

External Review Draft

Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen

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Abbreviations

α -HBDH	Alpha-hydroxybutyrate dehydrogenase
γ H2AX	Phosphorylated form of histone H2AX (phosphorylation of H2AX at serine 139)
ξ	Chi potential
π	Pi, approximately equal to 3.14159
σ_g	Geometric standard deviation
μ g	Microgram(s)
μ g/g	Microgram(s) per gram
μ g/kg	Microgram(s) per kilogram
μ g/L	Microgram(s) per liter
μ L	Microliter(s)
μ m	Micrometer(s)
μ m ² /cm ³	Micrometer(s) squared per centimeter cubed
4-MBC	4-methylbenzylidene camphor
ACGIH	American Conference of Governmental Industrial Hygienists
ACROS	Acros Organics
AFM	Atomic force microscopy
Al ₂ (SO ₄) ₃ · 16H ₂ O	Alum
Al ₂ O ₃	Aluminum oxide, also known as alumina
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
As(III)	Arsenite
As(V)	Arsenate
AST	Aspartate aminotransferase
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BAuA	German Occupational Safety and Health (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin)
BBB	Blood brain barrier
BET	Brunauer, Emmett, Teller method of calculating surface area
BrdU	Bromo-deoxy-uridine
BUN	Blood urea nitrogen
BW	Body weight
C ₆₀	Fullerene
Ca ²⁺	Calcium cation
CCOHS	Canadian Centre for Occupational Health and Safety
CE	Capillary electrophoresis
CEA	Comprehensive environmental assessment
CK	Creatinine kinase
cm ²	Centimeter(s) squared
cm ³	Centimeter(s) cubed
CMD	Count median diameter
CPC	Condensation particle counter
CREM	Council for Regulatory Environmental Modeling
CVD	Chemical vapor deposition
DIN	Deutsches Institut für Normung (German Institute for Standardization)

DLS	Dynamic light scattering
DMA(V)	Dimethylarsinic acid
DMEM	Dulbecco's Modified Eagle's Medium
DPPC	Dipalmitoyl phosphatidylcholine
EC3	Estimated concentration required to induce a threshold positive response, where stimulation index equals 3
EC50	Effective concentration 50; the concentration at which 50% of subjects show a response
EDS	Electron-dispersive X-ray analysis
E-FAST V2.0	Exposure and Fate Assessment Screening Tool Version 2.0
EHS	Environmental health and safety
ELISA	Enzyme-linked immunosorbent assay
ELPI	Electrical low pressure impactor
EM	Electron microscopy
EN	European Norm
EPA	U.S. Environmental Protection Agency
EU	European Union
EWG	Environmental Working Group
F344	Fischer 344
FDA	U.S. Food and Drug Administration
FE-SEM	Field emission-type scanning electron microscopy
FeTiO ₃	Ilmenite
FFF	Field flow fractionation
FHD	Flame hydrolysis deposition
FIFFF	Flow field flow fraction (also known as flow FFF)
g	Gram(s)
g/kg	Gram(s) per kilogram
GFAP	Glial fibrillary acidic protein
GGT	γ-Glutamyltransferase
GSD	Geometric standard deviation
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GST	Glutathione-S-transferase
H ₂ O ₂	Hydrogen peroxide
H ₂ SO ₄	Sulfuric acid
HBSS	Hank's Basic Salt Solution
HCl	Hydrochloric acid
HEPA	High efficiency particulate air
HPLC	High performance liquid chromatography
hprt	Hypoxanthine-guanine phosphoribosyltransferase (gene)
HRTEM	High resolution transmission electron microscopy
Hz	Hertz
i.p.	Intraperitoneal
i.v.	Intravenous
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
IC ₂₀ , IC ₂₅	Inhibitory concentration at which organisms show 20%, 25% inhibition in measured endpoints
ICP	Inductively coupled plasma
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
IEP	Isoelectric point

IFN- γ	Interferon-gamma
IL-10	Interleukin-10
IL-1 β	Interleukin-1 β
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8 (KC)	IL-8 = interleukin-8, KC = chemokine (CXC motif) ligand 1 (CXCL1)
ILSI	International Life Sciences Institute
IOAA	(U.S. EPA) Immediate Office of the Assistant Administrator
ISO	International Organization for Standardization
ITT	Isopropyl titanium trisostearate
K ⁺	Potassium cation
kg	Kilogram(s)
L	Liter(s)
LC ₅₀	Lethal concentration 50; the concentration at which 50% of subjects died
LDH	Lactate dehydrogenase
LIBD	Laser-induced breakdown detection
LOEC	Lowest observed effect concentration
LOEL	Lowest observed effect level
LPS	Lipopolysaccharide
m ²	Meter(s) squared
m ² /g	Meter(s) squared per gram
m ³	Meter(s) cubed
MARA	Microbial array for risk assessment (assay)
MCL	Maximum contaminant level
mg	Milligram(s)
mg/cm ²	Milligram(s) per centimeter squared
mg/kg	Milligram(s) per kilogram
mg/L	Milligram(s) per liter
mg/m ³	Milligram(s) per meter cubed
mg/mL	Milligram(s) per milliliter
Mg ²⁺	Magnesium cation
MgCl ₂	Magnesium chloride
micro-TiO ₂	Microscale titanium dioxide
mL/kg/day	Milliliter(s) per kilogram per day
mm	Millimeter(s)
mM	Millimolar
MMA(V)	Monomethylarsonic acid
MMAD	Mass median aerodynamic diameter
MPPS	Maximum penetrating particle size
mSv	Millisevert
MTC	Microbial Toxic Concentration, in microbial array for risk assessment (MARA) assay
MTP	Microsomal triglyceride
Na ⁺	Sodium cation
NaCl	Sodium chloride
NAG	Nacetyl- β -glucosaminidase
Nano-TiO ₂	Nanoscale titanium dioxide
Nano-TiO ₂ F-1R	Nanoscale titanium dioxide a formula containing nano-TiO ₂ that is 3% anatase and 97% rutile
NCEA	(U.S. EPA) National Center for Exposure Assessment
Nano-TiO ₂	Nanoscale titanium dioxide
ng/mL	Nanogram(s) per milliliter

