phase composition (anatase/rutile) and addition of dopants. The techniques can be used to synthesize coatings on almost any base material (textiles, plastics, metals and glass). As an example, coatings used for self-cleaning windows are typically PVD or CVD coatings with a thickness of 15-25 nanometers. The coatings deposited by PVD/CVD methods are usually extremely well adhering compared to e.g. paints.

Sol-gel is a wet-chemical technique often used to produce titanium dioxide thin films from a solution of small molecules. The 'sol' (solution) is gradually evolved towards the formation of a gel-like system containing a solid and a liquid phase. The liquid phase is removed by a drying process and often followed by a heat treatment or firing process. By tailoring the chemical structure of the primary precursor and carefully controlling the processing variables, nanocrystalline products with a very high level of chemical purity can be achieved. In sol–gel processes, titanium dioxide is usually prepared by the reactions of hydrolysis and polycondensation of titanium alkoxides, which are then transformed into an oxide network. The precursor sol can be deposited on a substrate to form a film by e.g. dip coating or spin coating or simply be painted/sprayed onto the surface (West 1999; Kelly *et al.* 2000; Ohring 2002).

2.4 Physical and chemical properties and use titanium dioxide

One of the main differences between nano-sized titanium dioxide and conventional titanium dioxide is the much greater surface are of a given mass or volume of nanoparticles compared to an equivalent mass or volume of conventional particles. This greater surface of the nano-sized titanium dioxide affords a greater potential for many properties significant to the bulk material, including catalytic activity and UV absorption at certain wavelengths (United States EPA 2009), see section 2.6.2 and section 2.6.3. This survey focuses primarily on nano-sized anatase titanium dioxide, meaning particles in the 1 to 100 nm size range. Where information is not specific to nano-sized titanium dioxide, the term ’titanium dioxide’ is used without the prefix ‘nano-sized’, implying that the physical and chemical properties are the same for nano-sized and conventional.

Titanium dioxide is stable in aqueous media and is tolerant of both acidic and alkaline solutions. It is inexpensive, recyclable, reusable and relatively simple to produce. Other important features include inertness, good thermal stability and resistance to UV degradation (IARC 2010). Titanium dioxide is insoluble in water, hydrochloric acid, dilute sulphuric acid and organic solvents, while it dissolves slowly in hydrofluoric acid and in hot concentrated sulphuric acid. It is nearly insoluble in aqueous alkaline media (United States EPA 2009). Anatase titanium dioxide is a metastable phase that transforms irreversibly to the stable rutile phase at a particular temperature, depending on the preparation method and morphology of the primary particles. Moreover, the rutile titanium dioxide is more resistant to UV light than the anatase (Mohammadi *et al.* 2008).

Another characteristic of significance is the aggregation or agglomeration of nano-sized titanium dioxide grades. Reported particle size values for aggregates and agglomerates are influenced by factors such as surface treatment, concentration, pH, medium characteristics and measurement technique. Using the same measurement technique, the mean particle size of three different titanium dioxide particles suspended in water and PBS have been found to differ by a factor ~10. In addition, the mean aggregated particle diameter of the commercial product P25 is reported to be 3.6 μm; i.e. roughly two orders of magnitude larger than the primary particles of P25, which is 21 nm. Despite the presence, and sometimes the predominance, of larger aggregates and agglomerates, several researchers investigating commercial titanium dioxide grades such as P25, have also found particles or aggregates with diameters below 100 nm (United States EPA 2009).

The major use of anatase titanium dioxide may be grouped in three categories based on the function of the titanium dioxide:

- Pigment
- Photocatalyst
- UV-A and UV-B protection

In the section below, a description of each function is presented to illustrate the many properties of titanium dioxide.

2.4.1 Pigment

Titanium dioxide is the most widely used white pigment because of its brightness and high refractive index, which determines the degree of opacity. The high refractive index, only surpassed by few other materials, allows titanium dioxide to be used at relatively low levels to achieve its opacifying effect. Rutile titanium dioxide has a slightly higher refractive index than anatase titanium dioxide (rutile: 2.73, anatase: 2.55). The opacity of light by titanium dioxide is maximized in particles that are 0.2-0.3 µm in diameter, and most commercial products that are used as pigments have a primary particle size within this range. Primary particles generally form aggregates and agglomerates[2] and are not normally found as discrete particles; consequently, the primary particles of the bulk material may be smaller than 0.2-0.3 µm in diameter and do often contain a range of particles, including some with nanoscale dimensions (United States EPA 2009). Most titanium dioxide in the anatase form is produced as a white powder; whereas various rutile grades are often off-white and can even exhibit a slight colour, depending on the physical form affecting light reflectance. According to WHO, anatase pigments may have a more blue tone than the rutile form and are less abrasive (IARC 2010).

Coatings of titanium dioxide used as a pigment

All commercially produced titanium dioxide pigments are coated using aqueous precipitation techniques. Most coatings consist of a multilayer inorganic structure, and in some cases an organic treatment is deposited on the surface. The number and the types of layers combined with the particle size distribution are parameters differentiating one grade from another. The coatings are applied to improve the particle dispersibility, dispersion stability, photostability, opacity and gloss. A list of common grades of titanium dioxide pigment (rutile titanium dioxide) is given in Image 6 below. The types of coating described in the image are expected to be similar for anatase titanium dioxide pigments; however, titanium rutile pigments generally contain 1–15 w% of coating, whereas anatase pigments contain 1–5 w% of coatings (IARC 2010).

Titanium dioxide can act as a photocatalyst and participate in the breakdown of organic matter (see section 2.4.2). However, the photoactivity of the titanium dioxide can be diminished by coating the particle, thereby enhancing the photostability of the pigment. In order to be effective, the coating material must have a large band gap and the coating barrier must completely encapsulate the titanium dioxide particle. Silica fulfils these requirements and is, consequently, commonly used as a coating for titanium dioxide (United States EPA 2009; IARC 2010).

Application of titanium dioxide as a pigment in liquid colorants may result in the formation of flocculates (titanium dioxide particles coming out of suspension), which can reduce the opacity and change the colour. An alumina coating is very effective in retarding flocculation and thereby enhancing dispersibility and dispersion stability (IARC 2010).

Pigmentary titanium dioxide can be made hydrophobic, hydrophilic or neither by depositing organic compounds on the surface. The surface properties depend on the desired in-use performance properties of the titanium dioxide, and the most commonly used organic compounds are polyols (trimethylolpropane (TMP) and trimethylolethane (TME)), esters, siloxanes and silanes such as trimethoxy-caprylylsilane (IARC 2010).

[2] Clustering of particles into a single entity of such particles

Surface treatment type	Composition, range (wt %)	Application
Alumina/TMP	Al_2O_3, 1.0–5.5 Total carbon, <0.3	Paint/coatings
Alumina/zirconia/TMP	Al_2O_3, 1.0–5.0 ZrO_2, 0.3–1.0 Total carbon, <0.3	Paint/coatings
Alumina/silica/siloxane	Al_2O_3, 1–6 SiO_2, 0.3–3 Total carbon, <0.3	Plastics
Alumina/silica/TMP	Al_2O_3, 1.0–6.0 SiO_2, 0.5–13.0 Total carbon, <0.3	Paint/coatings/plastics
Alumina/TME	Al_2O_3, 1.0–3.5 Total carbon, <0.3	Paint/coatings
Alumina/zirconia/TME	Al_2O_3, 1.0–5.0 ZrO_2, 0.3–1.0 Total carbon, <0.3	Paint/coatings
Alumina/silica/TME	Al_2O_3, 1.5–5.0 SiO_2, 1.5–3.5 Total carbon, <0.3	Paint/coatings
Alumina/silica/silane	Al_2O_3, 1.0–6.0 SiO_2, 0.3–3 Total carbon, <0.3	Plastics

IMAGE 6
TYPES OF COATING USED FOR COMMON GRADES OF TITANIUM DIOXIDE PIGMENT (RUTILE TITANIUM DIOXIDE). TME, TRIMETHYLOL ETHANE; TMP, TRIMETHYLOL PROPANE (IARC 2010)

2.4.2 Photocatalyst

Titanium dioxide is a natural semiconductor with photocatalytic properties. The electrons in titanium dioxide can easily become excited by energy adsorbed from UV radiation (impinging photons with energies equal to or higher than its band gap >3.0 eV) (Chen *et al.* 2007). The excited electrons result in the generation of electron-hole pairs ($h^+ e^-$). In this reaction, h^+ and e^- are powerful oxidizing and reducing agents, respectively. The electron–hole pairs may recombine in a short time or take part in chemical reactions, depending on the reaction conditions and molecular structure of the semi-conductors, and the strong oxidation power of h^+ enables it to generate reactive oxygen species (ROS) (Chen *et al.* 2009b). Examples of photocatalytic generation of ROS and conversion of nitric oxide (NO) to nitric acid are illustrated in the below equations:

1. The photocatalytic reaction begins with the generation of the electron-hole pair
$$TiO_2 \xrightarrow{hv} h^+ + e^-$$
2. The h^+ may react with OH^- dissociated from water to form the hydroxyl radical
$$h^+ + OH^- \rightarrow OH^{\cdot}$$
3. The e^- may react with molecular oxygen to form superoxide anion
$$e^- + O_2 \rightarrow O_2^-$$
4. The superoxide anion may further react with H^+ dissociated from water to produce $HO_2^{\cdot}$ radicals
$$H^+ + O_2^- \rightarrow HO_2^{\cdot}$$
5. NO can be oxidized to NO_2 by $HO_2^{\cdot}$ radicals
$$NO + HO_2^{\cdot} \rightarrow NO_2 + OH^{\cdot}$$
6. The NO_2 may be further oxidized to nitric acid by reacting with hydroxyl radicals
$$NO_2 + OH^{\cdot} \rightarrow HNO_3$$

Similarly, the generation of ROS can be exploited in the photocatalytic decomposition of organic matter. As illustrated in the above equations, the photodecomposition process usually involves one or more ROS or intermediate species such as hydroxyl radicals, hydrogen peroxide (H_2O_2) or superoxide (O_2^-) (Chen *et al.* 2007). Since the reactions are governed by a free radical mechanism, it implies that the catalysis process is not selective (Carp *et al.* 2004).

Reactive Oxygen Species

Oxidative stress induced by reactive oxygen species- (ROS-) generation in nanoparticle systems is thought to be the main mechanism of their antibacterial and self-cleaning activity. In particular, many previous studies have explored the photogeneration of ROS on the surfaces of metal-oxide nanoparticles. The general principle is that when illuminated by light with photoenergy greater than the band gap, the electrons (e^-) of nanoparticles are promoted across the band gap to the conduction band, which creates a hole (h^+) in the valence band. Electrons in the conduction band and holes in the valence band exhibit high reducing and oxidizing power, respectively. The electron can react with molecular oxygen to produce superoxide anion ($O2^{\bullet -}$) through a reductive process. The hole can abstract electrons from water and/or hydroxyl ions to generate hydroxyl radicals ($^{\bullet}OH$) through an oxidative process. Singlet oxygen ($^1O_2$) is mostly produced indirectly from aqueous reactions of $O2^{\bullet -}$. $^{\bullet}OH$ is a strong and nonselective oxidant that can damage virtually all types of organic biomolecules, including carbohydrates, nucleic acids, lipids, proteins, DNA, and amino acids. $^1O_2$ is the main mediator of photocytoxicity and can irreversibly damage the treated tissues, causing biomembrane oxidation and degradation. Although $O_2^{\bullet -}$ is not a strong oxidant, as a precursor for $^{\bullet}OH$ and $^1O_2$, $O2^{\bullet -}$ also has significant biological implications. Consequently, these three types of ROS ($O_2^{\bullet -}$, $^{\bullet}OH$, and $^1O_2$) contribute to the major oxidative stress in biological systems. (Li *et al.* 2012).

The photoreactivity of titanium dioxide has been found to depend on surface area of the catalyst and the availability of the active sites. Therefore, particle size is an important parameter for the photocatalytic efficiency. The smaller the particles are, the higher the surface-to-weight ratio is, and, hence, the more active sites there are and thereby increased catalytic activity may be exerted. Much work has been carried out in order to find the optimum particle size of titanium dioxide to obtain the maximum photocatalytic oxidation rate of organic substances. According to some literature, the optimum particle size is approx. 10 nm; however, an optimum size may vary with the exact type of catalyst, organic compound and process parameters (Carp *et al.* 2004). Moreover, surface properties like crystalline structure, pore size, density of OH groups and surface acidity significantly affect the photocatalytic efficiency. The surface characteristics may be modified by a number of different pre-treatments of the photocatalyst, including sulphation and halogenation (Mohammadi *et al.* 2008).

Both the anatase and rutile crystal structures can act as photocatalysts; however, it is widely accepted that the anatase polymorph generally shows significantly higher photocatalytic or photoreactive effect than the rutile polymorph (Carp *et al.* 2004; Chen *et al.* 2007). The mechanism responsible for this behaviour is a controversial subject, and considerable research is in progress to gain a better understanding into this phenomenon. At present, there are several hypotheses seeking to explain the higher photocatalytic activity of the anatase (higher Fermi level, indirect band gap, excitation electron mass), but the discussion is on-going (Banerjee *et al.* 2006). Other studies, on the other hand, claim that a mixture of anatase (70-75 w%) and rutile (25-30 w%) titanium dioxide is more photoreactive than the pure anatase (United States EPA 2009).

In conclusion, the anatase polymorph is more desirable when it comes to harvesting the photocatalytic effect, and the smaller the particle size the more reactions can take place. As a consequence, it is very likely that photocatalytic consumer products contain nano-sized anatase titanium dioxide.

Doping of photocatalytic titanium dioxide
Contrary to titanium dioxide used as pigments, photocatalytic titanium dioxide grades are generally not coated since this would inhibit the desired photoreactivity. Instead, various dopants have been widely studied to improve the photocatalytic performance of rutile, anatase and mixtures of rutile/anatase titanium dioxide on the degradation of various organic substances. By doping titanium dioxide, the band gap may be reduced and hence allow for photoactivation by visible light, which is less energetic than UV light. This allows photoreactivity of titanium dioxide to be offered not only outdoor, but also indoor based on the radiation from indoor lighting, thereby expanding the range of possible applications. Impregnation, co-precipitation, vapour deposition and sol-gel methods are commonly used for introducing the dopants (Carp *et al.* 2004), and cationic doping (transition and rare earth metals), anionic doping and metal oxide doping have been extensively studied (Banerjee 2011). It has been found that the presence of a metal ion in the titanium dioxide matrix significantly influences the photoreactivity of titanium dioxide. Doping with small amounts of Fe^{3+}, Mo^{5+}, Ru^{3+}, Os^{3+}, Re^{5+}, V^{4+} or Rh^{3+} (0.1-0.5 w%) may significantly increase the photoreactivity, while Co^{3+} and Al^{3+} doping may decrease the photoreactivity (Chen *et al.* 2007). However, the photophysical mechanism of doped semiconductors is not fully understood and many controversial results are reported in literature (Carp *et al.* 2004).

2.4.3 UV-A and UV-B protection
UV radiation is classified by wavelength into three types: UV-A (320-400 nm), UV-B (290-320 nm) and UV-C (200-290 nm). The shorter the wavelength, the more energy the UV radiation transmits. Approximately 10 % of the solar radiation that reaches the surface of the earth is UV radiation, and approximately 95 % of that is UV-A (United States EPA 2009).

Titanium dioxide scatters light in the UV-A and UV-B regions and, consequently, the degradation of a polymer (e.g. plastic, rubber or paint) is minimized when surface-treated titanium dioxide is incorporated into the matrix, thereby increasing product longevity.

The size of the titanium dioxide particles (both the primary particle size and the effective particle size of aggregates and agglomerates) affects the UV-A and UV-B transmittance and scattering efficiency of the particles. Consequently, the particle size affects the degree of protection against UV-A and UV-B radiation. In most cases, titanium dioxide particles are present in a size range rather than one specific size due to various primary particle sizes and to aggregation and agglomeration. Primary particles can cluster together to form aggregates and agglomerates, and they are the smallest units that are present in a liquid formulation such as sunscreen. Theoretical calculations suggest that a mean particle size of approx. 50 nm gives the optimum combined protection from outdoor UV-A and UV-B radiation. The particle size also determines the opacity of the formulation; titanium particles larger than 200 nm will e.g. leave a white hue on the skin if used in sunscreen. On the other hand, titanium dioxide particles smaller than 100 nm are generally not visible, and a formulation, e.g. a sunscreen, will appear invisible (United States EPA 2009).

Coatings of titanium dioxide used as sun filter
To increase the photostability of titanium dioxide and prevent formation of free radicals in sunscreen, the titanium dioxide used for sunscreen is most commonly coated. Coating titanium dioxide with silicon dioxide and aluminium (3.5 w%) can reduce photocatalytic activity by 99 % (United States EPA 2009; IARC 2010). Surface coatings for nano-sized titanium dioxide in sunscreen include combinations of inorganic oxides, simethicone, methicone, lecithin, stearic acid, glycerol, silica, aluminium stearate, dimethicone, metal soap, isopropyl titanium triisostearate (ITT), triethoxycaprylylsilane and C9-15 fluoroalcohol phosphate (United States EPA 2009; SCCS 2013). As seen from Image 7 and appendix 2, the most common coating materials for nano-sized

titanium particles used for sunscreen products are combinations of alumina and silica. An alternative technique for increasing photostability is by doping nano-sized titanium dioxide particles by embedding minute amounts of metals within them; metals such as manganese, vanadium, chromium and iron (United States EPA 2009). In addition to increasing the photostability, the surface coating influences the interaction of titanium dioxide with the dispersion medium, which can be water- (aqueous), oil- or silicone-based.

Particle name	Manufacturer	Crystal type	Average crystal size	Coating materials and concentrations
T805 Degussa20/80 RU/AN	Degussa	rutile/ anatase	21 nm	silicone dioxide <2.5%
T817 Degussa79/12/2 RU/AN/Fe	Degussa	rutile/ anatase	21 nm	silicone dioxide <2.5% (also doped with di-iron trioxide 2%)
UV-Titan M160	Kemira	rutile	17-20 nm	alumina 5.5-7.5%, stearic acid 10%
UV-Titan M212	Kemira	rutile	20 nm	alumina 5-6.5%, glycerol 1%
UV-Titan X161	Kemira	rutile	15 nm	alumina 8.5-11.5%, stearic acid 10%
UV-Titan X200	Kemira	rutile	20 nm	none
Eusolex T-2000	Merck	unknown	14 nm	alumina 8-11%, simethicone 1-3%
TTO 51A	Merck	rutile	35 nm	alumina 11%, silica 1-7%
TTO 51C	Merck	rutile	35 nm	alumina 11%, silica 1-7%, stearic acid 3-7%
MT-100 AQ	Mitsubishi/Tayca	rutile	15 nm	alumina 4-8%, silica 7-11%
MT-100 AR	Mitsubishi/Tayca	unknown	15 nm	alumina 4-8%, silica 7-10%
MT-100 T-L-1	Mitsubishi/Tayca	rutile	15 nm	alumina 3.3-7.3%, stearic acid 5-11%
MT-100SA	Mitsubishi/Tayca	rutile	15 nm	alumina 4-7.5%, silica 2-4%
MT100TV (or MT-100TV)	Mitsubishi/Tayca	rutile	15 nm	alumina 1-15% or 3-8%; aluminum stearate 1-13% or 1-15% or stearic acid 5-11%
MT100Z (or MT-100Z)	Mitsubishi/Tayca	rutile	15 nm	alumina 6-10%, stearic acid 10-16%
MT-500SA	Mitsubishi/Tayca	rutile	35 nm	alumina 1-2.5%, silica 4-7%
Mirasun TiW60	Rhodia	anatase	60 nm	alumina 3-7%, silica 12-18%
UV-Titan M262	Rhodia and Kemira	rutile	20 nm	alumina 5-6.5%, dimethicone 1-4%
Tioveil dispersions	Uniqema	rutile	10-28 nm	alumina 10.5-12.5% or 5-15% and silica 3.5-5.5%; alumina 5-15% and aluminum stearate 5-15%

IMAGE 7
LIST OF ULTRAFINE TITANIUM DIOXIDE GRADES USED IN SUNSCREEN (UNITED STATES EPA 2009) . A COMPREHENSIVE LIST OF ULTRA-FINE GRADES CAN BE FOUND IN APPENDIX 1

In one study, by Barker and Branch, it was investigated whether the surface coatings of nano-sized titanium dioxide in sunscreen was stable and effective. The investigators studied the weathering of paint in contact with sunscreen. The one sunscreen with nano-sized titanium dioxide that showed no appreciable effect on paint weathering used a 100 % rutile doped with manganese rather than surface-coated particles. On the other hand, all four sunscreens with an anatase/rutile mix released photocatalytically generated hydroxyl radicals that accelerated the weathering of the paint. (Barker *et al.* 2008). This study has led to a number of newspaper articles and blog posts debating the safety of using anatase titanium dioxide in dermal products (sunscreen and cosmetics), though without reaching a conclusion. In contrast to the above results, the recent SCCS opinion on titanium dioxide (nano form) found that all 15 ultra-fine titanium dioxide grades tested, including one pure anatase and two mixtures of rutile/anatase, were photocatalytically stable (results listed in appendix 2). SCCS does however stress that there is a concern in relation to stability of the coatings. Based on photocatalytic activity data measured in the formulations, there is an indication that either some of the material is not coated or that some of the coatings were not stable in the formulations and effects of photocatalytic activity could be seen at a later (SCCS 2013).

2.5 Commercial titanium dioxide

A large number of titanium dioxide grades are commercially available, and they are to a large extent distinguished by their use, i.e. as a pigment, as a photocatalyst and for UV protection. Some grades are the pure form (or close to) of either anatase or rutile titanium dioxide, while others are (intentional) mixtures of the two.

The possible impurities in titanium dioxide arise from the impurities in the mineral ores and solvent used in the manufacturing process. Anatase titanium dioxide pigments may contain rutile titanium dioxide, and anatase titanium dioxide produced by the sulphate process contains both phosphorous and sulphate concentrated at the surface of the particle. In addition, uncoated anatase titanium dioxide pigments retain about 0.3 % niobium pentoxide and 0.3 % phosphorus pentoxide from the ore and 0.2 % alumina that is added during manufacturing (IARC 2010). Titanium dioxide can be prepared at a high level of purity, and specifications for food use currently contain a minimum purity assay of 99.0 % (Kuznesof *et al.* 2006). Anticipated by-products of the flame hydrolysis method of titanium dioxide production include those resulting from chlorine contamination of the titanium dioxide (from the $TiCl_4$ precursor). Consequently, solutions of titanium dioxide synthesised by flame hydrolysis have been found to be acidic because of chloride ion artefacts on the particle surface; however, manufacturers state that a steam washing step during the manufacturing process removes hydrochloride acid adsorbed on the surface (United States EPA 2009).

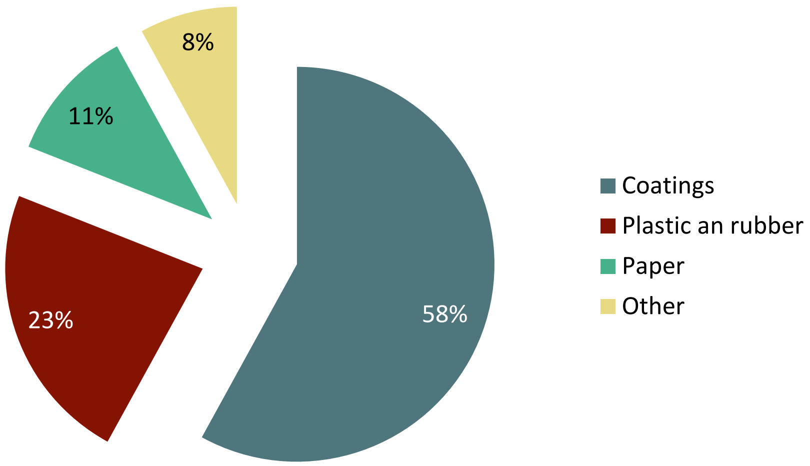

IMAGE 8
WORLD CONSUMPTION OF TITANIUM DIOXIDE PIGMENT BY END-USE IN 2005 (MASS-PERCENTAGES) (IARC 2010).

The majority of produced titanium dioxide is used for pigmentary purposes. World consumption of titanium dioxide pigment by end-use in 2005 was: coatings 58 %, plastic and tubes 23 %, paper 11 %, and other 8 %. The 8 % other include the use of titanium dioxide as a pigment in food and for cosmetics (IARC 2010). Several hundred commercial grades have been developed for various applications, and in Image 8, the frequently used coatings for rutile titanium dioxide pigments are listed. The type of coating is expected to be similar for anatase titanium dioxide pigments.

The rutile titanium dioxide pigment is commonly preferred over the anatase titanium dioxide pigment for paint and plastic products; especially for those exposed to outdoor conditions, since the anatase form is less stable than the rutile form. Further, the blue tone of anatase pigments is in many cases undesirable. On the other hand, the lower cost of anatase pigments favour the use of anatase titanium dioxide in products where the colour tone and the reduced abrasive properties are of less importance than cost, which may the case for plastics, paper and cheap low-gloss paints. Despite their lower price, anatase pigments account for only 10 % of the total global production (IARC 2010). In the database 'Substances in Preparations In the Nordic countries' (SPIN)[3], the total use of titanium dioxide in Denmark was estimated to 27,061.9 tonnes in 2011 with more than 4,600 different preparations. In contrast, the total use of anatase titanium dioxide in Denmark was

[3] The database, Substances in Preparations In Nordic Countries (SPIN), contains non-confidential information on substances from the Product Registries of Norway, Sweden, Finland and Denmark.

estimated to 107.3 tonnes in 2011 with almost 100 tonnes being used for paint, lacquers and varnishes. However, the total use varies greatly as illustrated in image 9 and Image 10.

According to the American Society for Testing and Materials (ASTM, 1988) D476–84 standard, four types of titanium dioxide pigments exist. Type I (minimum 94 % titanium dioxide) is an anatase pigment that chalks freely and is used in white interior and exterior house paints. The three remaining types are rutile pigments with a higher resistance to chalking (IARC 2010).

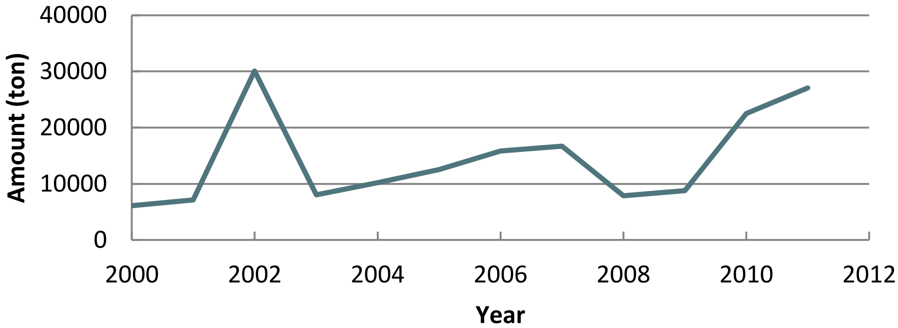

IMAGE 9
TOTAL USE OF TITANIUM DIOXIDE IN DENMARK BASED ON THE ESTIMATES FROM THE SPIN DATABASE

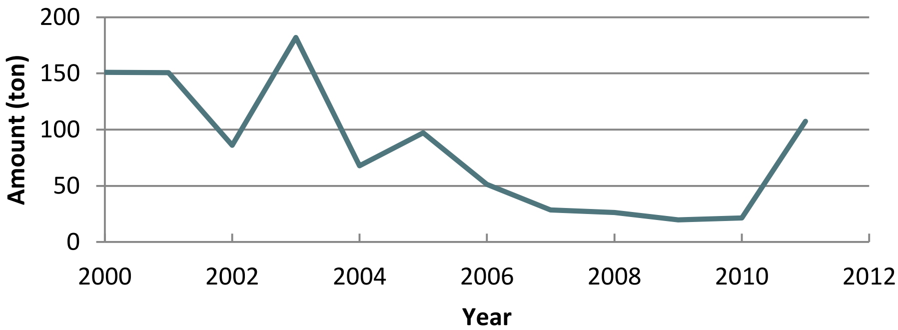

IMAGE 10
TOTAL USE OF ANATASE TITANIUM DIOXIDE IN DENMARK. BASED ON THE ESTIMATES FROM THE SPIN DATABASE

Ultrafine grades of titanium dioxide have a primary particle size of 10–50 nm, are most often coated and are used predominantly as ultraviolet blockers in sunscreen and plastics. Ultrafine grades of titanium dioxide transmit visible light while scattering light in the UV-A and UV-B region (Nie *et al.* 2009). A list of commercially available ultrafine titanium dioxide grades (with description of particle size, crystal form and coating) for use in sunscreen is presented in Image 7, and a more comprehensive list can be found in Appendix 2:. The titanium dioxide materials used in sunscreen products are reported to be composed of both anatase and rutile or a mixture of the two (SCCS 2013). Sunscreen manufacturers can purchase nano-sized titanium dioxide grades and formulate their own dispersion, or they can purchase ready-made 'pre-dispersions' (United States EPA 2009). The annual global production of nano-sized titanium dioxide was estimated to 10,000 metric tons with 65 % being used for personal care products (United States EPA 2009). This far, nano-sized titanium dioxide production has only represented a small fraction of the overall titanium dioxide production accounting for more than 4.5 million metric tons annually. The use of nano-sized anatase titanium dioxide in sunscreen is described in further details in section 3.3 and mapped in chapter 4.

Photocatalytic titanium dioxide grades are marketed by several manufacturers, with the anatase titanium dioxide being predominant. Further, a commercial titanium dioxide known as Degussa

P25 is used widely because of its relatively high levels of activity in many photocatalytic reaction systems. P25 is a mixture of anatase (approx. 80 %) and rutile (approx. 20 %) with a primary particle size of 21 nm. Relatively small quantities of titanium dioxide are used for non-pigmentary purposes, and it has been estimated that approx. 1,500 tonnes of titanium dioxide is used for photocatalytic purposes (IARC 2010).

3. Anatase titanium dioxide in consumer products

Anatase titanium dioxide is used industrially in a wide range of products, and as indicated in Chapter 2, the major applications may be grouped in three categories based on the function of the anatase titanium dioxide:

- Pigment
- Photocatalyst
- Physical sun filter in sunscreens

In this chapter, an overview of consumer product applications containing anatase titanium dioxide is given based on the above categorisation. From this overview, two relevant product groups have been selected for detailed mapping. The results of these studies are described in chapter 4.

TiO_2 function	Applications
Pigment	Paints, coatings, paper, plastic, rubber, fibre, ceramic, food, cosmetic
Photocatalyst	Paints, coatings, water treatment systems, air cleaners, construction materials
Physical sun filter	Sunscreens

TABLE 1
SUMMARY OF IDENTIFIED PRODUCT GROUPS CONTAINING ANATASE TITANIUM DIOXIDE.

3.1 Pigment

Anatase pigments are mainly used in indoor paints and in paper, ceramics, rubber and fibres manufacture, and approx. two-thirds of the total anatase supply is used for e.g. paper, low-priced emulsion paints or tiles and enamels because pigment quality is of less importance in these products. The remaining 33 % is used in applications for which its specific properties are highly valued, such as when a bluish tint is desired in some plastics (IARC 2010). According to the SPIN database, more than 90 % (mass) of the total anatase titanium dioxide supply is used for paints, laquers and varnishes.

The scattering of light by titanium dioxide is maximized in particles of 0.2-0.3 µm in diameter, and, according to WHO, most pigments have a particle size within this range. However, primary particles generally form aggregates and agglomerates and are not normally found as discrete particles; consequently, the primary particles of the bulk material may be smaller. This is clearly illustrated for pigmentary titanium dioxide used for food.

Food-grade titanium dioxide (referred to as E171) is purchased by the ton and is available as synthetic forms of anatase, rutile and as mixtures of the two forms. Titanium dioxide is widely used in e.g. dairy products, candy, gums, baked goods, seasonings and beverages and is present in concentrations varying between 0.0005 % and 0.04 %, as determined in a number of food products (Weir *et al.* 2012). Recently, it has been debated whether titanium dioxide is to be considered a nanomaterial when used in food and feed products, since the smaller particles may aggregate strongly to larger colloids (ELC 2009). The mean size of food-grade titanium dioxide has been determined to 110 nm, and 36 % of the particles by number are smaller than 100 nm (Weir *et al.* 2012), meaning that it is not unlikely that food grades of titanium dioxide may be termed a nanomaterial according to the Recommendation of the EU Commission on the definition of nanomaterials (2011/696/EU).

3.2 Photocatalyst

Photocatalysts based on titanium dioxide are mostly employed in self-cleaning surfaces and for water and air cleaning systems where it degrades or transforms various unwanted microorganisms or organic substances and pollutants into less harmful substances. Additionally, photocatalysts based on titanium dioxide can be used in solar cell applications (photovoltaic cells) and for production of hydrogen. On the Danish market, a number of photocatalytic products have been introduced where the photo-reactive properties of titanium dioxide are utilized to create radical species upon photo-excitation (as described in section 2.4.2). The free radicals decompose organic pollutants and bacteria on the surface and lead to clean surfaces, thereby exerting a self-cleaning or antibacterial effect.

3.2.1 Photocatalytic products

In the preliminary product screening of this survey, close to 90 photocatalytic products containing titanium dioxide were readily identified on the global market. For a large part of the identified products, the size distribution and/or the crystal form of titanium dioxide (anatase and/or rutile) are not specified; however, in order to obtain the designated product performance, it is most likely that nano-sized anatase titanium dioxide is contained in the product. In table 2, the identified products have been categorized into four product groups and assigned to the photocatalytic properties and the form in which titanium dioxide is present in the products. The table is non-exhaustive; nevertheless, it covers the main product groups.

Type of photocatalytic product	# Products identified	Self-cleaning	NOx/CO reducing	Antimicrobial	Presence
Paint (indoor/outdoor)	16	X	X	X	Dispersion
Coating (for plastic, glass, metal, textile, painted surfaces)	43	X		X	Dispersion /aerosol
Construction materials (tiles, windows, concrete, mortar)	19	X	X		Surface
Air cleaners	11		X	X	Surface

TABLE 2
OVERVIEW OF PHOTOCATALYTIC PRODUCT GROUPS, AND THE NUMBER OF PRODUCTS AS WELL AS THEIR EFFECTS, IDENTIFIED ON THE DANISH MARKET.

Paints

The use of anatase titanium dioxide in paint can be divided into two categories. Primarily, the use of titanium dioxide (anatase and or rutile) in paint is due to its light-scattering properties that ascribe it as a white pigment, and this application is covered by the pigment category described above.

Another application, the deliberate use of nano-sized anatase titanium dioxide in paint, has been developed to exploit the photo-reactive properties of nano-sized anatase titanium dioxide for decomposing organic substances and bacteria on the surface to achieve self-cleaning/antibacterial effects. Photocatalytic paints are frequently incorporated in different binders such as lime, polyorganic siloxane, silica sol-gel and organic binders (Chen *et al.* 2009b).
In the preliminary product screening, a total of 16 paint products that apply photo-reactive titanium dioxide particles (Table 2), were identified. The products include paints for indoor and outdoor, including various types of facades (bricks, wood, etc.).

A detailed mapping of photocatalytic paints on the Danish market is given in Chapter 4.

Coatings
The use of coatings for surfaces has the explicit purpose of protecting the treated surface from various physical stresses. Lately, a number of self-cleaning and self-disinfection coatings, have been brought to the market. Many of the commercially available coatings are aimed at the DIY market, where surfaces on buildings and metal, stone and glass can be coated to gain the self-cleaning property. In the preliminary product screening, a total of 43 coating products that contain photo-reactive titanium dioxide particles were identified (Table 2).

Construction materials
The major applications of titanium dioxide-based photocatalytic construction and building materials are air pollution remediation, self-cleaning and self-disinfection. For all products, the driving force is solar light. Construction and building materials are optimal media for applying the photocatalytic materials because large areas are exposed to light; however accumulation of contaminants on the surface have proven a general problem. Several pilot projects have been carried out to verify the effectiveness of photocatalytic construction and building materials(Chen *et al.* 2009b). Successful commercialization of self-cleaning surfaces that enable buildings to maintain their aesthetic appearance over time includes concrete elements, pavements, windows and roof tiles. Similarly, a number of self-disinfecting building materials have been commercialized to achieve an environment free of microorganisms. Finally, roofing felt and pavements enable of decomposing air pollutant (including volatile organic compounds and oxides such as NO, NO_2 and SO_2) have been marketed.

Air cleaners
A limited number of air cleaners using the combination of artificial UV light and titanium dioxide as a catalyst for the removal of pathogens, viruses and volatile organic compounds are marketed. In the preliminary product screening, a total of 11 air cleaners using artificial UV light and titanium dioxide as a catalyst were identified (Table 2), including products for professional application (e.g. offices).

The most common approach is to coat titanium dioxide onto a surface of an air-purification filter to increase the irradiated area (Yao *et al.* 2011). Specific to the air cleaners is that the nano-sized titanium dioxide has been deposited as a thin film to the surface of these products during manufacturing. Most thin films are synthesized using chemical vapour deposition or spray pyrolysis deposition as described in section 2.3.1.

3.3 Physical sun filter in sunscreen

The Scientific Committee on Consumer Safety (SCCS) has recently published an opinion based on the risk assessment of nano-sized titanium dioxide used as UV filter in cosmetic products (SCCS 2013). According to this opinion, sunscreen products containing nano-sized titanium dioxide are available on the European market with titanium dioxide concentrations ranging from 2 % to more than 10 %. The maximum concentration (by weight) of titanium dioxide in sunscreen that is allowed in the EU is 25 %, but this limit does not distinguish between nano-sized and conventional titanium dioxide, between anatase and rutile or between coated and uncoated. In 2006, the Australian Therapeutic Goods Administration (TGA) estimated that 70 % of sunscreens with titanium dioxide were formulated with nanoparticles (Tran *et al.* 2011).

Recently, a comprehensive study conducted by the National Measurement Institute (Australian Government) indicated that the primary form of titanium dioxide was anatase in 75 % of the tested sunscreens, including well-known brands such as Nivea and L'ôreal (NMI 2012). In a comparable study, five titanium dioxide sunscreens 'purchased over the counter' was analysed. It was found that one was pure rutile and the other four were anatase/rutile mixtures in which anatase was predominant (Barker *et al.* 2008).

A detailed mapping of sunscreen products on the Danish market is given in Chapter4.

4. Mapping of nano-sized anatase titanium dioxide in photocatalytic paints and sunscreens

The objective of the mapping has been to gather information on the prevalence of nano-sized anatase titanium dioxide in photocatalytic paints and sunscreens on the Danish market. The mapping comprises alone products marketed in Denmark or sold on Danish/Danish language internet pages.

The mapping has been carried out during November 2013. The degree of detail of the questionnaire responses varied a lot, and for some of the responses, only a few of the questions were answered. Some of the completed questionnaires were followed up by a telephone interview to clarify further. The full questionnaire scheme is seen in Appendix 1.