NHEERL	(U.S. EPA) National Health and Environmental Research Laboratory
NIOSH	National Institute for Occupational Safety and Health
nm	Nanometer(s)
NMR	Nuclear magnetic resonance
NMRI	Naval Medical Research Institute
NOEC	No observed effect concentration
NOSH	Nanoparticle Occupational Safety and Health (Consortium)
O ₂ ⁻	Superoxide radical anion
OC	Octocrylene
°C	Degree(s) Celsius
OECD	Organization for Economic Co-operation and Development
OH	Hydroxyl
· OH	Hydroxyl radical(s)
· OOH	Hydroperoxyl radical(s)
OM	Octyl methoxycinnamate
OPPT	(U.S. EPA) Office of Pollution Prevention and Toxics
OPT	Optical particle counter
ORD	(U.S. EPA) Office of Research and Development
ORISE	Oak Ridge Institute for Science and Education
OSHA PEL	Occupational Safety and Health Administration permissible exposure limit
OSP	(U.S. EPA) Office of Science Policy
<i>p</i>	Pink-eyed dilution
P25	AEROXIDE® P25
PAM	Pulse amplitude modulation
PBS	Phosphate buffered saline
PEC	Predicted environmental concentration
pH	Measure of acidity or alkalinity of a solution
pH _{pzc}	pH at the point of zero charge
PIGF	Placenta growth factor
PMN	Polymorphonuclear neutrophil
PNEC	Predicted no-effect concentration
PPE	Personal protective equipment
ppm	Part(s) per million
PTFE	Polytetrafluoroethylene
Pt	Platinum
PTM	Particle tracking model
<i>p^{un}</i>	Pink-eyed unstable
RLE-TN	Rat alveolar type II epithelial cell line
ROS	Reactive oxygen species
rPTM	Radius particle tracking model
RT-PCR	Reverse transcription polymerase chain reaction
s.c.	Subcutaneous
SAXS/WAXS	Small- and wide-angle X-ray scattering
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers
SCCP	Scientific Committee on Consumer Products
SCID	Severe combined immunodeficiency
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SiO ₂	Silicon dioxide
SMPS	Scanning mobility particle sizer

SOD	Superoxide dismutase
SPF	Sunburn protection factor
SPM	Scanning probe microscopy
St-C n	Sunscreen standard C from the Japan Cosmetic Industry
SWCNT	Single-walled carbon nanotube(s)
TEC	Threshold effect concentration
TEM	Transmission electron microscopy
TEOM®	Tampered element oscillating microbalance
TFF	Tangential-flow ultrafiltration
TGA	Australian Therapeutic Goods Administration
TGF- β	Transforming growth factor-beta
THF	Tetrahydrofuran
Ti	Titanium
TiCl ₄	Titanium tetrachloride
TiO ₂	Titanium dioxide
TiOSO ₄	Titanyl sulfate
TLV	Threshold limit value
TNF- α	Tumor necrosis factor-alpha
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TS	Technical Specification
TUNEL	Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling
U.S. EPA	U.S. Environmental Protection Agency
USP	U.S. Pharmacopeia
UV	Ultraviolet (light/radiation), wavelengths in the range of 10-400 nm
UV-A	Ultraviolet A, wavelengths in the range of 320-400 nm
UV-B	Ultraviolet B, wavelengths in the range of 290-320 nm
VEDIC	Video-enhanced differential interference contrast
WHMIS	Workplace Hazardous Materials Information System
Wt%	Weight percent
XAS	X-ray absorption spectroscopy
XPS	X-ray photon spectroscopy
XRD	X-ray diffraction
ZnO	Zinc oxide

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Preamble

1 Engineered nanoscale materials (nanomaterials) have been described in part as having at least one
2 dimension on the order of approximately 1 to 100 nanometers (nm) and unique or novel properties that
3 arise from their small size. This document is a starting point to determine what is known and what needs
4 to be known about selected nanomaterials as part of a process to identify and prioritize research to inform
5 future assessments of the potential ecological and health implications of these materials. Two specific
6 applications of nanoscale titanium dioxide (nano-TiO₂) are considered: as an agent for removing arsenic
7 from drinking water and as an active ingredient in topical sunscreen. These “case studies” do *not*
8 represent completed or even preliminary assessments, nor are they intended to serve as a basis for risk
9 management decisions in the near term on these specific uses of nano-TiO₂. Rather, the intent is to use
10 this document in developing the scientific and technical information needed for future assessment efforts.

11 The case studies are organized around the comprehensive environmental assessment (CEA)
12 approach, which combines a product life-cycle framework with the risk assessment paradigm. Risk
13 assessment relates exposure and effects information for a substance or stressor; CEA expands on this
14 paradigm by including life-cycle stages and considering both indirect and direct ramifications of the
15 substance or stressor. The organization of the document reflects the CEA approach: after Chapter 1
16 (Introduction), Chapter 2 highlights stages of the product life cycle (feedstocks, manufacturing,
17 distribution, storage, use, disposal), followed by Chapter 3 on fate and transport processes, Chapter 4 on
18 exposure-dose characterization, and Chapter 5 on ecological and health effects.

19 Each chapter and some sections of chapters have lists of questions that reflect information gaps in
20 that portion of the document. For the most part, these information gaps can be thought of as research
21 needs. Note that some of these needs are specific to the respective uses of nano-TiO₂ either as a water
22 treatment agent or as an ingredient in topical sunscreen. Other research needs may apply more broadly to
23 nano-TiO₂ irrespective of its application, and still other needs may apply even more widely to
24 nanomaterials in general.

25 Readers are encouraged to consider the questions listed throughout the document and offer specific
26 comments on how individual questions, or research needs, might be more precisely or accurately
27 articulated. If additional questions should be included or if information is already available to address
28 some of the questions posed here, readers are encouraged to provide such comments as well. These or
29 other comments on any aspect of the document should be submitted in writing in accordance with
30 instructions, including the specified time period, stated in a Federal Register notice appearing on or about
31 July 31, 2009 referring to Docket ID No. EPA-HQ-ORD 2009-0495.

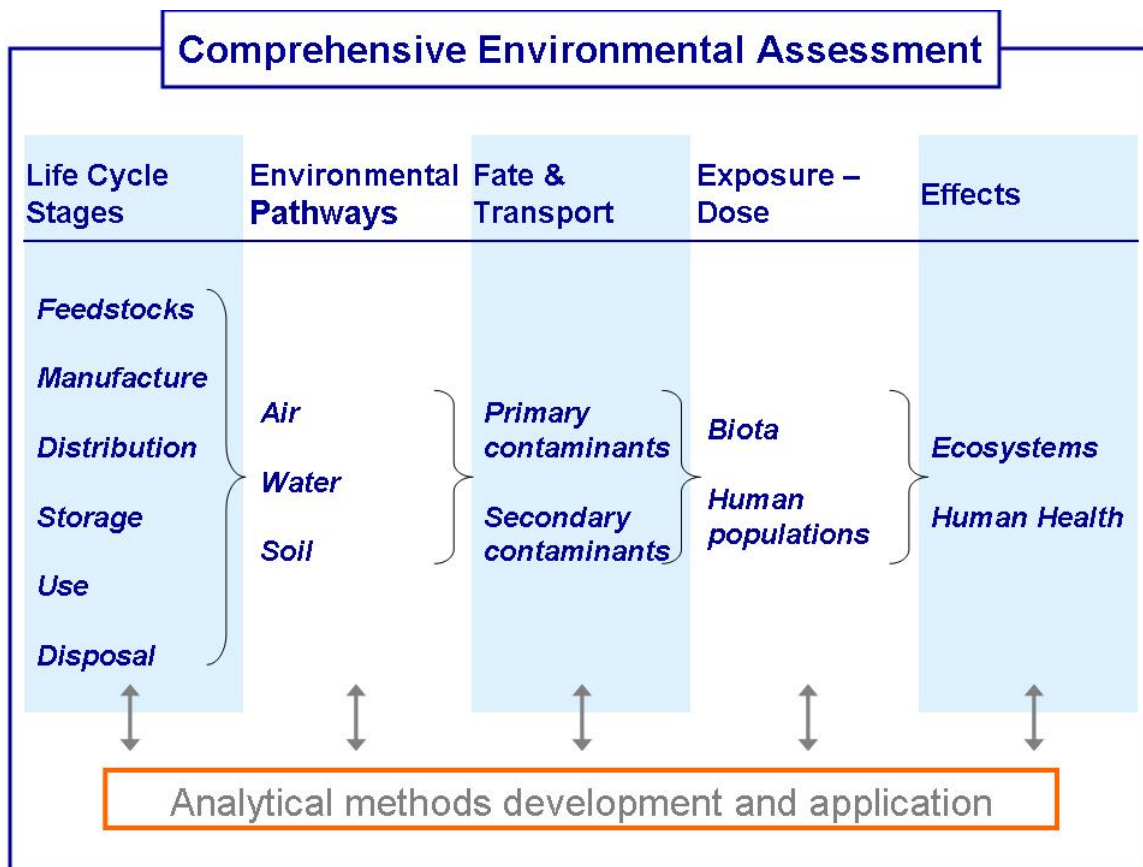
Chapter 1. Introduction

1.1. Background

1 Engineered nanoscale materials (nanomaterials) have been described in part as having at least one
2 dimension on the order of approximately 1 to 100 nanometers (nm) and unique or novel properties that
3 arise from their small size (National Nanotechnology Initiative, 2006). Like all technological
4 developments, nanomaterials offer the potential for both benefits and risks. The assessment of such risks
5 and benefits requires information, and given the nascent state of nanotechnology, much remains to be
6 learned about the characteristics and impacts of nanomaterials before such assessments can be completed.
7 This document is a starting point to identify what is known and, more importantly, what *needs* to be
8 known about selected nanomaterial applications – in this case, for nanoscale titanium dioxide (nano-TiO₂)
9 – to assess their potential ecological and health implications.

10 The complex properties of various nanomaterials make evaluating them in the abstract or with
11 generalizations difficult if not impossible. Thus, this document focuses on two specific uses of nano-
12 TiO₂, as a drinking water treatment and as topical sunscreen. These “case studies” do not represent
13 completed or even preliminary assessments; rather, they present the structure for identifying and
14 prioritizing research needed to support future assessments of nano-TiO₂ and an approach to study other
15 nanomaterials.

16 The case studies follow the comprehensive environmental assessment (CEA) approach, which
17 combines a product life-cycle framework with the risk assessment paradigm (Davis and Thomas, 2006;
18 Davis, 2007). In essence, risk assessment relates exposure and effects information for a given substance
19 or stressor, and CEA expands on this paradigm by including life-cycle stages and considering both
20 indirect and direct ramifications of the substance or stressor. Figure 1-1 illustrates the principal elements
21 in the CEA approach. The first column of Figure 1-1 lists typical stages of a product life cycle:
22 feedstocks, manufacturing, distribution, storage, use, and disposal (including reuse or recycling, if
23 applicable). The second column lists environmental pathways or media (air, water, soil) to which
24 nanomaterials or associated materials (e.g., manufacturing by-products) might be released at various
25 stages of the life cycle. Within these media, nanomaterials or associated materials can be transported and
26 transformed, as well as interact with other substances in the environment, both natural and anthropogenic.
27 Thus, a combination of primary and secondary contaminants can be spatially distributed in the
28 environment (column 3, Figure 1-1).



Source: Adapted from Davis and Thomas (2006) and Davis (2007).

Figure 1-1. Basic structure of comprehensive environmental assessment (CEA) as a framework for identifying and prioritizing research efforts.

1
 2 The fourth column of Figure 1-1, exposure-dose, goes beyond characterizing the occurrence of
 3 contaminants in the environment, as exposure refers to actual contact between a contaminant and
 4 organisms (i.e., biota¹ as well as human populations). Under the CEA approach, exposure
 5 characterization can involve aggregate exposure across routes (e.g., inhalation, ingestion, dermal);
 6 cumulative exposure to multiple contaminants (both primary and secondary); and various spatiotemporal
 7 dimensions (e.g., activity patterns, diurnal and seasonal changes). Dose is the amount of a substance that
 8 actually enters an organism by crossing a biological barrier. Conceptually, dose links exposure with the
 9 last column of Figure 1-1, which refers to ecological and human health effects that can result when an
 10 effective dose reaches a target cell or organ in a receptor organism or, in an ecological context, when a
 11 stressor is at a sufficient level to cause an adverse response in a receptor. “Effects” encompass both
 12 qualitative hazards and quantitative exposure-response relationships.

¹ The term biota is used here to refer to all organisms other than humans.

1 CEA involves the elaboration and synthesis of information from the elements in all five columns
2 depicted in Figure 1-1 to systematically evaluate the direct and indirect ramifications of a nanomaterial
3 and its by-products. Underlying the CEA elements are analytical methods that make detection,
4 measurement, and characterization of nanomaterials in the environment and in organisms possible. Not
5 reflected in Figure 1-1 is an essential ingredient in making CEA effective – the inclusion of diverse
6 technical and stakeholder perspectives to ensure that a holistic view is maintained. As an assessment or as
7 a framework for developing a research strategy, CEA is a collective process that requires numerous
8 participants and contributors.