4.1 Photocatalytic paints

In the mapping, five companies have been identified as marketing photocatalytic paint products; three major international companies offering a full product range of paints, coatings, lacquers, etc. for a wide range of applications, and two small/medium-sized companies focusing on niche paint products, primarily photocatalytic paints for indoor and outdoor, including various types of facades (e.g. brick and wood). All five manufacturers/importers of photocatalytic products submitted the questionnaire, and, in three cases, a subsequent thorough telephone interview was conducted.

Overall, the two specialized companies responded that they sell photocatalytic paint products based in titanium dioxide, while one large company responded that their photocatalytic product is not based on titanium dioxide. Further, the three large companies have in the past offered photocatalytic paints based on titanium dioxide, but have withdrawn the products. This means that the present photocatalytic paints commercially available on the Danish market is offered by small companies focusing narrowly on niche products such as the photocatalytic paints. It also means that none of the products are marketed through major DYI retailers, i.e. Silvan, Bygma, Bauhaus, etc., but rather via the internet. The main findings of the mapping of photocatalytic paints are summarized in table 3.

	Yes	No
Do you sell paint products with a photocatalytic effect?	3	2
Is the photocatalytic effect (if present) based on titanium dioxide?	2	1
Can the titanium dioxide used (if present) be termed as a nanomaterial?	1	1

TABLE 3
OVERVIEW OF THE MAPPING RESULTS OF PHOTOCATALYTIC PAINTS. NOT ALL QUESTIONS ARE RELEVANT FOR ALL COMPANIES, AND, FOR THIS REASON, THE ANSWERS DO NOT SUM TO FIVE.

The overall prevalence of photocatalytic paints is by one company stated to be higher on the European market compared to the Danish market. The reasons for the withdrawal of the photocatalytic paints based on titanium dioxide from the Danish market by three companies were based on a lack of customer interest and limited sales, making the Danish market for photocatalytic paints a niche market. According to one respondent, customers are not willing to pay the additional cost of photocatalytic paint, and the photocatalytic paints withdrawn from the market had some drawbacks, which in some cases favoured the selection of non-photocatalytic paints. However, the one company is planning the introduction of improved photocatalytic products, implying a faith in the future of photocatalytic products.

One respondent specifies that the titanium dioxide used for photocatalytic paints on the German market is composed combinations of doped anatase and rutile titanium dioxide a with particle size of the rutile being 0.2-0.4 µm and 1.0-2.0 µm for the anatase. Consequently, the respondent states that the photocatalytic titanium dioxide is not to be considered as a nanomaterial. However, it is not known whether the size distributions are for the aggregated/agglomerated particles or for the primary particles[4]. According to the respondent, the concentration of the photocatalytic titanium dioxide varies between 5 % and 10 % in their products.

The two small companies market specialty paint products, including photocatalytic paints. Both companies have experienced an increase in sales of photocatalytic paints and expect this trend to continue. The photocatalytic titanium dioxide was by one of the companies stated to be anatase and nano-sized. According to the respondent, the concentration of the photocatalytic titanium dioxide is approximately 6%.

The remaining companies were not able or willing to inform of either size or crystal form of the titanium dioxide used in their product; however, it is assumed likely that the concentrations given by other respondents are representative.

Highlights of nano-sized anatase titanium dioxide in photocatalytic paints

Based on the mapping (chapter 4) and international literature and reviews (chapter 2-3), the use of nano-sized anatase titanium dioxide in photocatalytic paints can be summarized to:

- Photocatalytic paints are only marketed by a few companies in Denmark, and the number of companies has been decreasing.
- The companies offering photocatalytic paints have experienced increasing sales of the paints.
- Photocatalytic paints represent a minor share of the total market for paints.
- The photocatalytic paints generally contain 5-10 % anatase titanium dioxide.
- The applied anatase titanium dioxide is likely to have a primary particle size in the nano range; however, the mean aggregated particle diameter of titanium dioxide in the products is likely to be larger than 100 nm.

[4] As previously described in section 2.5, the mean aggregated particle diameter of the product P25 is reported to be 3.6 µm in the finished product, roughly two orders of magnitude larger than the primary particles of P25, which are 21 nm

4.2 Sunscreen

The Danish sunscreen market consists of more than 30 different brands, including large international brands, smaller brands targeted at narrow customer segments and purely Danish brands. Manufacturers most often produce a number of different brands (including private label), and, likewise, importers and retailers offer several brands. 11 companies were approached (see list in table 4), covering differently sized companies as well as differing target customers, and, in addition, the Danish cosmetics trade association and senior consultant Daniel Minzari (IPU) was interviewed as an expert who has recently tested sunscreen products on the Danish market. The trade association were not able to offer useful information. Most responses were obtained from Danish companies, while the international companies with large brands are underrepresented in the results. This means that only minor fraction of the sunscreen products available on the Danish market are represented in the mapping, which in turn may have the consequence that the mapping does not reflect the actual prevalence of nano-sized anatase titanium dioxide.

Company type	Contacts (approached/ responses)	Questionnaires (sent/response)	Interview	Titanium dioxide (now/previously)
Danish manufacturers	5 / 3	5 / 2	2	0 / 2
International manufacturers	4 / 1	2 / 1	0	1 / -
Danish retailers	2 / 2	2 / 2	1	0* / 2*
Expert	1 / 1	0	1	N/A

** based on own brands alone. N/A: Not applicable.*

TABLE 4
OVERVIEW OF THE MAPPED COMPANIES AND ANSWERS OBTAINED FROM QUESTIONNAIRES AND INTERVIEWS. SOME COMPANIES GAVE NO RESPONSE AT ALL, SOME BOTH RESPONDED TO THE QUESTIONNAIRE AND WERE INTERVIEWED, AND YET OTHERS GAVE INFORMATION BY EITHER COMPLETING THE QUESTIONNAIRE OR BEING INTERVIEWED.

The overall conclusion is that only one company, that took part in the sunscreen mapping, markets sunscreen products containing titanium dioxide as a physical sun filter in Denmark. No data of the crystal form, size or coating were made available. The company is an international manufacturer with one product line of 15 sun protection products, all containing titanium dioxide, and another product line with three sun protection products containing titanium dioxide. Supplementing with product data obtained from the company's web page, the lists of ingredients state that most of their sunscreens contain nano-sized titanium dioxide. Moreover, several sunscreens from international manufacturers, including L'Ôreal, were recently tested by the Australian NMI and found to contain nano-sized titanium dioxide (see section 3.3).

The Danish manufacturers focus on sunscreen products with the Nordic Ecolabel 'Svanen', which (since 2011) does not allow nanomaterials. Consequently, none of the Danish manufacturers offer sunscreen with titanium dioxide. This has not always been the case, and two Danish manufacturers state that titanium dioxide has been used in their sunscreen products previously, meaning that the use of titanium dioxide as a sun filter has declined due to the focus on nanomaterials in cosmetics. Further, two of the companies specify that they may reintroduce sunscreens containing nano-sized titanium dioxide if allowed for use in sunscreens with the Nordic Ecolabel. The substitute for titanium dioxide in sunscreen products is chemical sun filters.

The Danish retailers, including the major Danish retailer Matas, have based their responses solely on their own brands plus one international brand, for which Matas is the exclusive distributor in Denmark. Thus the large international brands, which the retailers offer along with their own brands, are not considered in the conclusions at all. Both retailers state that none of their sunscreen products contain titanium dioxide. In addition, the one retailer, like the Danish manufacturers, states the reason that the Nordic Ecolabel does not allow the use of nano-sized titanium dioxide, but gives the information that the products have contained the titanium dioxide sun filter previously.

The international company marketing sunscreen containing titanium dioxide was not willing to inform of the characteristics due to business confidentiality, meaning that there is no information about the particle size, concentration or coating of the titanium dioxide particles used in the specific products. However, a Danish manufacturer was able to report that a titanium dioxide sun filter previously used was the UV-TITAN M161 ULTRAFINE which is rutile titanium dioxide particles in the nanoscale (average size of approx. 17 nm) coated with aluminium and added in a concentration of approx. 1 %.

Daniel Minzari, engineer and senior consultant at IPU – Technology Development, has recently tested seven randomly picked sunscreens (that contain titanium dioxide) available on the Danish market. He found that the primary crystal form of titanium dioxide was anatase in two of the sunscreens, whereas the primary form of titanium dioxide was rutile in the remaining five. Using a photocatalytic activity assay, the photocatalytic activity was measured in one sunscreen containing anatase titanium dioxide and one containing rutile titanium dioxide. The sunscreen containing rutile titanium dioxide exhibited negligible photocatalytic activity, while the sunscreen containing anatase titanium dioxide showed a significant photocatalytic activity. The particle size of the sun filter was not measured in this study. The findings are in good agreement with previous findings as described in section 2.6.1.

Highlights of nano-sized anatase titanium dioxide in sunscreen

Based on the mapping (chapter 4) and international literature and review (chapter (2-3), the use of nano-sized anatase titanium dioxide in sunscreens can be summarized to:

- Many Danish brands have the Nordic Ecolabel 'Svanen' which states that the products cannot nano-sized particles. Therefore, many Danish sunscreen products do not contain titanium dioxide.
- Overall, nano-sized titanium dioxide is used abundantly in sunscreens, and transparent sunscreens with a physical sun filter are likely to contain nano-sized titanium dioxide.
- Sunscreens using a physical sun filter often contain between 2 % and 10 % titanium dioxide.
- National and international studies have found that the titanium dioxide materials used in sunscreen products are composed of rutile, anatase or a mixture of the two types. However, the exact proportion of anatase and rutile titanium dioxide used for sunscreen cannot be estimated based on this survey.
- All titanium dioxide used in sunscreen is coated to improve photostability, dispersibility, dispersion stability and to prevent formation of free radicals. The use of anatase titanium dioxide in sunscreen with regard to generation of radical species has been debated. SCCS recently found all tested nano-sized grades (rutile, anatase or a mixture of the two types) to be photostable; however, SCCS further stresses that from data on other measures of photocatalytic activity determined in formulations, the stability of the coatings is less obvious

5. Toxicity, exposure and risk with focus on anatase titanium dioxide

Titanium dioxide is as previously described a well-known and widely used material. It is commonly found as a UV filter in sunscreen products but has only recently attracted attention in this use, due to the potentially hazardous properties related to the nanoform of the material. Until the SCCS opinion was published in 2013 (SCCS, 2013), there has been no restriction regarding the percentage of titanium dioxide which was allowed in the **anatase** form in cosmetic products. In the 2013 Opinion it was concluded that use of titanium dioxide in its nanoform in concentrations up to 25 % and with a content of maximum 15 % anatase was considered safe. However, the Opinion from 2013 was updated in 2014 (SCCS, 2014)) and the amount of anatase $TiO2$ considered safe was further reduced to 5% of the contained nano $TiO2$ (SCCS, 2014). This means a maximum of 25% titanium dioxide in the nanoform as UV filter in the sunscreen products and up to 5% of this amount in the **anatase** crystal form, i.e. 1.25 % anatase in the product.

As background for the present report, the most recent projects, reviews and evaluations addressing nano-sized titanium dioxide have been reviewed with the specific objective of identifying available toxicity information for the **anatase** form alone or in combination with **rutile**. Coating of the nanoparticles is also considered as far as the available literature allows to make conclusions. The key conclusions from these reviews and papers from the open literature are described in this chapter. Further, for the product types identified/chosen based on the previous activities in this project, exposure and risk assessments are addressed based on the available data.

It should, however, be emphasised that there is still uncertainties in the risk assessment of nanomaterials and not yet a standardised framework for evaluation and interpretation of toxicity and exposure data. Knowledge gaps include information on the validity of the available test regimes when used for nanomaterials, a more complete understanding of the role of physical–chemical properties by mode-of-action category, and relevance of the assessment factors used in risk assessment of bulk chemicals.

Currently most toxicity data available for nanomaterials are from *in vitro* tests which are generally designed and validated for chemical substances in non-nano forms and therefore need to be accompanied by other supporting evidence, in order to demonstrate that the nano-specific properties have been properly considered. Another issue is the quality of available data and the lack of adequate physico-chemical characterisation including information on crystallinity in the case of nano-sized titanium dioxide in many studies. Keeping these uncertainties in mind, "a positive toxic response in the available tests is still considered valid for risk assessment as it would indicate "a hazard potential" as concluded in the SCCS opinion on titanium dioxide (SCCS, 2013).

5.1 Absorption, distribution, metabolism and excretion of nano-sized titanium dioxide

5.1.1 Oral absorption

A recent report providing an overview of systemic absorption of nanoparticles by oral exposure (Danish EPA 2013b) concludes that there are very few well-performed *in vivo* studies on the absorption of titanium dioxide nanoparticles. Four studies were identified, three studies in rats and one in mice. Results from these studies are summarised in Table 5.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Wang *et al.* 2012)	Anatase, nearly spherical, OH-group on the surface, 75 ± 15 nm	Rats, male Sprague-Dawley, 3 weeks old and 8 weeks old Comparative oral toxicity study in young and adult rats 0, 10, 50, 200 mg/kg bw/day for 30 days	Endpoints: Body and organ weight (liver, kidney, spleen, testis, lung, heart, brain) Blood biochemistry, histology, histamine, IgE, elemental content, TEM. Findings: Liver and heart injury in young rats, slight liver and kidney injury in adult rat. Reduced intestinal permeability, especially in adult rats. Reductive stress based on different mechanisms in young and adult rats. Enhanced no. of mast cells in young rats. No particles found in the liver of young and adult rats. Aggregation of TiO_2 NPs in the GI tract	Young rats seem more susceptible to NP exposure than adult rats. Low absorption from the gastro intestinal tract, no translocation of the NPs located in the mucosa of the stomach and the small intestine into the systemic circulation. Necessary to consider age difference when setting up recommended daily intake of TiO_2 NPs in food.
(Onishchenko *et al.* 2012)	Anatase Rutile TiO2, micron size	Rats, male Wistar Administration of 50 mg/cm³ into isolated loop of rat small intestine, 3 hour exposure, only rutile. 28 days subacute intragastric intubation of 1 or 100 mg/kg	Transmission electron microscopy. 3 hour exposure caused no appreciable morphological changes in enterocyte ultrastructure. Accumulation in the liver.	Short-term (3 h) exposure to TiO_2 NP (rutile) in high concentrations was not toxic for the rat intestinal epithelium. Titanium accumulation in the liver indicating possible penetration of rutile TiO_2 through the GI barrier at the high dose. Low absorption of anatase TiO_2.
(Wang *et al.* 2007)	TiO_2, 25 nm TiO_2, 80 nm TiO_2, 155 nm (No information on crystallinity)	Mice, CD-1 Oral gavage. 5 g/kg bw, single dose	Endpoints: Body and organ weight (liver, kidney, spleen) Blood biochemistry, histology, histamine, IgE, Ti content. Increased liver coefficients in female mice in the 25 and 80 nm groups. Changes of biochemical parameters (ALT/AST, BUN, and LDH), demonstrated that TiO_2 particles induced the significant lesions of liver and kidneys in female mice.	No obvious acute toxicity was observed. TiO_2 particles induced the significant lesions of liver and kidneys in female mice. No significant size-dependent toxic effects, but gender dependent effects were obvious.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Jani *et al.* 1994)	Rutile, 500 nm	Rats, female Sprague Dawley Oral gavage, 12.5 mg/kg bw, 10 days	Endpoints: Body and organ weight, histology, Ti content. Findings: Particle uptake in the GI tract takes place primarily via Peyer´s patches and are translocated to the mesentery network. Some particles enter general circulation and are taken up by liver and spleen.	Results indicate that insolubility and particulate nature of materials does not guarantee non-absorption from the gut.

TABLE 5
ORAL ABSORPTION OF TITANIUM DIOXIDE

Wang *et al.* (2012) investigated the distribution of titanium dioxide nanoparticles (**anatase** crystals with hydroxyl groups on the surface) following administration by gavage in young and adult rats. The results indicated a very low absorption from the gastro intestinal tract and that there was no translocation of the nanoparticles located in the mucosa of the stomach and the small intestine into the systemic circulation. The administered doses were up to 200 mg/kg bw, and the highest dose thereby 2000 times higher than the potential human exposure by the oral route (estimated at 0.1 mg/kg bw/day) (Wang *et al.* 2012). A possible explanation of the low absorption as suggested by the authors is aggregation of the **anatase** form.

Penetration of **anatase** titanium dioxide nanoparticles, rutile titanium dioxide nanoparticles or micro-sized titanium dioxide particles (the food additive E 171) into enterocytes, after their administration into isolated loops of rat small intestine, was investigated in an *in situ* experiment by (Onishchenko *et al.* 2012). The study demonstrated that at least a part of the titanium dioxide nanoparticles present in the intestinal lumen and located on the surfaces of microvilli as well as individual titanium dioxide nanoparticles present in the enterocyte apical part and in the cytoplasm had crystal structure. Water dispersions of nanoparticles (3 hour exposure of the isolated loops to high concentrations) caused no appreciable morphological changes in enterocyte ultrastructure.

In a separate 28-day study, Onishchenko *et al.* (2012) investigated the absorption and distribution of titanium dioxide nanoparticles in rats. The animals received a low dose (1 mg/kg bw/day) and a high dose (100 mg/kg bw/day) of water suspensions of **anatase** titanium dioxide nanoparticles, rutile titanium dioxide nanoparticles or micro-sized titanium dioxide particles (the food additive E 171) for 28 days by gavage. The content of titanium was measured in the liver. The results indicated that titanium dioxide nanoparticles are more readily absorbed than micro-sized particles (E 171). The absorption is also dependent of the crystallinity of the particles, with the rutile form being the best absorbed. Titanium was only detected in the liver after exposure to doses 1,000 times higher than the expected human exposure (Onishchenko *et al.* 2012).

Another study by Wang *et al.* (2007) investigated the distribution of titanium dioxide nanoparticles (25 and 80 nm, crystal form not specified) and fine titanium dioxide (155 nm) particles in CD-1 mice administered a single large dose of 5 mg/kg bw by gavage. The results indicated that titanium dioxide nanoparticles are absorbed from the gastrointestinal tract after oral exposure to extremely high doses and distributed to liver, spleen, lung and kidneys. The nanoparticles initiated an inflammatory response in the liver, with liver damage indicated by a rise in serum transanimases. Hepatic necrosis was observed in histopathological investigations. Markers of cardiac damage in serum were also observed to be elevated by the titanium dioxide nanoparticle exposure (Wang *et al.* 2007). It is not clear which crystal form is used and in which form titanium dioxide is absorbed (ions, particles or both). Furthermore, it is also highlighted that it is not clear whether absorption is dependent on size because the tissue concentrations resulting from dosage of the two nano-sized suspensions appeared to be contradictory (high tissue Ti for larger titanium dioxide nanoparticles). A hypothesis offered in (Danish EPA 2013b) is that aggregation of the pristine nanoparticles has occurred and therefore influences the intestinal uptake. Accumulation was observed in the kidneys of 25 and 80 nm particles, but not "fine" particles (155 nm). The relevance of the study results for importance of crystallinity for titanium dioxide toxicity is limited as is the relevance for human exposure situations because the high exposure levels are not considered realistic.

The uptake of **rutile** titanium dioxide particles (not strictly nano-sized, nominal size 500 nm) was investigated in a relatively old study by Jani *et al.* (1994) in female rats receiving doses of 12.5 mg/kg bw by oral gavage for 10 days. Histological examination showed that titanium dioxide particles were present in all the major lymphoid tissue associated with the gut (GALT) and demonstrated that 500 nm titanium dioxide particles were translocated to systemic organs; mainly the liver and to a lesser extent the spleen. 4 % of the administered dose was found in the colon, 2.86 % in Peyer's patches the mesentery network and nodes and 1.4 % in the liver. It was calculated that absorption of 6.5 % of the total dose of titanium dioxide particles in the 500 nm size range administered orally over 10 days takes place. In conclusion the study results indicate that a minor part of the rutile form of titanium dioxide with a nominal size of 500 nm was absorbed and were translocated to mainly liver and spleen in its particulate form after oral exposure (Jani *et al.* 1994).