9 Other efforts have been made to assess the potential risks of nanomaterials by incorporating a life-
10 cycle perspective (e.g., Environmental Defense - DuPont Nano Partnership, 2007; Shatkin, 2008; Thomas
11 and Sayre, 2005) or by using collective expert judgment methods (e.g., Kandlikar et al., 2007; Morgan,
12 2005), primarily in a risk management context. Although the present document differs somewhat from
13 these other efforts in its purpose, namely to aid in developing a research strategy for the comprehensive
14 environmental assessment of nanomaterial risks, all of these endeavors complement and reinforce one
15 another.

1.2. How to Read this Document

16 The intent of this document is to identify systematically what is known and what needs to be
17 known about nano-TiO₂ to conduct an adequate assessment of such nanomaterials in the future. The goal
18 is not to provide an actual comprehensive environmental assessment or to state conclusions regarding
19 possible ecological or health risks related to nano-TiO₂.

20 This document is organized around two case studies of nano-TiO₂ using the CEA approach as a
21 basic framework. Although the differences between the applications of nano-TiO₂ as a water treatment
22 agent versus a topical sunscreen are important, the information currently available does not allow
23 complete differentiation between the two. For example, the ecological and health effects of nano-TiO₂ are
24 described in a single chapter without regard to whether the source of nano-TiO₂ is water treatment or
25 sunscreen. However, where distinctions are possible or seem likely (e.g., in life-cycle stages such as
26 manufacturing and use), the discussion of water treatment is presented first, followed by discussion of
27 sunscreen. In some sections, the discussions are not strictly parallel, reflecting the availability of data.

28 Also important to note is that these case studies have been developed without a specific regulatory
29 objective in mind. Although the topics selected for consideration, water treatment and sunscreen, might
30 be of interest in various policy and regulatory contexts, this document is not intended to serve as a basis
31 for risk management decisions in the near term on these specific uses of nano-TiO₂. Rather, the intent is

1 to use this document in developing the scientific and technical information needed for future assessment
2 efforts as input to policy and regulatory decision-making.

3 Focusing on only two examples of nano-TiO₂ applications obviously does not represent all the
4 possible ways in which this nanomaterial could be used or all the issues that different applications could
5 raise. Rather, by considering the commonalities and differences between two applications of nano-TiO₂,
6 research needs can be identified that apply not only to these specific applications but generally to nano-
7 TiO₂ and perhaps even more broadly to other nanomaterials. Also, additional case studies will be
8 developed for other applications and nanomaterials so that this process can continue and research
9 strategies to support assessment efforts can be further refined.

10 When implemented, a CEA is intended to be comparative, examining the relative risks and benefits
11 of different technological options, for example. Ultimately, a CEA of nano-TiO₂ for water treatment or
12 for topical sunscreen would seek to compare these options against current water treatment practices or
13 sunscreen ingredients. However, it is beyond the scope of this document to describe the various
14 alternatives to nano-TiO₂ for these applications, given that the immediate objective is to identify and
15 prioritize research needs related to nano-TiO₂ as illustrated by the two cases under consideration. Readers
16 seeking comparative assessments of topical sunscreen products, with or without nano-TiO₂, may wish to
17 consult evaluations by the Scientific Committee on Consumer Products (SCCP) (2007) and the
18 Environmental Working Group (EWG) (2009). The EWG analysis in particular takes a broad view that is
19 consistent with the CEA approach in referring to the product life cycle and noting potential ecological as
20 well as human health considerations.

21 That this draft document is a work in progress also should be noted. New, pertinent information
22 seems to appear daily, and readers are encouraged to provide information bearing on the case studies and,
23 in particular, to identify additional research needs and refine the questions listed throughout this
24 document. The document, however, is not intended to provide an exhaustive review of the literature, and
25 focuses instead on findings most clearly relevant to assessment objectives.

26 Finally, the information presented in this document was obtained from a variety of published and
27 unpublished sources, including corporate Web sites and personal communications, as well as inferences
28 based on information about other materials or applications.

1.3. Terminology

29 This document focuses on nano-TiO₂ particles primarily in the size range of 1 to 100 nm. Where
30 information is not specific to nanoscale particles, TiO₂ may be referred to without the “nano” prefix. To
31 make an explicit distinction between the nanoscale material and other forms of TiO₂ not having the

1 special characteristics of nano-TiO₂, the term “conventional” is used.² Even so, conventional materials
2 will often contain a range of particle sizes, including a fraction with nanoscale dimensions. Conversely,
3 as discussed in more detail below, in many circumstances primary nanoscale particles can aggregate or
4 agglomerate into secondary particles with dimensions greater than 100 nm. However, it is not clear that
5 once a cluster of primary nano-TiO₂ particles exceeds 100 nm their properties become like those of
6 conventional TiO₂. For example, inhalation of nano-TiO₂ (20 nm diameter) induced more pulmonary
7 inflammation in the rat than inhalation of fine TiO₂ (about 250 nm diameter) at a similar mass
8 concentration, even though particles in both groups had similarly sized agglomerates (0.71 micrometer
9 [µm] mass median aerodynamic diameter [MMAD] nano; 0.78 µm MMAD fine) (Oberdörster et al.,
10 1994; Oberdörster, 2000). Additional analysis revealed that effects were similar when expressed on the
11 basis of surface area. Whether the constituent primary particles necessarily remain agglomerated or
12 aggregated if conditions change also is not clear. As will be discussed under Fate and Transport (Chapter
13 3), disaggregation can occur under some conditions. Given these considerations, this document does not
14 use 100 nm as a definitional hard line in considering what might be relevant to an evaluation of nano-
15 TiO₂. This view is consistent with a statement by the European Commission (2008) that extends the term
16 nanomaterial to encompass “nanostructured materials,” defined by the International Organization for
17 Standardization (ISO) (Technical Specification [TS] 27687) as “[a]ggregates and agglomerates, often
18 existing at a micro size, [that] may have some of the behaviour and effects of their smaller sub units, e.g.,
19 due to an increased surface area.”

20 Degussa AEROXIDE[®] P25 (hereafter referred to as P25) is a commercial-grade, uncoated nano-
21 TiO₂ product that has been studied extensively and referenced in the literature and is therefore often
22 mentioned in later sections of this document. As discussed below, however, P25 does not represent all
23 nano-TiO₂ preparations and should not be equated with the generic term nano-TiO₂.

1.4. Conventional TiO₂

24 Although this document focuses on nano-TiO₂, highlighting some facts about conventional
25 titanium dioxide (TiO₂) first is instructional. Also known as titania, TiO₂ has been used commercially
26 since the early 1900s in numerous consumer and industrial applications, particularly coatings and
27 pigments. TiO₂ is a naturally occurring mineral that can exist in three crystalline forms, known as rutile,
28 anatase, and brookite, and in amorphous form. Rutile is the most common form of TiO₂ found in nature.

² The terms “bulk” and “pigmentary” are also often used to distinguish conventional from nanoscale TiO₂. Additionally, terms such as ultrafine, PM-0.1, micronized, and attenuation-grade have been used to denote nanoscale particles, but usually in a particular context or field of specialization such as aerosols and air pollution.

1 Elemental titanium is also found in ilmenite (FeTiO_3) and other minerals and ores, and TiO_2 can be
2 produced by processing of these minerals and ores. TiO_2 is insoluble in water, hydrochloric acid, nitric
3 acid, and ethanol, but soluble in hot concentrated sulfuric acid, hydrogen fluoride, and alkali (NRC,
4 1999). TiO_2 is used to increase the whiteness or opacity of many consumer products, such as paints,
5 coatings, plastics, paper, printing inks, roofing granules, food, medicine, toothpaste, cosmetics, and skin
6 care products, including topical sunscreens. In the United States, surface-mining operations in Virginia
7 and Florida produce concentrated titanium-containing minerals (ilmenite and rutile) suitable as feedstock
8 for TiO_2 production (U.S. Geological Survey, 2009). Other countries that produce significant amounts of
9 titanium ores include Australia, Canada, China, India, Norway, and South Africa (U.S. Geological Survey,
10 2009).

11 With exposure to ultraviolet (UV) radiation (wavelengths less than ~ 400 nm), pure TiO_2 is
12 photocatalytic. Studies suggest anatase and rutile have different photocatalytic properties, with anatase
13 being the more reactive (Sayes et al., 2006; Uchino et al., 2002). In applications such as paints, coatings,
14 and cosmetics, where chemical stability is required, the photocatalytic properties of TiO_2 are often
15 suppressed by coating the particles with silica and alumina layers. On the other hand, the photocatalytic
16 properties of TiO_2 are increasingly exploited in a number of other experimental and commercial
17 applications, including degradation of organic compounds, microbiological organism destruction, and
18 conversion of metals to less soluble forms in waste water, drinking water, and indoor air. For more
19 information on conventional TiO_2 , please see the article by Diebold (2003) and the bulletin published by
20 the National Institute for Occupational Safety (NIOSH) (2005).

1.5. Nano- TiO_2

21 One of the main differences between nano- TiO_2 and conventional TiO_2 is the much greater surface
22 area of a given mass or volume of nanoparticles compared to an equivalent mass or volume of
23 conventional TiO_2 particles. To illustrate, a 5-nm particle would have a volume of 65 cubic nm ($\frac{4}{3} \pi r^3$)
24 whereas a 500-nm particle would have a volume of 65,000,000 cubic nm. Therefore, one million 5-nm
25 particles would be required to equal the volume of a 500-nm particle. The surface area of a 5-nm particle
26 equals approximately 80 square nm ($4 \pi r^2$), whereas the surface area of a 500-nm particle equals
27 approximately 800,000 square nm. Multiplying the surface area of the 5-nm particle by one million (the
28 number of 5-nm particles needed to equal the volume of a 500-nm particle) yields a total surface area of
29 approximately 80,000,000 square nm, which is 100-fold greater than the surface area of the 500-nm
30 particle. This greater relative surface area of the nano- TiO_2 particles affords a greater potential for

1 properties such as catalytic activity and UV absorption at certain wavelengths (Shao and Schlossman,
2 1999).

3 Such properties have led to the development or use of nano-TiO₂ for a wide variety of applications,
4 including self-cleaning surface coatings, light-emitting diodes, solar cells, disinfectant sprays, sporting
5 goods, and the subjects of this document, water treatment agents and topical sunscreens. Before
6 considering specific applications of nano-TiO₂, some fundamental issues related to characterization of this
7 material should be noted.

8 Not all nano-TiO₂ is the same. Commercially available brands of nano-TiO₂ can vary in particle
9 size, surface area, purity (e.g., due to doping, coating, or quality control), surface characteristics,
10 crystalline form, chemical reactivity, and other properties (see Table 1-1). Nano-TiO₂ is available in pure
11 anatase, pure rutile, and mixtures of anatase and rutile. In general, anatase nano-TiO₂ is more
12 photocatalytic than the rutile form, and nanoscale rutile is less photoreactive than either anatase and rutile
13 mixtures or anatase alone (Sayes et al., 2006). However, a mixture of 79% anatase and 21% rutile nano-
14 TiO₂ (P25) was found to be more photocatalytic than 100% anatase nano-TiO₂ in some instances
15 (Coleman et al., 2005; Uchino et al., 2002), but less effective in others (Nagaveni et al., 2004). Such
16 contrasts point to the role of other factors in accounting for the behavior and effects of nano-TiO₂. For
17 example, surface treatment of nano-TiO₂ can change nano-TiO₂ activity, including photoreactivity.
18 Aeroxide T805, which is nano-TiO₂ that has been treated with trialkoxyoctyl silane on the surface, has
19 very low surface reactivity (Degussa, 2003). Similarly, surface coatings of silicone and other compounds
20 are used to decrease nano-TiO₂ photoreactivity so that nano-TiO₂ can be used to protect human skin,
21 plastic, and other objects from UV radiation.

Table 1-1. Examples of nano-TiO₂ physicochemical properties.

Agglomeration / aggregation status in the relevant media	Particle size and size distribution	Shape / aspect ratio (e.g., width and length)
Bulk density / particle density	Photocatalytic activity	Surface area / specific surface area
Composition / surface coatings	Pore density	Surface charge / zeta potential
Crystal structure / crystallinity (crystalline phase, crystallite size)	Porosity	Surface chemistry
Dustiness	Purity of sample	Surface contamination
Octanol-water partition coefficient	Radical formation potential	Surface reactivity
	Redox potential	Water solubility

Source: Data from Department for Environment, Food, and Rural Affairs (2007); Powers et al. (2006); Powers et al. (2007); Warheit et al. (2007c); and Organisation for Economic Co-operation and Development (OECD) (2008).