Agglomeration of titanium dioxide was seen in the suspensions administered to the animals in most studies, and therefore the authors of the Danish EPA report conclude that it remains a question which size distribution and form the experimental animals were actually exposed to (Danish EPA 2013b).

The study by Onishchenko *et al.* (2012) investigating the influence of crystal structure of nanoparticles on absorption after oral exposure seems to indicate that the **rutile** form is better absorbed than the **anatase** form, although absorption is generally reported to be low (Onishchenko *et al.* 2012). This finding is noteworthy since the **anatase** form in general is considered more toxic than the rutile form.

Results from an *in vitro* study, investigating three pathways for uptake of titanium dioxide nanoparticles in a model using the human derived Caco-2 cell line, indicate that titanium dioxide nanoparticles as such can cross the epithelial lining of the gastro-intestinal tract at low levels by transcytosis[5] after exposure to concentrations of 10 μg/ml and above (Koeneman *et al.* 2010).

The overall conclusions drawn in (Danish EPA 2013b) and the studies referred above regarding the influence of physical and chemical properties on the absorption of nano-sized titanium dioxide are that low absorption seems to be the rule, that agglomeration / aggregation decreases the absorption and that the **rutile** form is better absorbed than the **anatase** form following oral intake/administration.

5.1.2 Dermal penetration and absorption

Another recent report provides a comprehensive overview of dermal absorption of nanomaterials (Danish EPA 2013a). The report defines dermal absorption, permeation and penetration as follows:

- Dermal absorption is defined as the transport of a compound from the outer surface of the skin into the skin and into the body and for those chemicals that are absorbed, the vast majority is by passive diffusion following Ficks Law. Therefore, skin absorption implies that the compound becomes systemically available.
- Skin permeation is the diffusion of a compound into a certain skin layer.

[5] A mechanism for transcellular transport in which a cell encloses extracellular material in an invagination of the cell membrane to form a vesicle, then moves the vesicle across the cell to eject the material through the opposite cell membrane by the reverse process. Also called vesicular transport.

- Skin penetration is the diffusion into deeper layers.

Nano-sized titanium dioxide is in general one of the most widely studied nanoparticles when it comes to dermal absorption and penetration, due to the extensive use in cosmetics (not the least as UV absorber in sunscreen). Studies are available with the **anatase** form, the rutile form or a mixture of both.

According to (Danish EPA 2013a), titanium dioxide nanoparticles are generally reported to penetrate no further than the *stratum corneum*. However, deeper penetration into the basal cell layer and even dermis is also reported. Actual penetration is, however, often reported as being a very small fraction or infrequent. In relation to titanium dioxide, the crystalline structure also plays a role. As mentioned in the Danish report, studies are available using the **anatase** form, the **rutile** form or a mixture.

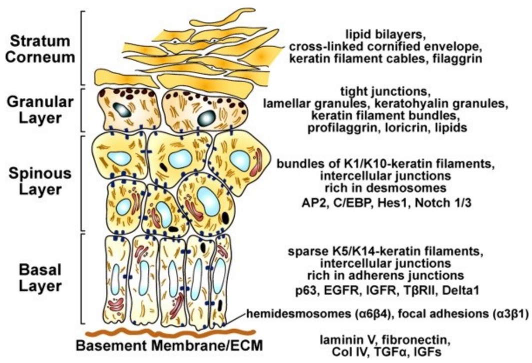

IMAGE 11
EPIDERMAL DIFFERENTIATION (REPRODUCED FROM DANISH EPA (b), POLAND *ET AL.* (2013) REFERRING FUCHS (2008))

The authors were not able to find any studies which appear to systematically evaluate the role of crystal form in titanium dioxide absorption into the skin and could therefore not conclude on the influence of the crystal form (Danish EPA 2013a).

The Scientific Committee on Consumer Safety (SCCS) summarises a number of studies on dermal and percutaneous absorption in their opinion on titanium oxide in the nanoform (SCCS 2013). The reviewed studies cover a range of nanomaterials and a variety of experimental conditions from *in vitro* to *ex vivo* and *in vivo* also including human volunteers as well as intact and UV-damaged skin. In conclusion, the results suggest that titanium dioxide nanoparticles, when applied to skin in a sunscreen formulation, are likely to stay largely on the skin. Only minimal penetration to the outer layers of *stratum corneum* is detected, although a few reports have suggested the possibility that titanium dioxide nanoparticles may penetrate deeper to reach *stratum granulosum*, e.g. in human foreskin grafts transplanted onto SCID[6] mice or to dermis of minipigs treated with nano-sized titanium dioxide (Gontier *et al.* 2008) .

Results from a study with skin from weanling Yorkshire pigs exposed to UV-B radiation (sunburn simulation) demonstrated that UV-B-sunburned skin slightly enhanced the *in vitro* or *in vivo* penetration of rutile titanium dioxide (uncoated mixture of **anatase** and rutile and dimethicone/methicone copolymer-coated rutile titanium dioxide) present in the sunscreen formulations into the *stratum corneum*. The penetration was, however, considered minimal, and there was no evidence that nano-sized or submicronised titanium dioxide penetrated the intact epidermis to any significant extent or evidence of systemic absorption (Sadrieh *et al.* 2010).

Senzui *et al.* (2010) investigated skin penetration of four different types of **rutile** titanium dioxide (T-35, 35 nm, non-coating; TC-35, 35 nm, with alumina/silica/silicon coating; T-disp, 10 x 100 nm, mixture of alumina coated and silicon coated particles, dispersed in cyclopentasiloxan; T-250, 250 nm, non-coating). The penetration was determined with *in vitro* intact, stripped, and hair-removed skin of Yucatan micropigs to study the effect of dispersion and skin conditions. No penetration was observed regardless of titanium dioxide type in intact and stripped skin. The concentration of Ti in skin was found to be significantly higher when TC-35 was applied on hair-removed skin. SEM-EDS

[6] scid=severe combined immunodeficiency. A strain of mice lacking in T and B lymphocytes and immunoglobulins, allowing human skin to be grafted without any rejection.

observation showed that Ti penetrated into vacant hair follicles (greater than 1 mm below the skin surface), however, it did not penetrate into dermis or viable epidermis. There was no significant difference observed between the coated and uncoated forms (Senzui *et al.* 2010).

The conclusions regarding dermal absorption and penetration of titanium dioxide nanoparticles drawn in the SCCS opinion (SCCS 2013) are consistent with other reviews and studies published in the open literature, demonstrating that nanoparticles do not penetrate into the viable epidermis or dermis cells of healthy skin and skin exposed to UV-B radiation. SCCS (2013) refers to a study by Pinheiro *et al.* (2007) investigating the titanium dioxide permeation in psoriatic skin and showing that psoriasis had limited effect on the permeation profile. In this study it was shown that the nano-sized titanium dioxide in a sunscreen formulation penetrated into deeper areas of the *stratum corneum* than in healthy skin, but did not reach living cells in either psoriatic or healthy skin. Source and concentration of the particles were not specified.

Study summaries from investigations of dermal penetration of nanosized **anatase** or **rutile** titanium dioxide are presented in Table 6. Most results support the conclusion that only minimal penetration is observed in studies with nanosized titanium dioxide in the **anatase** and **rutile** form in both healthy and UV-damaged skin.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2,
(Senzui *et al.* 2010)	T-35: Rutile, T-35, 35 nm, non-coating TC-35: Rutile, 35 nm, with alumina / silica / silicon coating T-disp: Rutile, 10 x 100 nm, mixture of alumina coated and silicon coated particles, dispersed in cyclopentasiloxan T-250: Rutile, 250 nm, non-coating	Stripped, hairless, Yucatan micropigs 10% TiO_2 suspensions 2 µl/cm², 24 hours	SEM-EDS observations Ti concentration measured by ICP-MS Statistical analysis	No penetration into dermis or viable epidermis
(Monteiro-Riviere *et al.* 2011)	Rutile (14-16 nm)	In vitro flow-through diffusion cells, 50 µl sunscreen formulation, 10 hours In vivo, pig skin, 250 µl sunscreen formuation, UVB-exposed, dosing at 24 and 48 hours	Dermal absorption through UV-damaged skin In vivo: Penetration of both normal and UVB-exposed skin up to 20 cell layers	UVB-sunburned skin slightly enhanced the in vitro or in vivo SC penetration Minimal penetration into upper epidermal layers No evidence of systemic absorption
(Sadrieh *et al.* 2010)	P25: anatase/rutile (uncoated) nano Rutile (coated) nano Rutile (uncoated) submicron sized	Topical application, minipigs, 40 % suspension, 2 mg/cm² 4 times/day, 5 days / week, 4 weeks Quantitative analysis	Measurable but insignificant amount of TiO_2	No significant penetration through intact normal epidermis, regardless of particle size and type Particles found primarily in stratum corneum and upper follicular lumens (uncoated TiO_2)
(Bennett *et al.* 2012)	P25 (anatase/rutile) (27±4 nm)	Photoinduced transdermal penetration of porchine skin 200 µl of 1000 mg L^{-1} TiO_2: Light or dark 180 min UV exposure, TiO_2 application every 30 min.	Natural and artificial light facilitated disaggregation of NPs and increased penetration Irradiated TiO_2 penetrated skin and yielded up to 370 mg/TiO_2 kg^{-1} in skin after 5 hours exposure Unirradiated skin: 130	The results indicate that TiO_2 nanoparticle clusters can be disaggregated by natural and artificial light, and that the effect is related to light intensity. Pig skin thicker than human skin ⇒ NP penetration of human skin may be more

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2,
			mg TiO_2 kg^{-1}	significant

TABLE 6
DERMAL PENETRATION OF NANOSIZED ANATASE AND RUTILE TITANIUM DIOXIDE

In the study by Bennett *et al.* (2012) photoinduced transdermal penetration of titanium dioxide nanoparticles (P25 TiO2: approx. 80 % **anatase** and 20 % rutile) through pig skin grafts exposed to sunlight and other light sources was investigated. The results indicated that titanium dioxide nanoparticle clusters can be disaggregated by natural and artificial light, and that the effect is related to light intensity. It was demonstrated that light facilitates increased nanoparticle penetration and that sunlight facilitates nanoparticle penetration deeper into the skin and possibly into the viable layers of the skin (Bennett *et al.* 2012). This has however not been confirmed by other identified studies.

Other studies to support the conclusions regarding possible penetration of in particular anatase titanium dioxide nanoparticles into viable layers of the skin have not been identified.

Although there is no conclusive evidence at present to indicate penetration of titanium dioxide nanoparticles through the skin to viable cells of the epidermis, the SCCS opinion highlights that a number of studies have shown that nano-sized titanium dioxide particles can penetrate into the outer layers of the stratum corneum, and enter hair follicles and sweat glands. On this basis it is recommended not to use titanium dioxide with high photocatalytic activity in sunscreen formulations in order to avoid deposition of photocatalytic active titanium dioxide in these areas and possible generation of reactive oxygen species (ROS).

(Danish EPA 2013a) and (SCCS 2013) emphasise that limited studies are available studying the influence of damaged and flexed skin on the penetration/absorption of nanomaterials.

5.1.3 Pulmonary absorption

Data specific for the **anatase** form have not been identified. The comprehensive review performed in the ENRHES project identified no studies investigating the systemic absorption of titanium dioxide nanoparticles following inhalation (Stone *et al.* 2010). More recently, a review reports on quantitative data available from rodent studies (Shi *et al.* 2013). In a study by Muhlfeld *et al.* (2007) with rat lungs exposed to an aerosol containing titanium dioxide nanoparticles (crystal form not indicated), it is suggested that a small fraction of titanium dioxide nanoparticles (20 nm; 1 and 24 h; dose ranges differed according to compartment size) is transported from the airway lumen of adult male WKY/NCrl BR rats to the interstitial tissue and subsequently released into the systemic circulation (Muhlfeld *et al.* 2007).

Another experiment by Li *et al.*, (2010) studying intratracheal instillation of 3 nm titanium dioxide in mice, found that at 28 days after instillation, a small fraction of pulmonary titanium dioxide nanoparticles was able to access the blood circulation and reach extra-pulmonary tissues such as liver and kidneys. The results showed that instilled nano-sized titanium dioxide could induce lung damage, and change the permeability of alveolar-capillary barrier and also indicated that titanium dioxide nanoparticles may pass through the blood-brain barrier (BBB), and induce the brain injury through oxidative stress response (Li *et al.* 2010).

Wang *et al.* (2008) reported on translocation of titanium dioxide nanoparticles to the central nervous system based on a study with intranasally instilled titanium dioxide nanoparticles (80 nm rutile, 155 nm ***anatase***; 500 µg/ml; 2, 10, 20, and 30 days) in female mice. The results provided preliminary evidence that nasal instilled titanium dioxide nanoparticles could be translocated into the central nervous system and cause potential lesion of the brain, and that the hippocampus would be the main target within brain. It should however be mentioned that the dose is very high (Wang *et al.* 2008).

In a study in mice Hougaard et al. (2010 and 2011 (erratum)) found 21 % of the inhaled dose was retained in the lung 25 days after exposure. Results were obtained following eleven days of inhalation of TiO2 nanoparticles. Mice were exposed 1 hr/day at 40 mg TiO2/m3 corresponding to the 8-hr time weighted average (TWA) occupational exposure limit according to Danish Regulations (2007).

Although only few studies regarding pulmonary absorption are available, Shi *et al.* (2013) suggests that titanium dioxide nanoparticles can translocate from the lung into the circulatory system to systemic tissue and from the nasal cavity into sensory nerves to the nervous system. Data also suggest that the rate of nanoparticle migration to the circulatory system is low (Shi *et al.* 2013). Available evidence does not allow identifying any possible difference between the anatase and rutile forms with or without coating.

5.1.4 Distribution

Stone *et al.* (2010) report that nanoparticles have been shown to distribute to the liver, spleen, lungs and kidneys following oral absorption, and that the hippocampus is the main accumulation target for titanium dioxide nanoparticles absorbed following nasal installation (Stone *et al.* 2010).

Results from a study by (Fabian *et al.* 2008) with rats administered a single intravenous injection of 5 mg/kg bw of P25 that consisted of both **anatase** and **rutile** forms (70/30), without surface coating, showed no detectable levels of titanium dioxide in blood cells, plasma, brain or lymph nodes and no changes in the cytokines and enzymes measured in blood samples. The highest retention of titanium was found in the liver.

SCCS concludes regarding studies investigating distribution, that the limited available evidence suggests that if titanium dioxide nanoparticles become systemically available, they may accumulate mainly in the liver with a very slow clearance (SCCS 2013). It should also be noted that the intravenous exposure level was high, which may influence organ distribution by damaging the integrity of the endothelial barrier (Fabian *et al.* 2008).

In a two week acute toxicity study by Chen *et al.* (2009) where mice were administered different doses between 0 and 2593 mg/kg bw of 80 nm and 100 nm **anatase,** titanium dioxide nanoparticles by intraperitoneal injection, examination of particle distribution demonstrated that at 1, 2, 7, and 14 days post-exposure accumulation of titanium dioxide nanoparticles (80 nm, 100 nm, **anatase**) was highest in the spleen, followed by the liver, kidneys and lung in a decreasing order (Chen *et al.* 2009a).

Available evidence does not allow identifying any possible difference between the **anatase**, rutile or coated forms but indicates that the main target organ if nano-sized titanium dioxide becomes systemically available seems to be the liver.

5.1.5 Metabolism and elimination

No specific literature has been identified regarding metabolism and elimination of titanium dioxide nanoparticles, except as noted above, the clearance of nano-sized titanium dioxide distributed to the liver seems to be slow.

5.1.6 Summary on ADME

In summary, it can be concluded that absorption, distribution, metabolism, and excretion (ADME) of titanium dioxide nanoparticles may be influenced by factors such as exposure routes and particle size, particle agglomeration and surface coating. The most frequently investigated exposure routes in the toxicokinetic studies of titanium dioxide nanoparticles are pulmonary instillation, lung inhalation, dermal and oral administrations. Limited absorption into the body can occur through the lung and gastro-intestinal tract, but no specific information is available regarding the magnitude of the absorption and transport for the purpose of systemic risk assessment (Shi *et al.* 2013). **Rutile** titanium dioxide seems to be better absorbed orally than the **anatase** form. For dermal and inhalation administration, available evidence does not allow identifying any possible difference in absorption between the **anatase** and **rutile** forms.

Most studies on dermal absorption do not provide evidence to indicate that titanium dioxide nanoparticles alone or in sunscreen formulations pass the *stratum corneum* of intact skin into the human body under normal conditions resulting in systemic absorption. A few studies indicate that titanium dioxide nanoparticles penetrated slightly deeper into UV-damaged and psoriatic skin, but did not reach viable layers (Danish EPA 2013a). Further studies might be performed on damaged and/or flexed skin.

Titanium dioxide nanoparticles injected intravenously or intraperitoneally have been found in different organs, such as liver, spleen, kidneys, lung, lymph nodes and brain. The same distribution pattern was found after oral administration of large doses (Wang *et al.* 2007). Titanium dioxide nanoparticles may have the potential to penetrate the blood-brain barriers (BBB) and blood-placenta barriers (Shi *et al.* 2013).

No specific information on metabolism and elimination has been identified, except that clearance of nano-sized titanium dioxide distributed to the liver seems to be slow.

5.2 Toxicity of nano-sized titanium dioxide

Toxicity information from recent reviews of titanium dioxide, including the **anatase** form, is summarised in the following together with information from the original literature and selected, more recent studies. Reviews include:

- Stone *et al.*, 2009: Engineered Nanoparticles: Review of Health and Environmental Safety
- Johnston *et al.*, 2009: Identification of the mechanisms that drive the toxicity of TiO_2 particulates: the contribution of physicochemical characteristics
- SCCS opinion on Titanium Dioxide (nano form) COLIPA No S75, 2013, which is based on the risk assessment of nano-sized titanium dioxide (TiO_2) for use as a UV filter in sunscreen formulations
- Danish EPA Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials, 2011 – including titanium dioxide.
- Shi *et al.*, 2013: Titanium dioxide nanoparticles: a review of current toxicological data.
- NIOSH (National Institute of Occupational Health), 2011: Occupational exposure to titanium dioxide

Results with coated titanium dioxide are also summarised, where relevant. A typical coating used in cosmetics is trimethoxy-caprylylsilane.

Acute toxicity

No acute oral toxicity studies with the **anatase** form alone have been identified. Results from studies referred by (SCCS 2013) submitted by the applicant with **anatase**/rutile mixtures and titanium dioxide (85 % **anatase** and 15 % rutile) coated with trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane show low acute oral toxicity with LD_{50} values above 2150 mg/kg (single dose study).

There are only a limited number of studies on acute dermal toxicity of titanium dioxide and none for which the pure **anatase** form of nano-sized titanium dioxide is used or other nano-form of titanium dioxide. Based on results from absorption/penetration studies with titanium dioxide nanoparticles, no systemic toxicity is expected when titanium dioxide nanoparticles are applied to healthy or UV-damaged skin.

Several studies referred to in a Danish EPA survey (Danish EPA 2012) involving nano-sized titanium dioxide show clear evidence that the nano-form is considerably more toxic than micro-sized titanium dioxide. It was also found that the crystallinity of titanium dioxide nanoparticles is thought to influence the toxicity, with the anatase form expected to be more toxic than the rutile form. The pulmonary response to titanium dioxide is reported to be inflammation, epithelial damage, and increased permeability of the lung epithelium, oxidative stress and cytotoxicity as well as morphological alteration within the lung.

Chen *et al.* (2006) exposed mice, via intratracheal instillation (0.1 and 0.5 mg per mouse), to nano- (19-21 nm, rutile) and microsized (180-250 nm, crystal form not specified) forms of titanium dioxide and determined their pulmonary toxicity 3 days, 1 week or 2 weeks post exposure. In mice exposed to titanium dioxide nanoparticles (size 19-21 nm), toxicity (inflammation and histological changes in the lung) was observed at the lowest dose of 100 µg per mouse, and the authors conclude that the results of the study indicate that single intratracheal instillation of 0.1 mg nano-sized titanium dioxide can induce severe pulmonary inflammation and emphysema in the mouse lung. No significantly pathological changes were seen using the same dose of micro-sized titanium dioxide (180–250 nm) (Chen *et al.* 2006).

In an inhalation study with mice exposed to **anatase** (5 nm) and a mixture of **anatase** and **rutile** (21 nm) nanoparticles for 4 hours it was found that the smaller 5 nm titanium dioxide particles did not induce a greater inflammatory response than 21 nm titanium dioxide particles (Grassian *et al.* 2007). It was however observed that the 21 nm titanium dioxide particles evoked a larger inflammatory response in the mice than the 5 nm particles through inhalation. The same result was observed when mice were exposed through nasal instillation. Overall it was concluded that the data suggested that there are differences in the nature of the nanoparticle agglomeration state between 5 and 21 nm particles which may perhaps be an equally important factor as the surface and other physical characteristics of the primary nanoparticles in determining their toxicity.

Summaries of other studies investigating the effects of short-term inhalation or intratracheal instillation are presented in Table 7.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Sayes *et al.* 2006)	Anatase, NP Anatase/rutile, NP Rutile, NP	Human dermal fibroblast (HDF) Human lung epithelial cells (A549) Cytotoxicity (LDH, viability, mitochondrial activity (MTT)), inflammation, IL-8 production UV illumination	Increased cytotoxicity Increased ROS Increased IL-8 production Response dependent on crystal form	Dose-response and time-dependent cytotoxicity Anatase 100 times more toxic than rutile Cytotoxicity and and inflammation only at high doses (1500 µg/ml) Most cytotoxic NPs most effective at ROS production
(Simon-Deckers *et al.* 2008)	Anatase (95%) P25, Anatase (75%) Rutile (100%) Anatase (100%) (different size and surface area)	Human pneumocytes (A549)	Cytotoxicity and intracellular accumulation	All NPs internalised in cells Toxicity low and depending to some extent on chemical composition, crystalline structure and size
(Grassian *et al.* 2007)	Anatase (5 nm)	Inhalation (4 hour	Inflammation (BALF	21 nm particles more

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
	Anatase/rutile (21 nm)	exposure) in mice Inhalation: (5 nm: 0.77-7.35 mg/m^3; 21 nm: 0.62-7.16 mg/m^3) Nasal instillation (5 nm: 0.1-0.6 mg/ml; 21 nm: 0.5-3.0 mg/ml) (24 hour exposure) Observations immediately or 24 hours post exposure)o	cells & cytokines) Cytotoxicity (BALF LDH) Lung Permeability (BALF protein) Lung histopathology Inhalation: Increased no. of macrophages. No changes in total protein, LDH and histopathology Nasal instillation: neutrophil infiltration, increased IL-1β, IL-6, protein and LDH for 21 nm NPs only	toxic than 5 nm. Data suggest agglomeration to be the reason.
(Warheit *et al.* 2007)	uf-1: Rutile, ultrafine (alumina coating) uf-2: Rutile, ultrafine (silica and alumina coating) uf-3: Anatase/rutile, ultrafine F-1: Rutile (fine) 300 nm in water	Rats Intratracheal installation of 1 or 5 mg/kg Evaluation of bronchoalveolar lavage (BAL) fluid inflammatory markers, cell proliferation, and by histopathology at post-instillation time points of 24 h, 1 week, 1 and 3 months	No sustained adverse pulmonary effects of uf-1, uf-2 or F1. Uf-3: significant increase in BAL fluid LDH and MTP values; increases in lung parenchymal cell proliferation indices; macrophage accumulation	Rutile/anatase rutile Inhaled rutile ultrafine-TiO_2 particles are expected to have a low risk potential for producing adverse pulmonary health effects. Pulmonary inflam-mation/cytotoxicity responses: uf-3 > F-1 = uf-1 = uf-2
(Warheit *et al.* 2006)	Rutile (300 nm) Anatase, rods (200×35 nm) Anatase, dots (10 nm)	Rats Intratracheal instillation (eval. 24 hours, 1 week, 1 month, 3 months post exposure) 1 or 5 mg/kg	Inflammation BALF parameters Cell proliferation methods Lung histopathology Transient inflammatory and cell injury effects at 24 h post exposure - not different from the pulmonary effects of larger sized TiO_2 particle exposures	No differences in the pulmonary responses to anatase nanodots or rods versus anatase fine particles (due to method of exposure rather than effects of nanoscale particulates
(Kwon *et al.* 2012)	Anatase/rutile (primarily anatase)	Rats (Wistar) Inhalation (2 weeks, 6 hours/day, 5 days/week) 11.39±0.31 mg/m^3	Inflammation (BALF, total protein, LDH) No significant difference in BALF, LDH, enzyme activity or levels of IL-4, IL-6, IL-10 compared to controls. Mild degeneration / regeneration of olfactory epithelium in nasal cavity Lung infiltration	Concentration dependent toxicity Conc. too low to activate immune system cells Small particles (20 nm) more toxic even at low particle number
(Silva *et al.* 2013)	P25: Anatase 81% / rutile 19 % (24 nm) Anatase spheres (28 nm) Anatase nanobelts	Rats (Sprague-Dawley) Intratracheal installation: 20, 70, 200 µg 6 animals per dose Eval: 1 and 7 days post exposure	Inflammation (BALF, total protein, LDH) Lung histopathology: Changes in lung parenchyma	No significant correlation between NP persistence and BAL inflammation for spherical anatase TiO_2 nanobelts the most inflammatory with LOEL of 200 µg

TABLE 7
EFFECTS OF SHORT-TERM INHALATION AND INTRETRACHEAL INSTILLATION OF TITANIUM DIOXIDE

There is clear evidence that inhalation of titanium dioxide nanoparticles is more toxic than inhalation of microsized titanium dioxide, and that the effect is dose dependant. As indicated by the results presented above acute and sub-chronic inhalation exposure to titanium dioxide nanoparticles can result in inflammatory responses, epithelial hypertrophy and hyperplasia at high exposure doses. There seems to be a difference in sensitivity between species, with the rat being most sensitive (possibly due to the lung overload to which the rat is more sensitive than humans as well as mice). The crystalline form may influence toxicity, with the **anatase** form being more toxic than the rutile form (see e.g. Danish EPA 2012). As illustrated by the study by Grassian *et al.* (2007) several factors in addition to characteristics such as crystalline form and surface area play a role in the toxicity exerted by the nanoparticles, e.g. the nanoparticle agglomeration state (Grassian *et al.* 2007).

Skin irritation

On the basis of primarily two unpublished studies submitted with titanium dioxide nano-materials (85 % **anatase** and 15 % **rutile**, coating: trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane) available for the SCCS opinion, the SCCS concludes that it appears that the titanium dioxide nanomaterials are either mild or non-irritant to skin. There is no information on further characterization of the test substances. The primary irritation index was estimated to be zero and 0.3, and the materials were regarded as non-irritant on rabbit skin (SCCS 2013).

Two studies using ultrafine grade materials showed the mean irritation scores of 0.3 and 1.58-1.92 during 5 day repeat applications on rabbit skin, but the proportion of nano-scale fraction in the materials used has not been reported (SCCS 2013).

As mentioned in relation to the classification of **anatase** titanium dioxide, 24 out of 229 industry notifiers have suggested a classification based on skin irritation (Skin Irrit 2). There is no information on the background for this classification which is also not substantiated by the SCCS opinion and the literature identified within the scope of this project.

Eye irritation

On the basis of two unpublished studies with titanium dioxide nanomaterials (85 % **anatase** and 15 % **rutile**, coating: trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane) available for the SCCS opinion, SCCS concludes that nano-sized titanium dioxide can be regarded as having a low eye irritation potential. There was no information on the further characterisation of the test substances (SCCS 2013).

No other studies have been identified.

As it is the case in relation to skin irritation, 24 out of 229 industry notifiers have suggested a classification of **anatase** based on eye irritation (Eye Irrit 2). There is no further information on the background for this classification which is also not substantiated by the SCCS opinion and the literature identified within the scope of this project.

Skin sensitisation

Results from two studies with guinea pigs exposed to titanium dioxide nanomaterials (85 % **anatase** and 15 % **rutile**, coating: trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane) show that titanium dioxide nanomaterials can be regarded as non-sensitising. There was no information on the further characterisation of the test substances (SCCS 2013).

In a study with **rutile** titanium dioxide coated with alumina/silica, the nanomaterial was classified as a weak sensitizer according to the Magnusson-Kligman classification (that considers 0-8 % response a weak sensitizer category). There was no information on characterisation (particle size distribution) of the tested materials to indicate what proportion was in the nano-scale (SCCS 2013).

Subchronic, repeated dose toxicity

Most in vivo studies addressing repeated dose toxicity of **anatase** or **rutile** titanium dioxide nanoparticles are investigating consequences of nasal instillation or inhalation exposure. Two oral gavage studies have been identified.

Based on results from a subchronic 60 day oral (gavage) study in mice exposed to **anatase** titanium dioxide nanomaterials (primary particle size 5 nm), the SCCS concludes that a **LOAEL of 5 mg/kg bw/d** may be derived based on impaired neurofunction and behaviour at all dose levels (SCCS (2013) referring Hu *et al.*, 2010).

Results from another subchronic 30 day oral (gavage) study in mice exposed to **anatase** titanium dioxide nanomaterials with a primary particle size of 5 nm, a NOAEL of 62.5 mg/kg bw/d was suggested based on body weight reduction, increased coefficients of the liver, kidney, spleen and thymus, and serious damage to liver function at doses ≥ 125 mg/kg bw/d (SCCS (2013) referring Duan *et al.*, 2010)).

In a 30 days study it has been shown that both **rutile** and **anatase** titanium dioxide nanoparticles after nasal installation of 500 μg/mouse every other day for a total of 30 days can bypass the blood-brain barrier and can be translocated (via the olfactory nerve) to the brain, where they accumulate within the cerebral cortex, thalamus and hippocampus (main target). This resulted in morphological alterations and loss of neurones in the hippocampus, induction of oxidative stress and initiation of inflammation (Danish EPA (2012) referring Wang *et al.*, 2008).

In two comparative 13-week inhalation studies (Bermudez *et al.* 2002; Bermudez *et al.* 2004) demonstrated species differences in pulmonary clearance and inflammatory response to inhaled and deposited titanium dioxide independent of particle size. Rats were particularly susceptible to high lung burdens showing progression of histopathological lesions, while mice and hamsters showed minimal initial lesions which recovered during the post-exposure period.

Results from repeated dose toxicity studies involving the **anatase** or **rutile** crystal forms or a mixture are presented in Table 8.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Hu *et al.* 2010)	Anatase	Mice (CD-1) Intragastric adm. 60 days 0, 5, 10, 50 mg/kg bw/day	Impaired spatial recognition memory Calcification in neurocytes Change in trace elements and neurotransmitters Inhibiton of enzymatic activities Neurochemical changes in brain	TiO_2 translocates to the CNS Damage of spatial recognition memory behaveour LOAEL of 5 mg/kg bw/d derived by SCCS (2013)
(Duan *et al.*, 2010)	Anatase (5 nm)	Female mice (CD-1) Intragastric adm. 30 days 0, 62.5, 125, 250 mg/kg bw/day	Body weight and organ weight coeffiients, blood chemistry, liver function Body weight reduction, and increased coefficients of the liver, kidney, spleen and thymus Serious damage to liver function Increase in blood parameters and	Liver toxicity at high doses (NOAEL estimated at 62.5 mg/kg bw/day)

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
			disruption of liver function	
(Wang *et al.* 2008)	Anatase (155 nm) Rutile (80 nm)	Mice (CD-1) Nasal installation 500 µg NP suspension every other day for 30 days Examination of brain tissue	Enzyme activity (gluthathione peroxidise, catalase, SOD, glutathione-S-transferase) and lipid peroxidation (MDA): • Increased MDA • Increased catalase • Decreased SOD Protein oxidation (protein carbonyl formation): • Increased protein oxidation	TiO_2 translocates to the CNS Hippocampus main target Oxidative stress Anatase > rutile
(Hext *et al.* 2002)	TiO_2, pigment grade P25, ultrafine	Female rats, mice and hamsters 13 weeks exposure Inhalation 6 hours/day, 5 days/week Pigment grade: 10, 50 250 mg/m³ Ultrafine: 0.5, 2, 10 mg/m³	Endpoints: Lung burdens, indicators of pulmonary inflammation, oxidant damage, cellular proliferation and pathological responses Lung overload, reduces clearance rates and pulmonary inflammation High clearance rate in hamsters	Species dependent pulmonary response Rat > mice > hamster
(Bermudez *et al.* 2002)	Rutile (1.40 µm)	Female rats, mice, hamsters 13 weeks exposure Inhalation 6 hours/day, 5 days/week 10, 50, 250 mg/m³	Inflammation (BALF & Histology) Lung particle burden Cytotoxicity (LDH) and permeability (protein) Inflammatory response evident in all species, but was most severe and persistent in rats (least severe in hamsters). Increased LDH & protein.	Impaired lung clearance at high conc. in rats and mice Hamsters able to clear particles more efficiently. Species differences, and dose dependent effects observed.
(Bermudez *et al.* 2004)	P25 (anatase/rutile) (21 nm)	Female rats, mice, hamsters 13 weeks exposure Inhalation 6 hours/day, 5 days/week 0.5, 2.0, 10 mg/m³	Inflammation (BALF and histology) Lung particle burden Cytotoxicity (LDH) and permeability (protein) 10 mg/m³:pulmonary overload in rats and mice Increased cellular infiltration (macrophages and neutrophils) dependent on species Increased LDH and protein (not hamsters) Retained particle burden decreased with time post-exposure correlating with the increased neutrophilic response	Pulmonary overload in rats and mice at 10 mg/m³ (Rapid lung clearance in hamsters) Retained particle burden decreased with time post-exposure correlating with the increased neutrophilic response. Findings dependent on (rats>mice>hamsters) and particle concentration.

TABLE 8
REPEATED DOSE TOXICITY STUDIES INVOLVING ANATASE TITANIUM DIOXIDE

In summary the systemic toxicity following repeated dose exposure to titanium dioxide nanoparticles seem to be restricted to the organs where particles accumulate over time. Impaired lung clearance and lung overload is observed in particular in rats at high dose levels associated with chronic inflammation, pulmonary damage and lung tumours (see also study by Heinrich UF *et al.* (1995) in Table 12).

Mutagenicity/genotoxicity

There is indication of genotoxicity in different *in vitro* and *in vivo* tests with the pure **anatase** form and mixtures of **anatase** and **rutile** titanium dioxide. In an *in vitro* micronucleus test in human epidermal cells, the anatase titanium dioxide nanoparticles induced an increase in the number of cells with micronuclei, which indicates that titanium dioxide nanoparticles in the **anatase** form are genotoxic (clastogenic and/or aneugenic) in the human cell line A431 (SCCS 2013).

Results from a modified Comet assay with 99.5 % **anatase** titanium dioxide, the titanium dioxide nanoparticle caused a significant concentration-dependent induction of DNA damage. Effects were reported to be statistically significant at the two highest testing concentrations, which were not cytotoxic after 6 or 24 h treatment in the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) or NRU (Neutral Red Uptake) assay. Significant cytotoxicity for both concentrations was found in these assays after 48 h treatment. Uptake of nanoparticles into the A431 cells was shown by TEM analysis. Particles were observed mostly in the cytoplasm, but occasionally also in the nucleus. Oxidative stress in the cells was indicated from the significant depletion of glutathione, induction of lipid peroxidation and reactive oxygen species generation (SCCS 2013).

Based on results from alkaline Comet assays with **anatase** titanium dioxide in mammalian lung cells and mammalian liver cells it was concluded that the **anatase** titanium dioxide nanoparticles have a genotoxic potential in mammalian lung cells and caused small but significant increases in DNA damage in liver cells (SCCS 2013).

Trouiller *et al.*, (2009) investigated the genotoxicity, oxidative DNA damage and inflammation of nano-sized titanium dioxide in an *in vivo* study in mice. The crystal structure was a mixture of 75 % **anatase** and 25 % **rutile** titanium dioxide with a particle size of 21 nm. Male mice were dosed for 5 days in the drinking water with doses corresponding to 0, 50, 100 250 and 500 mg/kg bw. Pregnant dams were dosed in drinking water with 500 mg/kg bw for 10 days at gestation days 8.5 to 18.5 post-coitum. In male mice, nano-sized titanium dioxide induced DNA single strand breaks measured by the comet assay at the highest dose tested (500 mg/kg/bw) and micronuclei in peripheral blood. DNA double strand breaks (DSB) were measured by λλ-H2AX immunostaining assay in bone marrow, which was the most sensitive of the assays involved and showed an increase in DSB in a dose-dependent manner. Oxidative DNA damage (8-OHdG) was measured in the liver at the highest dose tested, and a pro-inflammatory response, measured as changes in cytokine expression, was seen in peripheral blood. *In utero* exposure of foetuses via the mother caused an increase in large deletions in offspring (Trouiller *et al.* 2009).

This *in vivo* study shows that the investigated mixture of **anatase** and **rutile** nano-sized titanium dioxide administrated orally is systemically distributed to different tissues such as blood, bone marrow, liver and even the embryo, where it can induce genotoxicity at relatively high exposure levels. The inflammatory response and oxidative damage in liver indicate that the mechanism behind the observed genotoxicity may be due to a secondary response following inflammation and oxidative stress.

Based on the study with titanium dioxide (75 % **anatase** and 25 % **rutile**; 21 nm) the authors suggest that these data raise a concern about a potential risk of cancer or genetic disorders especially for people occupationally exposed to high concentrations of titanium dioxide nanoparticles and that it might be prudent to limit ingestion of titanium dioxide nanoparticles through non-essential drug additives, food colours, etc. (Trouiller *et al.* 2009). The recommendation is not specifically related to the **anatase** form.

NIOSH (2011) concludes that the particle size influenced oxidative DNA damage in cultured human bronchial epithelial cells, which was detected for 10- and 20-nm but not 200-nm diameter **anatase** titanium dioxide. In the absence of photoactivation, an **anatase**-rutile mixture (50/50; size 200 nm) induced higher oxidative DNA damage than did the pure **anatase** or rutile (200 nm each). Overall, NIOSH concludes that these studies indicate that titanium dioxide (no specific reference to crystal form) exhibits genotoxicity (DNA damage) under certain conditions, but not mutagenicity (genetic alteration), in the assays used (NIOSH 2011).