1 External factors can also influence photoreactivity. Krishna and coauthors (2006), for example,
2 found that the presence of fullerenes, which scavenge photogenerated electrons, enhances the
3 photocatalytic efficacy of nano-TiO₂. Likewise, Komaguchi and colleagues (2006) saw significant
4 increases in photocatalytic efficiency of P25 after exposure to an oxidizing environment.

5 Photocatalytic nano-TiO₂ is preferred for water treatment, and photostable nano-TiO₂ is preferred
6 for sunscreen use. Some sunscreens, however, contain photoreactive nano-TiO₂. Although pure uncoated
7 and undoped anatase TiO₂ is photocatalytic, and uncoated and undoped rutile TiO₂ is generally
8 photostable, there is no quick way to identify the photoreactivity of other nano-TiO₂. For example,
9 although doped rutile nano-TiO₂ can be extremely photostable (Reisch, 2005), rutile nano-TiO₂ produced
10 by a certain specific powder-preparation method can be highly photocatalytic (Kim et al., 2003b).
11 Similarly, not all coatings decrease nano-TiO₂ photoreactivity.

12 Due to various degrees of porosity, nano-TiO₂ particles with the same diameter can differ in surface
13 area. Because nano-TiO₂ reactivity and consequently behavior and effects are influenced by many nano-
14 TiO₂ physicochemical properties, two nano-TiO₂ products with the same reported (but limited) parameters
15 should not be assumed in fact to be equivalent. For instance, a manufacturer might use the same core
16 nano-TiO₂ for surface-treated and untreated nano-TiO₂, and both might have the same particle size and
17 surface area, but differ in reactivity, as in the case of P25 and Aeroxide T805.

18 Another characteristic of significance is the aggregation or agglomeration of nano-TiO₂ particles.³
19 According to one industrial manufacturer of nanoscale titania produced through flame hydrolysis (see
20 Section 2.2 for a description of this manufacturing technique and others), “tests and calculations have
21 shown that free primary particles with dimensions of less than 100 nm only exist in [flame] reactors for a
22 few milliseconds” (Degussa, 2009). Aggregates of nano-TiO₂, sometimes referred to as “colloidal,” are
23 often roughly an order of magnitude greater in size than primary particles (Dunphy Guzman et al., 2006;
24 Kormann et al., 1988; Lecoanet et al., 2004). The mean aggregated particle diameter of P25 is about
25 3.6 μm, with the smallest 4% of particles having an average diameter of 160 nm (Klaessig, 2006). After
26 being subjected to sonication for 10 minutes, the smallest 15% of P25 particles averaged an agglomerate
27 diameter of 160 nm, while the 50th percentile diameter was 1.6 μm, roughly two orders of magnitude
28 larger than the reported primary particle size of P25, which is 21 nm (Degussa, 2007; Wahi et al., 2006).
29 Ridley et al. (2006) observed that a suspension of uncoated nano-TiO₂ anatase from Ishihara Techno
30 Corporation (Osaka, Japan) with primary particles of 4-nm diameter consisted mainly of aggregates in the

³ Aggregation generally involves fusion or sintering of particles, while agglomeration involves a weaker bond. Use of these terms, however, has not been standardized, and in many cases the degree of bonding is unknown. Consequently, the terms are often used together in this document where it is not clear which would be more appropriate.

1 1- to 30- μm diameter range, and that these size ranges persisted even under sonication and other
2 conditions that would favor disaggregation.

3 Despite the presence, and sometimes the predominance, of such large particles, several researchers
4 investigating laboratory-synthesized anatase and commercial nano-TiO₂ products such as P25 have also
5 found free particles or aggregates with diameters less than 100 nm in varying amounts, depending on
6 synthesis method, temperature, solution pH, and the presence of buffers (Jiang et al., 2009). Moreover,
7 some preparations are specifically designed to generate dispersed particles (e.g., Seok et al., 2006), which
8 would be important in using nano-TiO₂ as a catalyst.

9 The pH_{pzc} of a nanoparticle (the pH at the “point of zero charge,” where the net electric charge at
10 the particle surface is zero) has important ramifications for aggregation, because at that pH particles will
11 fail to electrostatically repel each other. In laboratory studies, the size range of aggregates and the
12 presence of free nano-TiO₂ particles (synthesized on-site, ranging from 5 to 50 nm) were found to be pH-
13 dependent: when the solution pH differed from the pH_{pzc} of the particles, the aggregates tended to be
14 smaller (Dunphy Guzman, pers. comm., 2007; Dunphy Guzman et al., 2006). Sampled aggregates ranged
15 up to 150 nm in size, and contained an estimated 8 to 4,000 nanoparticles (Dunphy Guzman et al., 2006).
16 The pH_{pzc} also depends at least in part on the crystallinity of the nano-TiO₂ particles: Finnegan et al.
17 (2007) reported pH_{pzc} values of ~5.9 for rutile and ~6.3 for anatase.

18 Coatings and surface treatments also affect particle aggregation/agglomeration behavior. A
19 preliminary report by Wiench and colleagues indicated that coated nano-TiO₂ particles (rutile, size 50 x
20 10 nm, surface area of 100 square meters per gram [m^2/g]; coatings included combinations of aluminum
21 hydroxide, hydrated silica, and various polymers) had slower agglomeration and sedimentation rates and
22 a larger fraction of primary nanoparticles remaining in the sample compared with uncoated particles (20
23 to 30 nm, anatase/rutile 80/20, surface area 48.6 m^2/g) (Wiench et al., 2007).

24 The complexity of nano-TiO₂ characterization is illustrated in Table 1-2, from Warheit et al.
25 (2007a). The chemical composition of three different types of ultrafine TiO₂ manufactured by DuPont
26 was determined by X-ray fluorescence. The cores of all three types of nano-TiO₂ were TiO₂, but the
27 crystalline form and the surface coating of alumina or silica differed. Each type of particle was said to
28 exhibit a mean diameter of approximately 140 nm but with (unspecified) fractions of the size distributions
29 below 100 nm. The chloride ions on the surface of the particles were neutralized during production.
30 (Other effects these materials cause are described in Chapter 5.) As shown in Table 1-2, the surface area,
31 crystallinity, chemical reactivity, surface coating, particle size distribution, and pH varied for the
32 materials, all three of which were nominally nano-TiO₂.