Summaries of genotoxicity studies involving the anatase form of titanium dioxide are presented in Table 10.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Jugan *et al.* 2012)	Anatase (12, 25, 140 nm, spherical) Rutile(20 nm, sphecical) Rutile (68 nm, elongated)	Comet assay Micronucleus assay Gamma-H2AX immunostaining 8-oxoGuanidine H2-DCFDA Gluthathione content Antioxidant enzymes activities	Genotoxic potential in A549 cells: Induction of single strand breaks and oxidative lesions in DNA Oxidative stress Impairment of cell ability to repair DNA	Anatase 12 nm, 25 nm and rutile 20 nm > Anatase 140 nm and rutile 68
(Bhattacharya *et al.* 2009)	Anatase (91 nm)	Cell culture: Human diploid fibroblast (IMR-90) Human bronchial epithelial cells (BEAS-2B) Test: Alkaline Comet assay: 10 and 50 µg/cm² 8-OH-dG using ELISA: 5 and 10 µg/cm² Exposure: 24 t	No induction of DNA breakage DNA adduct formation Elevated amounts of free radicals	Indirect genotoxicity
(Dunford *et al.* 1997)	TiO_2 extracted from sunscreens Anatase, rutile and anatase/rutile (no information on coating)	Human cells (MRC-5 fibroblast) Oxidation of organic material (phenol) Plasmid DNA (in vitro) Comet assay (MRC-5 cells) (sunlight illumination)	Oxidation of organic materials by hydroxyl radicals and strand breaks in plasmid DNA. DNA damage suppressed by free radical quenchers (DMSO and mannitol)	DNA oxidative damage Anatase > rutile
(Falck *et al.* 2009)	Anatase < 25 nm Anatase < 5 µm Rutile 10×40 nm Rutile < 5 µm	Human bronchial epithelial cells (BEAS-2B)	Alkaline Comet assay Micronuclei assay 10, 20, 40, 60, 80 and 100 µg/cm² 24, 48, 72 hours	Cytotoxicity: Anatase > rutile DNA damage: Anatase (nano) and rutile (fine) > rutile (nano) Induction of micronuclei: only anatase (slight)

TABLE 9
GENOTOXICITY STUDIES INVOLVING THE ANATASE FORM OF TITANIUM DIOXIDE

Carcinogenicity

IARC has evaluated and categorised titanium dioxide (even the microform – if exposure is high enough) to be a Class 2B carcinogen (possibly carcinogenic to humans) (IARC 2010).

Nano-sized titanium dioxide has been studied in two 2-stage skin carcinogenicity studies with mice and rats.

Non-coated and alumina and stearic acid coated titanium dioxide (spindle shaped, crystal form not specified) have been studied in the two-stage mouse skin carcinogenicity studies with CD1 mice and a transgenic mouse strain (rasH2). No promoter activity was found in this study (Furukawa *et al.* 2011).).

The same result was obtained in a study with non-coated and silica-coated rutile titanium dioxide in mice although it was considered difficult to draw very firm conclusions from the study with silica coated titanium dioxide due to lack of positive controls and very high tumour activity in the "initiated" mice (Sagawa *et al.* 2012).

Non-coated rutile titanium dioxide studied in the 2 two-stage rat skin carcinogenicity study did also not show tumour promoter activity (Sagawa *et al.* 2012). As highlighted in SCCS (2013) it is however difficult to draw any conclusion because of little experience with the model and no use of positive controls.

As none of these studies with spindle shaped and rutile forms of titanium dioxide resulted in any significant treatment related increases in skin tumour production both with and without coating, SCCS (2013) concludes that titanium dioxide nanoparticles do not possess promoter activity for mouse skin carcinogenesis, most likely due to the inability to penetrate through the epidermis.

A short overview of the skin carcinogenicity studies is presented in Table 12.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Furukawa *et al.*, 2011)	TiO_2 coated and uncoated, spindle shaped: long axis 50–100 nm, short axis 10–20 nm)	Two stage mouse skin carcinogenicity test (promoter potential) Mice, female (CD1) 5, 10, 20 mg	Survival rate, general conditions, body weight: no changes Macroscopic observation of nodules No dose-dependent findings	Titanium dioxide nanoparticles do not possess promoter activity for mouse skin carcinogenesis
(Sagawa *et al.* 2012)	Rutile, coated (35 nm) Rutile, uncoated (20 nm)	Two stage mouse skin carcinogenicity test 0, 50, 100 ml; rasH2 mice: 5 times / week, 8 weeks CB6F1 mice: 5 times / week, 40 weeks CD1 mice: 2 times / week, 52 weeks (uncoated)	No significant increase in squamous cell papilloma's or squamous cell carcinomas	Uncoated rutile titanium dioxide nanoparticles do not promote skin tumours in mice
(Sagawa *et al.*, 2012)	Rutile, uncoated (20 nm)	Two stage rat skin carcinogenicity test 0, 100, 200 ml; Hras128: 2 times / week, 28 weeks SD rats: 2 times / week, 40 weeks	No identified treatment related results No skin penetration observed	Model not considered adequate – no conclusion can be drawn

TABLE 10
SKIN CARCINOGENICITY STUDIES WITH ANATASE AND RUTILE TITANIUM DIOXIDE

Linnainmaa *et al.* (1997) investigated the in vitro cytotoxicity and the induction of micronuclei of two ultrafine titanium dioxide samples in a rat liver epithelial cell (RLE) assay with and without UV light treatment. The results suggested that the ultrafine particles did not have a direct clastogenic potential (Linnainmaa *et al.* 1997).

Theogaraj *et al.* (2007) investigated the photo-clastogenic potential of eight different classes of ultrafine titanium dioxide particles in Chinese hamster ovary (CHO) cells in the absence and presence of UV light at a dose of 750 mJ/^{cm2}. None of the titanium dioxide particles tested induced any increase in chromosomal aberration frequencies either in the absence or presence of UV and the studies show that ultrafine titanium dioxide particles did not exhibit photochemical genotoxicity in the test system (Theogaraj *et al.* 2007).

Study summaries are presented in Table 11.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	To xicity of TiO_2
(Linnainmaa *et al.* 1997)	Anatase (20 nm) Rutile, coated (20 nm) Anatase, pigmentary (170 nm)	Rat liver epithelial cells	Cytotoxicity, UV treatment: No chromosomal damage (induction of micronuclei)	No clasogenic potential. Possible carcinogenic potential of TiO_2 not due to direct chromosome-damaging effect.
(Theogaraj *et al.* 2007)	Anatase/rutile (80/20: 21 nm) Anatase/rutile (80/20; 21 nm), doped Anatase (alumina coated) 60 nm Rutile (alumina coated) 15, 20, 20-22 nm	Chromosome aberration Chinese hamster ovary cells (CHO-WBL) UV light 3 hours	Photo-clastogenicity: No induced chromosome aberration	Eight different rutile and anatase forms of titanium dioxide with different surface treatments (five surface-treated and three without surface treatment) are not activated to photogenotoxins by solar simulated (UVA+ UVB) light.

TABLE 11
CLASTOGENIC POTENTIAL OF ANATASE AND RUTILE TITANIUM DIOXIDE

Lee *et al.*, (1985 and 1986) demonstrated development of lung tumours in a study investigating the pulmonary response of rats exposed to fine sized titanium dioxide in a two-year inhalation study. Rats were exposed to concentrations up to 250 mg/m^3 (Lee *et al.* 1985; Lee *et al.* 1986). The highest concentration is above what is considered generally acceptable inhalation toxicology practice today (NIOSH 2011).

Heinrich UF *et al.* (1995) investigated the carcinogenicity of titanium dioxide nanoparticles (anatase/rutile: 80/20) (15–40 nm) and found that these particles were tumorigenic in rats at a concentration of approximately 10 mg/m3 for 2 years, followed by a 6-month holding period. Titanium dioxide nanoparticles seem to have more carcinogenic potential in the rat than titanium dioxide fine particles on an equal mass dose basis. Lung tumours were also observed in mice but not significantly different from the control group (Heinrich UF *et al.* 1995).

Study summaries are presented in Table 12.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Lee *et al.* 1985; Lee *et al.* 1986)	TiO_2 (1.5 – 1.7 µm) No information on crystallity	Two-year inhalation study in rat 0, 10, 50 and 250 mg/m^3; 6h/day, 5 days/week	No abnormal clinical signs, body weight changes or excess mortality. Slight increases in pneumonia, tracheitis and rhinitis 10, 30 mg/m^3: no compound related lung tumours	Biological relevance of lung tumours and pulmonary lesions for man is considered negligible

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
			50, 250 mg/m^3: dose dependent dust cell accumulation 250 mg/m^3: Bronchioalveolar adenomas and cell cystic keratinizing squamous cell carcinomas Signs of overwhelmed lung clearance mechanism	
(Heinrich UF *et al.* 1995)	Anatase/rutile (80/20) (15-40 nm)	Two-year inhalation study in rat and mice 7, 11.6 and 10 mg/m^3 (Satelite groups: 3, 6, 12 and 18 months)	Inflammation and cytotoxicity (BALF LDH, protein) Histology (to assess Carcinogenicity) DNA adducts Lung particle burden and alveolar lung clearance	Rats: Increased mortality with TiO_2 and in the control group Alveolar lung clearance compromised by TiO_2 Increased protein, LDH in BALF Increased lung tumours

TABLE 12
CARCINOGENICITY STUDIES IN RAT - INHALATION

NIOSH (2011) has concluded that titanium dioxide is not a direct-acting carcinogen, but acts through a secondary genotoxicity mechanism that is not specific to titanium dioxide but primarily related to particle size and surface area. It is expected that carcinogenicity occurs following pulmonary particle overload and thus has a threshold, and that the effects are considered to be caused by the particle exposure rather than the specific chemical substance (NIOSH 2011). There are however also *in vitro* studies that demonstrate inflammation-independent particle effects and therefore a need to further investigate the influence of physico-chemical properties of particles and processes linked to neoplasm formation, including DNA damage, DNA repair, mutagenicity and proliferation (Knaapen *et al.* 2004).

Rats are more susceptible to lung overload compared to mice and hamsters as seen in several studies e.g. (Bermudez *et al.* 2002; Hext *et al.* 2002; Bermudez *et al.* 2004). Human relevance of findings from rat inhalation studies at doses causing lung overload is therefore debated and care has to be taken when interpreting such studies as e.g. discussed in the nano-specific REACH guidance updates, see Section 3.1.1 “Advisory note on the consideration of rat lung overload within inhalation toxicity assessment” of Appendix R7-1 “Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance” of ECHA (2012)[7].

Reproductive toxicity
No reproductive toxicity data relevant to the titanium dioxide nanoparticles were provided by the applicant for elaboration of the SCCS opinion. SCCS refers to a review of reproductive and developmental toxicity studies of, among others, titanium dioxide by Ema *et al.* (2010).

Ema *et al.* (2010) reviewed reproductive and developmental studies of manufactured nanomaterials including titanium dioxide. Two *in vivo* studies in mice are reported investigating subcutaneous injection of **anatase** titanium dioxide particles with a particle size of 25-70 nm and 20-25 m^2/g in surface area at doses of 100 µg/day in ICR mice. Mice were exposed at gestation days 6, 9, 12, and 15, and 3, 7, 10, and 14, respectively. In the first study, brain tissue from male offspring was obtained, total RNA was extracted from whole brain and gene expression was analysed. The results

[7] http://echa.europa.eu/documents/10162/13632/appendix_r7a_nanomaterials_en.pdf

demonstrated changes in gene expression related to development and function of central nervous system in male pubs. In the second study, male offspring were autopsied and lower body weights were found among offspring of dams exposed to titanium dioxide. Aggregates of titanium dioxide nanoparticles (100–200 nm) were detected in Leydig cells, Sertoli cells and spermatids in the testes of pups. In addition, disorganized and disrupted seminiferous tubules, tubule lumens with few mature sperm, and decreases in daily sperm production (DSP), epididymal sperm motility and numbers of Sertoli cells were observed in pups of the titanium dioxide-treated group. Titanium dioxide particles were detected in cells of the olfactory bulb and cerebral cortex of pups, and there were many cells positive for caspase-3, an enzymatic marker of apoptosis, in the olfactory bulb of pups in the titanium dioxide-exposed group. The authors note that although the possibility of adverse effects of titanium dioxide nanoparticles on brain development is noted, the behavioural effects of nanoparticles were not investigated. There was a lack of description on the maternal findings in this report (Ema *et al.* 2010).

Based on these two *in vivo* studies and other available studies not specifically referring to the **anatase** form, SCCS concludes that, overall, the information on this endpoint is patchy and inconclusive (SCCS 2014).

As shown in Table 14 Hougaard *et al.* (2010) observed that nano-sized UV Titan dusts induced long term lung inflammation in time-mated adult female mice and that gestationally exposed offspring displayed moderate neurobehavioral alterations (Hougaard *et al.* 2010).

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Hougaard *et al.* 2010)	Rutile, modified (Zr, Si, Al; coated: polyalcohol	Time-mated mice (C57BL/6BomTac) Inhalation: 1 h/day; 42 mg/m^3 aerosolised powder (1.7×10^6 n/cm^3, peak size 97 nm) GD: 8-18	Lungs: 38 mg Ti/kg (day: 5) BALF: lung inflammation (5 and 26-27 days after exposure termination)	Long term lung inflammation in time-mated adult female mice Gestationally exposed offspring displayed moderate neurobehavioral alterations (direct and indirect mechanisms)

TABLE 13
STUDY ON PRE-NATAL EXPOSURE IN MICE

Photo-induced toxicity

Nano-crystalline titanium dioxide generates reactive oxygen species (ROS) quite efficiently, particularly under ultraviolet (UV) illumination. In a study characterising the toxicity of nano-sized titanium dioxide under ambient (e.g., no significant light illumination) conditions in human dermal fibroblasts and human lung epithelial cells, it was concluded that **anatase** titanium dioxide was 100 times more toxic than an equivalent sample of **rutile** titanium dioxide and that the toxic response of nano-sized titanium dioxide increased substantially with UV illumination (Sayes *et al.*, 2006). The cellular responses exhibited classic dose-response behavior, and the effects increased with time of exposure. Cytotoxicity and inflammatory responses were examined based on viability stains and inflammatory indices with biological endpoints, including lactate dehydrogenase (LDH) release, metabolic (mitochondrial) activity and production of inflammation mediators (interleukin-8 [IL-8]).

Effects (cytotoxicity and inflammation) were only observed at relatively high concentrations (100 mg/ml) of nano-sized titanium dioxide (**anatase**/**rutile**). The most cytotoxic nanoparticle samples were also the most effective at generating reactive oxygen species; *ex vivo* ROS generation under UV illumination correlated well with the observed biological response. Based on these data, the authors suggest that nano-sized titanium dioxide samples optimized for ROS production in photocatalysis are also more likely to generate damaging ROS in cell culture (Sayes *et al.* 2006).

Both *in vitro* and *in vivo* tests of engineered nanoparticles (e.g. carbon nanotubes, titanium dioxide and quantum dots) indicate that reactive oxygen species (ROS) production is related to their toxicity. In a study proposing a method for investigating the dependence of size and crystal phase of nanoparticles on their oxidant generating capacity, the reactive oxidant species-generating capacity was demonstrated for titanium dioxide nanoparticles in nine different sizes (4-195 nm). The correlation between crystal phase and oxidant capacity was established using titanium dioxide nanoparticles of 11 different crystal-phase combinations but similar size, including pure **anatase** and pure **rutile** titanium dioxide (Jiang *et al.* 2008). The results showed that the oxidant reactivity (generation of ROS) exhibited by titanium dioxide particles with similar size but different crystal structures was highest for amorphous samples, followed by pure **anatase**, lower for **anatase/rutile** mixtures and lowest for pure rutile.

The phototoxic potential of titanium dioxide has been investigated in an *in vitro* study, where Balb/c 3T3 cells were incubated with both coated and uncoated titanium dioxide nanoparticles. No information on crystal form was given (SCCS 2013). The results showed that coated titanium dioxide did not show any cyto- or phototoxicity. P25, the uncoated form, was not cytotoxic up to the highest concentrations, but in the presence of irradiation, a viability reduction was observed. SCCS concludes that the study is indicative of the importance of coating on the phototoxic properties.

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
(Lu *et al.* 2008)	P25 (~21 nm) Anatase (>99%, 5-10 nm) Rutile (>98%, 50 nm)	Bovine serum albumin (BSA), UV exposure, TiO_2: 0.2-3 mg/ml) Mouse skin homogenate, UV radiation, TiO_2: 1.67 mg/ml.	Photocatalytic activity, formation of 3-nitrotyrosine (protein nitration) Optimum at physiological pH Photo-oxidative effect on proteins in mouse skin	Anatase>P25>rutile Relationship between photocatalytic protein nitration and cutaneous disease needs investigation
(Nakagawa *et al.* 1997)	Anatase (0.021 µm) In SCG only: Anatase (0.255 µm) Rutile (0.255 µm) Rutile (0.42 µm)	Effect of UV radiation: SCG mouse lymphoma cells L5178Y *Salmonella typhimurium* Mammalian mutation assay, L5178Y cells Chromosome aberration, CHL/IU cells	Genotoxicity: Positive Negative Negative Positive with UV light	Anatase 0.021 µm most postent
(Gurr *et al.* 2005)	Anatase (10, 20, 200 >200 nm) Rutile (200 nm)	Bronchial epithelial cell line (BEAS-2B)	TiO_2 (10, 20 nm): oxidative DNA damage without photocatalysis Anatase (200 nm): no induction of oxidative stress	Photocatalytic activity: Anatase > rutile Without light: Rutile > anatase
(Kermanizadeh *et al.* 2012)	Anatase (9 nm) Rutile (10 and 94 nm)	In vitro liver model Human hepatoblastoma cells (C3A)	Increased intracellular ROS levels Mild genotoxicity and interleukin 8 (IL8) protein production	Large increase in ROS level for both anatase and rutile
(Guichard *et al.* 2012)	Anatase, nano (14±4 nm) Anatase, micro (160±48) Rutile, nano (62±24; 10±2 nm) Rutile, micro (630±216 nm) P25, anatase/rutile	Syrian hamster embryo cells (SHE)	Cytotoxicity and genotoxicity. No significant induction of micronuclei formation.	Anatase and rutile nanoparticles more cytotoxic than microparticles. Anatase produced more intracellular ROS compared to rutile and microsized particles. Rutile microparticles

Paper	TiO_2 crystal form	Model, species, administration and dose	Endpoints, findings	Toxicity of TiO_2
	(25±6 nm)			were found to induce more DNA-damage than nanosized particles
(Tiano *et al.* 2010)	Anatase (10-20 nm) Rutile	In vitro tests: DPPH assay Deoxyribose assay Human dermal fibroblasts (HuDe) Cell viability assay Comet assay Intracellular ROS assay UV radiation	ROS generation under photo excitation	Anatase and anatase/rutile (coated and uncoated) are highly photoreactive In manganese doped rutile TiO2 free radical production is significantly reduced and it confers free radical scavenging behaveour.
(Pan *et al.* 2009)	Anatase (15+3.5 nm) Rutile Coated TiO_2 particles	Dermal fibroblast cells	Cytotoxicity and ROS generation Penetration of cell membranes ROS production	Anatase > rutile Rise in H_2O_2 level: Anatase ≈ 70% Rutile ≈ 24% Coated TiO_2 ≈ control
(Alarifi *et al.* 2013)	Anatase (50.4±5.6 nm)	Rat, intraperitoneal injection: 63, 126 and 252 mg TiO_2/ animal, 24 and 48 hours	Histological changes and apoptosis in hepatocytes	Effects possibly mediated by ROS and stress inducing atrophy and apoptosis
(Jiang *et al.* 2008)	11 samples (4-195 nm): Anatase Anatase/rutile Amorphous	Particle suspension incubated with fluorescent dye. Determination of ROS generating capacity	H_2O_2 generation ROS activity/surface area: S-shaped curve	ROS generating capacity: Amorphous > anatase > rutile

TABLE 14
PHOTOTOXIC PROPERTIES OF ANATASE TITANIUM DIOXIDE NANOPARTICLES

It has been shown that **anatase** titanium dioxide more effectively generates reactive oxygen species (ROS) because of surface reactivity. However, studies have also shown that in the absence of light, larger **rutile** particles can generate hydrogen peroxide, H_2O_2, and cause oxidative DNA damage whereas the same size **anatase** particles did not generate either effect (H_2O_2 production or DNA damage) but a combination of the **rutile/anatase** phase generated the most H_2O_2 and DNA damage (Gurr *et al.* 2005). These different studies show that nanoparticle (crystal) phase is important, but complex, in nanoparticle toxicity. This is also the case in relation and photocatalytic activity.

5.3 Classification of titanium dioxide

Titanium dioxide does not have a harmonised classification under the EU CLP Regulation[8]. There are 229 notifiers of industry self-classifications of **anatase** titanium dioxide (CAS No. 1317-70-0) in the ECHA Classification and Labelling Inventory. 201 notifiers suggest "No classification", without justification or the indication that "data is lacking". Out of the 28 remaining notifiers, 24 suggest a classification with Skin Irrit 2, Eye Irrit 2, Acute Tox 4 and STOT SE 3. Two notifiers suggest a classification with Carc. 2. These suggested classifications are based on information available to the industry and are not to be mistaken for official classifications approved by authorities.

For **rutile** titanium dioxide, 585 notifiers out of 621 suggest "not classified".

[8] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

IARC has categorised titanium dioxide is possibly carcinogenic to humans (Group 2B) (IARC 2010). This categorisation is general and not specifically related to the nanoform(s) and is based on exposure to a high dose.

Titanium dioxide will be evaluated under REACH in 2014 by France. The substance including its nanoform has been included on the CoRAP list (Community Rolling Action Plan) due to initial concerns for carcinogenicity, sensitization and vPvB properties. The first part of the evaluation will be completed in the spring of 2015 and may result in a request for additional test data to clarify the concerns mentioned above.

Information reviewed in the present report does not provide sufficient documentation for a classification of neither **anatase** nor **rutile** nano-sized and uncoated titanium dioxide according to the CLP criteria. Although many studies have been conducted, in particular *in vitro*, they are not always sufficient in order to evaluate the different endpoints and criteria properly due to differences in physico-chemical characteristics, lack of detailed information or limited knowledge about the mode of action.

The overall findings regarding the toxicity relevant for classification of anatase nanosized titanium dioxide are presented in Table 15.

Hazard	Toxicity
Acute toxicity	Mixtures of anatase/rutile titanium dioxide (85 % anatase and 15 % rutile) without coating and mixtures coated with trimethoxy-caprylylsilane or trimethoxy-n-octyl-silane show low acute oral toxicity with LD_{50} values above 2150 mg/kg (single dose tested). No in vivo tests are available on dermal toxicity of nanosized anatase titanium dioxide. Systemic toxicity is not expected based on results from absorption / penetration studies showing that nanotitanium dioxide is not likely to pass the *Stratum Corneum*. Mice exposed to the anatase crytal form by inhalation produced lung infiltration and showed that 21 nm particles were more toxic than 5 nm particles, possibly due to size dependent agglomeration. Several studies with nasal instillation are conducted showing a pulmonary inflammation response (neutrophil and macrophage infiltration). Available data are however not sufficient to conclude about classification. No classification.
Irritation/corrosion	Available studies indicate either mild or non-irritating effects to skin and eyes. No classification.
Sensitisation	Based on available studies of limited relevance and no specific data for the anatase form, nanotitanium dioxide is considered to have low or no sensitising potential. No classification.
Specific organ toxicity (single and repeated exposure)	Available data are not conclusive with regard to classification. Indication of liver and neurotoxic effects. No classification.
Mutagenicity/genotoxicity	Anatase nanotitanium dioxide has demonstrated the potential to cause DNA damage under certain conditions. Documentation is however not conclusive and sufficient to warrant classification. No clastogenic potential was identified for the anatase nanoform. No classification.
Carcinogenicity	Lung tumours are observed in a rat carcinogenicity study with a mixture of nanosized anatase and rutile titanium dioxide but are considered a result of particle overload and therefore not relevant for man. The studies behind the IARC categorisation of titanium dioxide in any form as a Group 2B carcinogen, are considered of little relevance to humans and therefore not relevant as background for CLP classification. Further investigation is needed. No classification.
Reproductive toxicity	No conclusive data are available for the anatase form. Neurobehavioral effects are observed in gestationally exposed offspring of mice exposed to modified rutile. No classification.

TABLE 15
CLASSIFICATION OF ANATASE NANOTITANIUM DIOXIDE

5.4 Exposure to anatase in consumer products

In general, relatively limited information is available in the literature on actual exposure to nano-sized titanium dioxide. Results from projects reviewing and/or estimating exposure to nano-sized titanium dioxide, e.g. NANEX[9] and ENRHES[10], address exposure to "nano-titanium dioxide" in general and not to specific crystalline forms.

Based on the results from the literature survey of consumer products containing **anatase** titanium dioxide and the mapping of specific products on the Danish market, direct exposure of consumers (apart from intake via food) is expected to occur primarily from use of cosmetics (sunscreen) and from application of paints and coatings. In addition, exposure to **anatase** titanium dioxide bound in matrices is possible through contact with paper, ceramics, rubber and fibres containing titanium dioxide as pigment as well as air filters coated with titanium dioxide.

Use of sunscreen and photocatalytic paints is selected for the further exposure and risk evaluation. Use of photocatalytic paints is currently limited in Denmark, but may increase in the future when products with improved properties become available.

Dermal exposure is the main exposure route in relation to sunscreens, but also oral exposure from the dermally applied products is possible as well as direct oral intake from use of sunscreen lip balm potentially containing anatase titanium dioxide. Because of evidence of inflammatory responses and indication of epithelial hypertrophy and hyperplasia when nano-sized titanium dioxide is inhaled at high doses, the use of nano-sized titanium dioxide in powders and sprayable products is in general not recommended (SCCS 2014), but could potentially result in inhalation exposure.

Photocatalytic stability depends on the stability of the coating applied to the nanomaterials. According to the information obtained from the survey of the Danish market, all anatase titanium dioxide in cosmetics is coated, and therefore no exposure to free radicals is expected. The 15 titanium dioxide nanomaterials evaluated by the SCCS were all reported to be photostable (SCCS 2013). SCCS does however stress that there is a concern in relation to stability of the coatings. Based on photocatalytic activity data measured in the formulations, there is an indication that either some of the material is not coated or that some of the coatings were not stable in the formulations and effects of photocatalytic activity could be seen at a later stage. The SCCS considers up to 10 % photocatalytic activity of a coated or doped nanomaterial, compared to the corresponding non-coated or non-doped reference, as acceptable, and indicates a maximum content of anatase of 5% of the titanium dioxide nanomaterial content.

Application of paints and coatings containing anatase titanium dioxide as pigment, photocatalyst or biocide may result in dermal and inhalation exposure. When applied using a paint brush or roller, the exposure will be mainly dermal, whereas spray painting may result in both dermal exposure and inhalation of aerosols.

Free anatase titanium dioxide particles would tend to agglomerate and exposure would partly be to titanium dioxide agglomerates. However, as stated in the SCCS Opinion on titanium dioxide in the nanoform (SCCS 2014) , the agglomerates may de-agglomerate under certain conditions of use, and risk assessment should therefore consider exposure to the nanoparticles directly.

However, in the case of spraying, it can be questioned whether exposure to free nanoparticles and/or their agglomerates/aggregates would take place, as the particles are bound in a droplet matrix. This could happen if the liquid part of the droplets easily evaporates.

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[9] http://nanex-project.eu/
[10] http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

Overall, it is considered that applying hazard data for exposure to free nanoparticles would (significantly) overestimate actual exposure.

It is not expected that the crystal form in itself would lead to different exposure levels.

According to the information obtained from the survey, most sunscreens contain between 2 % and 10 % titanium dioxide, and the Cosmetics Regulation allows titanium dioxide as UV filter in concentrations up to maximum 25 % in cosmetic products.

The overall conclusion from the SCCS based on the available evidence for titanium dioxide in the nano-form is, that the use of titanium dioxide nanomaterials with the characteristics similar to the reviewed products, at a concentration up to 25% as a UV-filter in sunscreens, can be considered to not pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin. Among the required characteristics are: i) a purity of ≥99.5%, ii) a median primary particle size based on the number-based size distribution of 30 to 100 nm or larger, iii) titanium dioxide particles that are composed of mainly the rutile form, or rutile with up to 5% anatase, iv) photostability in the final formulation, and v) are coated with one of the coating materials described in Table 1 of the SCCS opinion, and the coatings are stable in the final formulation and during use. Other cosmetic ingredients applied as stable coatings on TiO2 nanomaterials can also be used, provided that they can be demonstrated to the SCCS to be safe and the coatings do not affect the particle properties related to behaviour and/or effects, compared to the nanomaterials covered in this opinion. This conclusion from SCCS does however not apply to applications that might lead to inhalation exposure to titanium dioxide nanoparticles (such as powders or sprayable products).

Based on the survey, photocatalytic paints generally contain 5-10 % anatase titanium dioxide.

For the evaluation of the consumer exposure from products on the Danish market, the following (worst case) exposure scenarios are considered:

- Dermal exposure to a sunscreen containing 25% nano titanium dioxide. Although the maximum concentration of anatase titanium dioxide is 1.75% in the product (in accordance with (SCCS 2014) all 25% will be included in the evaluation.
- Inhalation exposure of an adult from spray application of a photocatalytic paint containing 10% anatase titanium dioxide used indoors.

Dermal exposure scenario

The worst case scenario selected for dermal exposure to sunscreen lotion/cream containing **anatase** titanium dioxide is based on daily application of a lotion/cream containing the maximum allowed concentration of 25% nanosized titanium dioxide (1.25% **anatase**) used as a UV filter. According to the survey, titanium dioxide used for sunscreens will in general be coated to prevent the formation of free radicals that may also have an adverse effect on the skin. However, the stability of coatings in different formulations is an area which needs further investigation.

The dermal scenario will as mentioned consider both the **anatase** and **rutile** forms, as available data do not strongly support to distinguish between the two forms in relation to all toxicity endpoints, and both forms should therefore be included in the evaluation.

Both *in vitro* and *in vivo* studies have indicated that nanosized titanium dioxide including the **anatase** form does not penetrate intact skin and reach viable layers, even when exposed to UV radiation. In addition it has been shown, that nanosized titanium dioxide does not reach viable layers of the skin when exposed to damaged skin such as psoriatic skin or sunburnt skin. Although no systemic absorption has been demonstrated, a calculation of the potential exposure is carried out for illustration.

The calculation of the external dose from dermal exposure is calculated using the following equation:

Total dermal load: $L_{der} = Q_{prod} \cdot A_{skin} \cdot C_{prod}$

External dermal dose: $D_{der} = \frac{L_{der} \cdot n}{BW}$

The parameters used for the calculation are shown in Table 16.

For a standard scenario involving dermal application of sunscreen cream/lotion, the surface area of the exposed skin and the mean number of events (application) per day are suggested based on SCCS Notes of Guidance (SCCS 2012). The amount of product used per application is based on recommendations from Danish EPA. Whereas SCCS recommends using an estimated daily amount of 17.5 g for the entire skin surface of an adult (corresponding to 1 mg/cm^2), the Danish EPA recommends for a "Danish summer" scenario to use 36 g product (EU Commission 2006) (corresponding to 2 mg/cm^2)and 2 times 36 g for a worst case summer scenario (corresponding to 4 mg/cm^2) during a vacation in the south. Using two applications per day, the applied amount is four times the amount assumed to be realistic by SCCS (2012).

Input parameter	Description	Value	Unit	Reference
Q_{prod}	Amount of product used per application	2	[mg/cm^2]	Danish EPA
C_{prod}	Concentration of rutile/anatase	25	[%]	Cosmetic Regulation
A_{skin}	Surface area of the exposed skin	17500	[cm^2]	SCCS, 2012
n	Mean number of events per day	2	[d^{-1}]	SCCS, 2012
BW	Body weight (woman)	60	kg	ECHA
Output	**Description**			
L_{der}	Dermal load		[mg/cm^2]	
D_{der}	External dermal dose		[mg/kg bw^{-1} d^{-1}]	

TABLE 16
PARAMETERS USED FOR DERMAL EXPOSURE ASSESSMENT

Based on the parameters in Table 16 the external dermal dose is calculated for the maximum allowed amount of nanosized titanium dioxide in the sunscreen irrespective of the crystal form as follows:

$$D_{der} = \frac{Q_{prod} \cdot A_{skin} \cdot C_{prod} \cdot n}{BW}$$

"Danish summer" scenario : 1 application of 2 mg/cm^2 (36 g per application) :

$$D_{der} = \frac{1 \cdot 17500 \cdot 25\% \cdot 2 \cdot}{60} \cdot mg \cdot kg_{bw}^{-1} \cdot d^{-1} = 146\ mg \cdot kg_{bw}^{-1} \cdot d^{-1}$$

"Worst case summer scenario": 2 applications of 2 mg/cm² (36 g per application):

$$D_{der} = \frac{2 \cdot 17500 \cdot 25\% \cdot 2}{60} \cdot mg \cdot kg_{bw}^{-1} \cdot d^{-1} = 292\ mg \cdot kg_{bw}^{-1} \cdot d^{-1}$$

Inhalation exposure scenario
NIOSH (2011) concludes that titanium dioxide and other poorly soluble; low-toxicity (PSLT) particles of fine and ultrafine sizes show a consistent dose-response relationship for adverse pulmonary responses in rats, including persistent pulmonary inflammation and lung tumors, when dose is expressed as particle surface area. In an inhalation scenario the exposure should therefore ideally be expressed as surface area rather that mass or number concentration of particles. This information is however not immediately available.

In a study describing a method developed to characterise nanoparticles that were produced under typical exposure conditions when using a consumer spray product, Chen *et al.* (2010) report that NIOSH has collaborated with the US Consumer product Safety Commission (CPSC) to characterise the aerosol from a new antimicrobial spray product containing titanium dioxide nanoparticles(Chen *et al.* 2010). In short the results are as follows:

1. The results of an experimental scenario indicated that most droplets were in coarse sizes (CMD=22 µm) immediately after spraying. The final aerosol contained primarily solid particles of nano-size (75 nm) and the mass concentration of the aerosol in the breathing zone was 3.4 mg/m³ equivalent to a number concentration of 1.6×10^5 particles/cm³ in a worst case scenario. The nanoparticle fraction of the aerosol amounted to 170 µg/m³, or 1.2×10^5 particles/cm³. Data on the exact composition of the spray product is not available due to confidentiality.

2. The worst-case lung burden for a human adult male after a 1-min spray indoors in a room with limited ventilation was estimated to be approx. 0.075 µg TiO_2/m² alveolar epithelium equivalent to a dose of 0.03 µg titanium dioxide in a rat. This dose is considered low compared to doses which have yielded systemic microvascular[11] dysfunction in rodent studies but raises a concern for potential harmful exposure in case of repetitive sprays conducted in a poorly ventilated environment according to the authors.

3. It is therefore concluded that the results suggest that consumers could be exposed to a significant concentration of airborne titanium dioxide nanoparticles while using titanium dioxide in a spraying application.

In the present scenario involving spray application of a photocatalytic paint containing 10% anatase titanium dioxide, it is suggested to use the mass concentration of the aerosol in the breathing zone measured at 3.4 mg/m³ by Chen *et al.* (2010) to define the exposure when calculating the risk characterisation ratio.

5.5 No effect levels for anatase titanium dioxide

5.5.1 Dermal

The Derived no Effect Level (DNEL) is based on the following equation:

$$DNEL = \frac{N(L)OAEL_{corr}}{AF_1\ x\ AF_2\ x \ldots AF_n} = \frac{N(L)OAEL_{corr}}{Overall\ AF}$$

[11] Microvascular refers to the portion of the circulatory system composed of the smallest vessels, such as the capillaries, arterioles, and venules, with an internal diameter of at most 100 microns.

$NOAEL_{corr}$ is the corrected NOAEL value, e.g. if a NOAEL has to be corrected for differences in absorption via different exposure routes (ECHA, 2012).

The assessment factors applied are listed in the table below. The assessment factors have been established on the basis of the default assessment factors of in the REACH guidelines.

Assessment factors applied for determination of DNEL are shown in table 6.

Parameter	Value	Assessment factor
Interspecies	Allometric scaling. Corrections for differences in metabolic rate per kg body weight (not relevant for local effects)	AS: 7 for mice 4 for rats
Interspecies	Remaining differences between different species	2.5
Intraspecies	Individual differences (for consumers)	10
Duration	Sub-acute to chronic	6

TABLE 17
ASSESSMENT FACTORS APPLIED FOR DETERMINATION OF DNEL

No dermal absorption is demonstrated in available studies with nano titanium dioxide, and consequently no relevant NOAEL or LOAEL values exist. Considering the absence of systemic exposure, the SCCS has concluded that the use of nanosized titanium dioxide in dermally applied cosmetic products "should not pose any significant risk to the consumer" (SCCS 2013). No quantitative risk assessment was carried out.

If a quantitative risk assessment is carried out based on the available information, a number of assumptions must be made. In the absence of a dermal study the **LOAEL of 5 mg/kg bw/day** based on impaired neurofunction and behaviour at all dose levels is based on a 60 days oral study (gavage) in female mice administered uncoated anatase titanium dioxide in doses of 0, 5, 10, and 50 mg/kg bw/d (SCCS 2013) could be used for deriving a DNEL. Indicators for impaired neurofunction included significantly altered levels of Ca, Mg, Na, K, Fe and Zn in brain, inhibition of certain enzymes, disturbance of the central cholinergic system and significantly decreased levels of monoamines neurotransmitters. No further details are available for the study, which was submitted by the applicant. This worst case scenario is highly conservative and not considered realistic based on available information, but it serves to illustrate if absorption through skin could result in an unacceptable risk as indicated by a risk characterisation ratio (RCR) > 1..

In order to convert the oral LOAEL into a dermal LOAEL the following equation from the REACH Guidance, Chapter R.8 is used, where ABS is the absorption:

$$\text{Corrected } dermal\ LOAEL_{dermal,corr} = LOAEL_{oral} \times \frac{ABS_{oral-mice}}{ABS_{derm-mice}} \times \frac{ABS_{derm-mice}}{ABS_{derm-human}}$$

$$= LOAEL_{oral} \times \frac{ABS_{oral-mice}}{ABS_{derm-human}}$$

With no evidence of actual systemic dermal absorption and little information on the oral absorption of nanosized titanium dioxide, the risk evaluation here is carried out as a calculation to show at which level of dermal absorption in humans compared to oral absorption in mice, the risk characterisation ratio would exceed the acceptable level (RCR=1).

The total assessment factor used in the calculation is 1050 based on a factor of 2.5 for general interspecies differences, 7 for allometric scaling between mice and humans, 10 for intra species differences and 6 for duration.

As this suggested scenario and the assumptions made are considered conservative no additional assessment factor is introduced for the extrapolation of LOAEL to NOAEL.

DNEL for anatase titanium dioxide is thus:

$$DNEL = \frac{oral\ LOAEL \times}{AF} = \frac{5 \times \frac{ABS_{oral-mice}}{ABS_{derm-human}}}{1050} \frac{mg}{kg_{bw} \times day^{-1}}$$

$$DNEL = 0.0048 \times \frac{ABS_{oral-mice}}{ABS_{derm-human}} \frac{mg}{kg_{bw} \times day^{-1}}$$

This DNEL can be used for comparison with the estimated dermal exposure.