Table 1-2. Characterization of three nano-TiO₂ particle types.^a

Particle Type	BET Surface Area (m ² /g)	Chemical Composition	Chemical Reactivity ^b	Median Particle Size and Size Range ^c		pH in Deionized Water
				in Water	in PBS	
Uf-A	18.2	98% TiO ₂ (100% rutile), 2% alumina	10.1	136 nm ± 35%	1990 nm ± 25%	5.64
Uf-B	35.7	88% TiO ₂ (100% rutile), 5% alumina, 7% silica	1.2	149.4 nm ± 50%	2669 nm ± 25%	7.14
Uf-C	38.5	92% TiO ₂ (79% rutile; 21% anatase), 7% alumina, 1% silica	0.9	140 nm ± 44%	—	4.80

^a BET – Brunauer, Emmett, Teller method of calculating surface area

PBS – Phosphate buffered saline

^b Chemical reactivity was tested using a Vitamin C (antioxidant) yellowing assay.

^c After sonication for 15 min at 60 Hertz (Hz).

Source: Modified with permission from Warheit et al. (2007a).

1 The characteristics of a nano-TiO₂ product might change over time. Using a custom-made anatase
2 nano-TiO₂ formulation (uncoated) with a range of particle sizes, Kolář et al. (2006) found that average
3 particle sizes increased over time, due to both agglomeration and re-crystallization (smaller particles
4 dissolving in the aqueous medium and their constituent molecules then adding to the mass of the larger
5 particles). Over the course of 8 years, average (mode) particle size increased from about 10 nm to about
6 14 nm. The investigators also observed that over time relative surface area decreased, light energy
7 absorbance characteristics changed, and perhaps most surprisingly, photocatalytic performance improved,
8 even as relative surface area decreased.

9 As discussed in greater detail in Chapter 5 (Section 5.1.1), these and other issues have been noted
10 in various recommendations for improving the characterization of nanomaterials in exposure and
11 ecological as well as health effects studies. In general, however, reports of toxicity and exposure studies
12 of nano-TiO₂, especially those conducted prior to the year 2000, have not been sufficiently attentive to the
13 issues described above. Manufacturers' literature often has been accepted as having described their
14 products under all conditions – an oversimplification at best. Additionally, attempts to characterize
15 nanoscale particle sizes and size distributions in relation to toxicity and exposure evaluations have been
16 prone to errors involving non-representative sampling, agglomeration during sample preparation,
17 contamination and degradation during product storage, measurement methods, and conditions under
18 which the study was conducted (Powers et al., 2007). Further, some particle characterization techniques
19 can affect measurement accuracy, suggesting that more than one technique might be necessary to describe
20 particle sizes accurately. Accurate characterization is clearly important if the behavior and effects of
21 nano-TiO₂ are to be understood, predicted, and related to other materials (both nanoscale and
22 conventional).

1.5.1. Water Treatment

1 This document assumes that nano-TiO₂ would be used specifically for arsenic removal in a
2 drinking water treatment facility. In addition to arsenic removal (Li et al., 2009), however, nano-TiO₂
3 could be used for disinfection of pathogens (Alrousan et al., 2009; Coleman et al., 2005; Li et al., 2008a;
4 Rincon and Pulgarin, 2003) or for remediation of ground water or waste water contaminated with various
5 organic and inorganic pollutants (Adams et al., 2004; Chen and Ray, 2001; Han et al., 2009; Kim et al.,
6 2003a; Lee et al., 2008; Lin and Valsaraj, 2003; Ryu and Choi, 2008; Xu et al., 2009b). The latter use
7 would pose rather different scenarios of environmental releases and fate and transport, and would add
8 considerably to the complexity of this document. Therefore, the case study of nano-TiO₂ for water
9 treatment has been limited to the consideration to arsenic removal in water treatment facilities.

10 Most of the relevant literature to date has reported laboratory tests of nano-TiO₂ as a photocatalytic
11 treatment for conversion of arsenite [As(III)] to arsenate [As(V)], a species that is more easily removed in
12 water treatment because of its lower solubility in typical drinking water treatment conditions (e.g., Dutta
13 et al., 2004; Ferguson et al., 2005; Pena et al., 2006). Although neither conventional TiO₂ nor nano-TiO₂
14 is known to have been used in a full-scale drinking water treatment plant, both conventional TiO₂ and
15 nano-TiO₂ as photocatalytic agents have been pilot-tested in drinking water treatment plants (Dionysiou,
16 pers. comm., 2009; Pichat, 2003; Purifics Solutions, 2008; Richardson et al., 1996).

17 For arsenic removal from water, both conventional and nanoscale TiO₂ have been developed to
18 photocatalytically oxidize arsenic and adsorb arsenic. Studies have shown that TiO₂ can oxidize As(III) to
19 As(V) and adsorb inorganic arsenic (Dutta et al., 2004; Fostier et al., 2008; Hristovski et al., 2007). The
20 mechanism for TiO₂ photocatalytic oxidation of As(III) has been suggested to be through the generation
21 of superoxide ions, and the major oxidant species might be hydroxyl radicals ($\cdot\text{OH}$) (Sharma and Sohn,
22 2009). Recently, nano-TiO₂ was shown to mineralize methylated arsenic and to adsorb methylated
23 arsenic (Xu et al., 2007; Xu et al., 2008). Both dimethylarsinic acid [DMA(V)] and monomethylarsonic
24 acid [MMA(V)] were readily mineralized to As(V) by transforming the methyl group into organic
25 compounds such as methanol, formaldehyde, and formic acid. Dimethylarsinic acid was
26 photocatalytically oxidized into MMA(V), which was subsequently oxidized into As(V). Hydroxyl
27 radicals could be the primary oxidant (Xu et al., 2007; Xu et al., 2008).

28 The mechanism of arsenic adsorption onto TiO₂ surfaces was through the formation of bidentate
29 inner sphere complexes for As(V), As(III), and MMA(V), and forming monodentate inner sphere
30 complexes for DMA(V) (Jing et al., 2004; Jing et al., 2005a; Jing et al., 2005b; Pena et al., 2006). In
31 ground water containing As(III), As(V), MMA(V), and DMA(V), nano-TiO₂ adsorbs As(III) and As(V)
32 most, followed by MMA(V), but almost no DMA(V) (Jing et al., 2009). The difference in competitive

1 adsorption could be due to lower stability of the monodentate surface structure formed between TiO₂ and
2 DMA(V) than that of the bidentate structure formed between TiO₂ and other arsenicals.