5.5.2 Inhalation

A specific DNEL for anatase titanium dioxide is difficult to derive based on the reviewed literature. In order to develop a scenario for photocatalytic paint, it is as an alternative suggested to use the NIOSH-recommended (NIOSH 2011) airborne exposure limit for ultrafine and nanoscale titanium dioxide of 0.3 mg/m3. NIOSH considers ultrafine nanotitanium dioxide a potential occupational carcinogen, and the REL is established for worker exposure as a time-weighted average (TWA) concentration for up to 10 hours per day during a 40-hour work week, representing the level that over a working lifetime is estimated to reduce the risk of lung cancer to below 1 in 1000.

Due to the conservative approach no further adjustment is made to derive a specific consumer-value, which normally involves introduction of an additional (or higher) assessment factor.

5.6 Health risk evaluation

5.6.1 Dermal

The risk characterisation ratio, RCR, shows the relation between the calculated absorption of the substance (the internal dose) and the Derived No Effect Level (DNEL) which is the exposure level below which no damaging health effects are expected. When RCR is below 1, the exposure is considered not to cause any risk. In this section RCR's are calculated on basis of the scenarios presented in section 5.4. In the following it is calculated at which level of human dermal absorption compared to oral absorption in the mice the RCR would exceed 1 and thus express an unacceptable risk.

Calculation of RCR for application of 2 mg/cm² skin per day (corresponding to the "Danish summer" scenario presented in section 5.4):

$$RCR_{dermal} = \frac{D_{der}}{DNEL} = \frac{146 \text{ mg/kg bw/day}}{0.0048 \times \frac{ABS_{oral-mice}}{ABS_{derm-human}} \text{ mg/kg bw/day}} = 30{,}417 \times \frac{ABS_{derm-human}}{ABS_{oral-mice}}$$

When inserting RCR=1, the Danish summer scenario indicates that an unacceptable risk may be expected if:

$$ABS_{derm-human} = 3.29 \cdot 10^{-5} \times ABS_{oral-mice}$$

Calculation of RCR for application of 2 4 mg/cm² product per day (worst case summer scenario presented in section 5.4):

$$RCR_{dermal} = \frac{D_{der}}{DNEL} = \frac{292 \text{ mg/kg bw/day}}{0.0048 \times \frac{ABS_{oral-mice}}{ABS_{derm-human}} \text{ mg/kg bw/day}} = 60{,}833 \times \frac{ABS_{derm-human}}{ABS_{oral-mice}}$$

When inserting RCR=1, the worst case summer scenario indicates that an unacceptable risk may be expected if:

$$ABS_{derm-human} \geq 1.64 \cdot 10^{-5} \times ABS_{oral-mice}$$

No estimate of oral absorption in mice has been identified. In rats available studies indicate that approximately 6 % orally administered bulk-sized titanium dioxide is absorbed whereas it has not been possible to estimate the absorption of the nanoparticles. The oral absorption of nano titanium dioxide is however considered very low (see section 5.1.1.)

Even though the read across from bulk to nano forms is not recommended without scientific justification it has been attempted to roughly estimate which extent of dermal absorption that could cause an unacceptable risk (RCR>1), if it is assumed that the effects seen after oral absorption in the rat of the bulk substance also could occur due to dermal uptake.

In other words, according to this evaluation, dermal exposure from the worst case summer scenario with application of 36 g sunscreen two times a day, could potentially lead to an unacceptable risk, if human dermal absorption is $1.64 \cdot 10^{-5}$ times the oral absorption in mice. If the oral absorption in mice is estimated at 6% as in the rat, the human dermal absorption should be $9.8 \cdot 10^{-5}$ %.

The majority of data currently available show a lack of dermal absorption of nano titanium dioxide into the dermis (see section 5.1.2). The only identified study which has made an attempt to quantify the absorption of coated nanosized titanium dioxide in sunscreens into minipig skin (which resemble human skin better than mice and rat skin), is Sadrieh et al., 2010., who used electron microscopy-energy dispersive x-ray analysis. The applied amount of sunscreen was 72 g/cm^2 pig skin with four applications per day, 5 days per week during four weeks. There was no sign of systemic absorption to internal organs, but an extremely low absorption of $8 \cdot 10^{-5}$ % of nano titanium dioxide to the dermis was observed. However, this may have been an artefact due to cross-contamination during tissue sectioning

Although these figures cannot be directly compared, the results from minipigs indicate strongly that there is no significant absorption of nanosized titanium dioxide through the normal skin. With the very low fraction of the dose applied to pig skin reaching the stratum corneum, the potential for further penetration and absorption would be very low.

The calculation of RCR for the worst case summer scenario shows that the human dermal absorption has to be $9.8 \cdot 10^{-5}$ % for the risk to be unacceptable. However, in light of the data gaps and uncertainties, which the calculation is based on an additional AF of 10 could be applied to the calculation as described in ECHA's Appendix R8-15 "Recommendations for nanomaterials". This would result in an absorption of $9.8 \cdot 10^{-6}$ % for the risk to be unacceptable and consequently a risk to consumers cannot be ruled out completely.

As **anatase/rutile** titanium dioxide is also present in other products, combined exposure should be considered in relation to risk assessment including all sources and exposure routes in order to obtain a full picture.

5.6.2 Inhalation

As no other relevant exposure data have been identified, the mass concentration of the aerosol in the breathing zone measured at 3.4 mg/m^3 by Chen *et al.* (2010) is used to calculate the exposure.

Using the recommended airborne exposure limit (REL) of 0.3 mg/m^3 suggested for workers by NIOSH as the derived-no-effect-level (DNEL) and comparing this to the mass concentration of 3.4 mg/m^3 and considering a concentration of nanosized titanium dioxide of 10% in the aerosol (as for the mapped paints and considered realistic worst case) results in an RCR of 1.13.

$$RCR_{inhalation} = \frac{10\ \% \times 3.4 \frac{\mathrm{mg}}{\mathrm{m3}} TiO2}{REL} = \frac{0.34 \frac{\mathrm{mg}}{\mathrm{m3}} TiO2}{0.3\ \mathrm{mg/m3}} = 1.13$$

This scenario involves significant precautionary assumptions, but indicates that there may be an unacceptable risk for the consumer. It should, however also be stressed that the REL is based on an average exposure of up to 10 hours per day during a 40-hour work week over a working lifetime, and representing the levels that over a working lifetime are estimated to reduce risks of lung cancer to below 1 in 1,000. For consumer exposure to photocatalytic paints a more realistic scenario would be expected to involve two working days of 8 hours per year. This scenario would reduce the risk from chronic exposure considerably as the annual exposure of consumers would be 125 times lower than the exposure of workers.

It should also be mentioned that the time-weighted average reference period for a full shift (working day) as applied by ECHA is 8 hours for occupational exposure. For comparison the Danish occupational exposure limit (8-hour time-weighted average) for titanium dioxide (not specifically in the nanosized forms) is 6 mg/m^3, calculated as Ti (60 %). In addition the present study the authors of this report do not find that the potential carcinogenic effects of **anatase** titanium dioxide are sufficiently documented by the available studies. Taking the modifying precautionary assumptions into consideration, the risk may be lower than indicated by the calculated example.

5.6.3 Uncertainties in risk assessment of nanomaterials

The health risk evaluation presented in 5.6.1 and 5.6.2 should be read in the light of the assumptions made and the many knowledge gaps and uncertainties which are still involved with risk assessment of nanomaterials. Among these uncertainties are:

- It is not known if available test methods are valid for nanomaterials.
- It is not known if the generally applied assessment factors are valid for nanomaterials.
- The information used for evaluation of nanomaterials today is based on data generated for many different forms of the particles with varying surface area, coating, crystal form. etc. which therefore cannot necessarily be compared.
- The quality of data varies significantly and test results with insufficient characterization of the nanoform may be included in the evaluations but may be less useful.

The results from this study will be carried over to the ongoing and more comprehensive study initiated by DEPA and investigating exposure and risk from consumer products on the Danish market.

6. Conclusions

Mapping of nanosized anatase titanium dioxide in consumer products

Titanium dioxide is used in a wide range of products, but in many cases it is not known whether the titanium dioxide in the specific product is nano-sized, if anatase, rutile or a mixture of the two crystal forms is used or the particles may be treated by e.g. coating. Titanium dioxide is mainly used as a white pigment, which may be both anatase and rutile, and have primary particle sizes in the micro or nano range. In general, nanoparticulate titanium dioxide is commonly used; however, often as part of a larger particle size distribution.

As a pigment, it is used in mass-produced products such as paper, paint and coatings as well as in e.g. food and cosmetics. Other uses of titanium dioxide are to harvest photocatalytic effects and as a physical UV filter. Photocatalytic effects may be improved by decreasing the primary particle size, meaning that nano-sized titanium dioxide exhibits effects superior to larger particles of titanium dioxide. Also, the anatase crystal form exhibits an improved photocatalytic effect compared to rutile, though, a mixture of the crystal forms may perform well, too. The photocatalytic effects are exploited in niche products, including paints with e.g. self-cleaning effects and in construction materials such as roof tiles and windows. As a UV filter, an important application is sunscreen, where the anatase and rutile crystal forms protects against radiation in different regions of the UV spectrum, and products may contain both forms of titanium dioxide. By using nano-sized titanium dioxide, the sunscreen will appear invisible. To reduce or hinder the undesired photocatalytic activity in sunscreen products, as well as other products where the photocatalytic effect is unwanted, the titanium dioxide particles are coated by e.g. silica or alumina compounds or doped with specific metals.

On the Danish market, very few paint products with photocatalytic activity based on titanium dioxide have been identified, and currently just two companies sell them. The paints contain 5-10 % titanium dioxide (a mixture of rutile and anatase or the pure anatase titanium dioxide), and the particle size is stated to be in the nano range or just above. The sale of the photocatalytic paints has been increasing, and this trend is expected to continue. For sunscreen products, a large number of companies are on the Danish market; however, the Danish producers offer mainly Eco-labelled products that are not allowed to contain nano-sized titanium dioxide. The one international company responding to the mapping offers a product line containing nano-sized titanium dioxide, and it is expected, that this is the case for many other brands as well. A test of sunscreen products containing titanium dioxide on the Danish market performed recently showed that two of seven products contained pure anatase titanium dioxide as a UV filter, while the remaining five were based on a mixture of anatase and rutile titanium dioxide. The particle size of the titanium dioxide in the sunscreens is not known.

Effects of anatase titanium dioxide and risks related to use in consumer products

Bulk titanium dioxide is generally considered safe when used in consumer products. Lately the increasing use of nanosized titanium dioxide in general, and in particular in the anatase crystal form, has raised concern due to its photoactive properties and potential to generate free radicals which may cause adverse effects if absorbed. However, in most consumer products like cosmetics, the photocatalytic effect is unwanted as it will also damage the products. The surface of the nanosized titanium dioxide particles is therefore coated in order to inhibit photo-generation of free radicals.

Nanosized anatase titanium dioxide has been shown to have low absorption, low acute toxicity by the oral route, and no systemic toxicity resulting from dermal exposure has been demonstrated due to lack of absorption. No irritation or sensitisation has been observed. When administered by gavage, **anatase** titanium dioxide has produced liver toxicity at high doses following sub-chronic exposure. Following nasal instillation the substance has been shown to bypass the blood-brain barrier and translocate via the olfactory nerve to the brain and result in morphological alterations and induction of oxidative stress. Inhalation of high doses of **anatase** titanium dioxide has been shown to cause inflammatory reactions and pulmonary overload of the lungs, in particular in the rat. Results from genotoxicity studies have indicated a certain genotoxic potential which seems to be more pronounced for the **anatase** crystal form compared to the **rutile** form. Development of lung tumours as demonstrated in rats exposed to high concentrations of a mixture of **anatase** and **rutile** titanium dioxide by inhalation, is believed to be a result of lung overload and therefore not relevant for humans.

A study investigating the influence of crystal structure of nanoparticles on absorption after oral exposure has indicated that the **rutile** form is better absorbed than the **anatase** form, although absorption is generally reported to be low(Onishchenko *et al.* 2012). This finding is noteworthy since the **anatase** form in general is considered more toxic than the rutile form.

Based on the available studies, there is no evidence of systemic exposure by dermal absorption from typical uses in sunscreen products. Limited penetration was also observed in the case of UV-B sunburnt and psoriatic skin. Titanium has been observed in vacant hair follicles, but no penetration into viable epidermis has been observed. The risk from dermal application of products containing nanosized titanium dioxide including the **anatase** form on intact, and also psoriatic and sunburnt skin is therefore considered very limited.

Carrying out risk evaluation based on very precautionary assumptions and using results from an oral study for putting dermal risk into perspective, shows that for a theoretical worst case dermal summer scenario with a sunscreen product, a risk cannot be excluded. However, it is emphasized that the estimate is made using very conservative assumptions.

Inhalation of nanoparticles, including **anatase** titanium dioxide gives rise to more concern, based on in vivo and in vitro studies demonstrating inflammation-driven effects. This has also lead the SCCS to emphasise that nanosized titanium dioxide should not be used in powders or sprays as it is generally recommended to avoid inhalation of particles. It is believed that particle overload of the lungs induces inflammation and ultimately lung damage. When used professionally the Danish occupational exposure limit for titanium dioxide in all forms including nanoforms is 6 mg/m^3 (as Ti) as an average for an eight hour working day.

Coating of nanoparticles inhibits the generation of reactive oxygen species (ROS) and thereby the phototoxicity. No information is available regarding the stability of the coating materials. In general there is no significant evidence to justify a clear distinction between the two crystal forms with regard to phototoxicity and ROS generation.

Defining the right dose metrics when establishing an exposure scenario is still an area for discussion and many studies lack sufficient detail to derive robust values for risk assessment. In order to calculate the risk from a scenario involving inhalation of nanotitanium dioxide from spray application of photocatalytic paint, an exposure limit recommended by the US National Institute for Occupational Safety and Health (NIOSH) for workers of 0.3 mg/m^3, and a measured concentration of 3.4 mg/m^3 of an aerosol (breathing zone) containing nanotitanium dioxide reported in the literature is used.

The calculation of the risk related to inhalation of nanosized **anatase** titanium dioxide based on a very conservative approach, indicating that consumers might be at risk in the described scenario for inhalation exposure. However, when adjusting for the most unrealistic assumptions in relation to a consumer scenario, e.g. that the recommended exposure level (REL) used to illustrate the DNEL in the calculation is based on an exposure scenario involving a worker exposure 125 times the realistic worst case consumer exposure, and taking uncertainties into consideration, no risk is validated.

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Appendix 1: Questionnaires

Questionnaires were sent by e-mail to selected companies producing and/or importing the selected product groups, i.e. photocatalytic paints and sunscreen, respectively. The wording of the short questionnaire is given below in a combined scheme, where the text for photocatalytic paints is given in blue and the text for sunscreen is given in green when the wording differs.

A follow-up interview of some of the respondents for more details was subsequently carried out.

Question 1	Yes	No
Do you sell paint products with a photocatalytic effect, e.g. self-cleaning, antimicrobial or NOx-reducing effects? Do you sell sunscreens with physical filter (titanium dioxide)?		

If your answer to question 1 is no, we don't need you to continue to answer the remaining questions.

Question 2
Which of your products with photocatalytic effect are based on titanium dioxide (TiO_2)? Which of your products contain titanium dioxide (TiO_2)?

Question 3
Which crystal form of titanium dioxide (anatase or rutile) is used in each of the given products? Are pure forms or mixes used? You may give trade names and producer of the titanium dioxide raw material, if this is relevant/easier.

Question 4
What is the particle size/size distribution of the titanium dioxide in your products? Can the titanium dioxide used be termed as a nanomaterial?

Question 5	Yes	No	Don't know
Is the titanium dioxide used in your products surface treated, coated or doped?			
Which type of surface treatment, coating or which material is the titanium dioxide doped with (e.g. silica or aluminium)?			

Question 6
In which concentration(s) are titanium dioxide included in each of your products? And if you know; how much anatase titanuim dioxide is included in each of your products (alternatively in the TiO_2 raw material)?

Question 7	Increase	Decrease	Not change
Did the sale of photocatalytic paint products in your company in the last five years: Did the sale of sunscreens (physical filter) in your company in the last five years:			
Any comments:			

Question 8	Increase	Decrease	Not change
What are your expectations for the sale of photocatalytic paint products in the future? What are your expectations for the sale of sunscreens (physical filter) in the future?			
Any comments:			

Appendix 2: Ultra-fine titanium dioxide grades for sunscreen

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

IMAGE 12
TEST INFORMATION ON COMMERCIALLY AVAILABLE TITANIUM DIOXIDE GRADES FOR SUNSCREENS AS GIVEN IN SCCS 2013

Material code	TiO2 purity/form	Coating material	Doping material	Form	Bulk density (g/cm3)	VSSA (m2 cm-3)
S75-A	> 99.5% Rutile	6% silica, 16% alumina	None	Oil dispersion	0.35	460
S75-B	> 99.5% Rutile	6% silica, 16% alumina	None	Aqueous dispersion	0.35	460
S75-C	> 99.5% Rutile	7.5% alumina, 9,5% aluminium stearate	None	Oil dispersion	0.31	220
S75-D	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Oil dispersion	0.58	300
S75-E	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Aqueous dispersion	0.58	300
S75-F	Anatase 85%, Rutile 15%	7.5% trimethoxycapryly lsilane	None	Hydrophobic powder	0.2	192
S75-G	Anatase 85%, Rutile 15%	None	None	Hydrophilic powder	0.13	213
S75-H	> 99,5% Rutile	6% alumina, 1% glycerin	None	Hydrophilic powder	0.31	260
S75-I	> 99,5% Rutile	7% alumina 10% stearic acid	None	Hydrophobic powder	0.28	300
S75-J	> 99,5% Rutile	6% alumina 1% dimethicone	None	Hydrophobic powder	0.31	260
S75-K	> 94% Rutile	6-8% aluminium hydroxide, 3.5-4.5% dimethicone/met hicone copolymer	None	Hydrophobic powder	0.12-0.28	426
S75-L	> 94% Rutile	6.5-8.5% hydrated silica, 2.5-4.5% aluminium hydroxide, 4.5-6.5% dimethicone/met hicone copolymer	None	Hydrophobic powder	0.07-0.2	426
S75-M	> 98% Rutile, <2% anatase	17% silica	None	Hydrophilic powder	0.09	260
S75-N	> 95% Rutile, <5% anatase	Alumina 10% simethicone 2%	1000 ppm Fe	Amphiphilic powder	0.16	400
S75-O	100% Anatase	Simethicone 5%	None	Hydrophobic powder	0.75	400

IMAGE 13
TEST INFORMATION ON COMMERCIALLY AVAILABLE TITANIUM DIOXIDE GRADES FOR SUNSCREENS AS GIVEN IN WEIR *ET AL.* 2012

Occurrence and effects of nanosized anatase titanium dioxide in consumer products

The use of photocatalytic active nano titanium dioxide (the special anatase form) in consumer products is limited on the Danish market. It is mostly used in sunscreen and paint. A conservative risk evaluation shows that it is unlikely that there is a risk associated with use of paint containing anatase titanium dioxide. In sunscreens more knowledge about the skin barrier is likely to exclude a minor theoretical risk that a conservative risk evaluation identifies.

Der er kun få forbrugerprodukter med fotokatalytisk aktivt nanotitandioksid (den særlige anataseform) på det danske marked. Det bruges mest i solcreme og maling. Anvendelsen af maling med anatase titandioksid udgør med stor sandsynlighed ikke en risiko. I solcreme vil mere viden om hudens barriere sandsynligvis kunne udelukke en teoretisk risiko som en konservativ risiko evaluering identificerer.

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