3 Photocatalytic oxidation is also the mechanism for TiO₂ degradation of organic pollutants in waste
4 water. Photocatalytic degradation is based on the formation of radicals (hydroxyl radicals, superoxide
5 radical anions [O₂⁻], and hydroperoxyl radicals [-OOH]), which serve as oxidizing species in the
6 photocatalytic oxidation process (Lu et al., 2009). Hydroxyl radicals, the most powerful oxidants TiO₂
7 produces in the photocatalysis, can act on organic contaminants present at or near the surface of TiO₂
8 (Bianco Prevot et al., 1999).

9 One generally accepted mechanism of nano-TiO₂ antimicrobial property is the generation of
10 reactive oxygen species (ROS), which can cause cell wall or cell membrane damage (Kühn et al., 2003;
11 Neal, 2008), such as lipid peroxidation (Maness et al., 1999). Although UV illumination increases
12 photocatalytic nano-TiO₂ toxicity to bacteria and fungi, photocatalytic nano-TiO₂ is also toxic in the dark
13 (Adams et al., 2006; Coleman et al., 2005). Because TiO₂ generates ROS (mainly highly reactive
14 hydroxyl radicals) in the presence of UV and oxygen (Reeves et al., 2008), mechanisms other than
15 oxidative stress might also contribute to nano-TiO₂ toxicity in the dark (and possibly also under UV), as
16 suggested by a recent study indicating that anatase nano-TiO₂ can generate carbon-centered free radicals
17 in the dark in the presence of dissolved oxygen (Fenoglio et al., 2009).

1.5.2. Sunscreen

18 Nano-TiO₂ formulations of sunscreen have proven popular because they appear transparent on the
19 skin; formulations using conventional TiO₂ or other inorganics such as zinc oxide (ZnO) (Schlossman et
20 al., 2006) create a milky white appearance. Nano-TiO₂ serves as a sunscreen in two ways, by absorption
21 and scattering, depending on the wavelength of UV light. UV-B wavelengths are in the range of 290–320
22 nm, and are primarily absorbed by nano-TiO₂; UV-A wavelengths are in the range of 320–400 nm, and are
23 primarily scattered by nano-TiO₂ (Shao and Schlossman, 1999). Optimal scattering is thought to occur
24 when the diameter of the particles is approximately half the wavelength of the light to be scattered
25 (Fairhurst and Mitchnick, 1997; Klaessig, 2009); also see Appendix A for more information on how nano-
26 TiO₂ particle size relates to UV-A and UV-B protection). Information on chemical and other properties of
27 topical sunscreens containing nano-TiO₂ can be found in Appendix A.

28 Conventional TiO₂ absorbs and scatters UV radiation, making it an effective active ingredient in
29 sunscreens. Like ZnO, TiO₂ is a “physical blocker” of UV radiation, as opposed to many chemically
30 active ingredients that serve as “chemical filters,” such as avobenzone and benzophenone, which in some
31 individuals can cause adverse skin reactions, including blisters, itching, and rash (U.S. EPA, 2006d).

1 Thus, sunscreens containing physical blockers have long been an attractive option to those with sensitive
2 skin. Apart from this niche market, the use of TiO₂ in sunscreen was historically limited because of
3 aesthetic considerations. Because conventional TiO₂ scatters visible light, it remains visible as a white
4 film when applied on skin. With the advance of technology to produce transparent nanoscale TiO₂
5 particles, which scatter very little visible light and therefore appear transparent when applied on skin,
6 nano-TiO₂ has entered the mainstream as an active ingredient in sunscreens and has also been added to
7 numerous other cosmetic products to provide UV protection. With exposure to UV radiation
8 (wavelengths less than ~ 400 nm), pure anatase nano-TiO₂ is photocatalytic. In sunscreen, however,
9 photocatalysis is an undesirable property that can be addressed by applying surface treatments to the
10 crystals, selecting a less photoreactive form (rutile), or adding antioxidant ingredients to the formula.

11 The maximum concentration of TiO₂ in sunscreen that the U.S. Food and Drug Administration
12 (FDA) allows is 25% (FDA, 1999), but this limit does not distinguish between conventional and nano-
13 scale TiO₂, between anatase and rutile, or between coated and uncoated particles. The concentrations
14 actually used, according to product labels, typically range from 2% to 15% (see Table A-1, Appendix A).
15 Europe, Australia, Canada, and South Korea also have approved the use of TiO₂ as a UV filter in
16 sunscreen with a maximum concentration of 25%. Japan does not regulate TiO₂ as a UV filter in
17 sunscreen (Oxonica, 2005; Risk & Policy Analysts Limited, 2004; Steinberg, 2007).

18 Some TiO₂-bearing sunscreens are explicitly labeled as containing nanoparticles. Others are
19 labeled as containing “micronized” TiO₂, a grade commonly used in cosmetics. “Micronized” implies a
20 particle size of about 1 micron (or micrometer, which is one order of magnitude larger than 100 nm), but
21 how precisely manufacturers use the term is unclear. Sometimes “micronized” is taken to imply a nano
22 size range (e.g., Shao and Schlossman, 1999), and sometimes it is considered distinct from nano (e.g.,
23 Environmental Working Group, 2008). In the latter case, TiO₂ with a mean particle size of several
24 micrometers is still very likely to include a significant fraction of particles in the nano size range. Even
25 sunscreens using pigment-grade TiO₂ likely contain a proportion of nano-sized particles. When
26 Consumer Reports tested seven leading national sunscreens labeled as containing ZnO or TiO₂ or both,
27 but with no indication on the container regarding the presence of nanoparticles, they found nanoparticles
28 in all seven products (Anonymous, 2007; La Farge, 2007). (They also confirmed the presence of
29 nanoparticles in an eighth brand labeled as containing nanoparticles.) No information was available,
30 however, on the quantities or sizes of the nanoparticles detected in any of these sunscreens (La Farge,
31 2007). Due to concerns over consumer acceptance of nanotechnology, some nano-TiO₂ sunscreens might
32 simply be labeled as containing “titanium dioxide.”

1.6. Analytical Methods

1 Sensitive and accurate analytical methods for nanomaterials are critical tools for nanomaterial risk
2 assessment, because measurement and characterization of nanomaterials, alone and in various media, are
3 required for properly assessing exposure, conducting toxicological studies, estimating dose-response
4 relationships, and understanding the behavior and effects of nanomaterials.

5 Section 1.4 addressed the aspects of characterization generally needed for nanomaterials,
6 particularly nano-TiO₂. This section provides a brief review of analytical methods that could be suitable
7 for nano-TiO₂, with a focus on currently available methods. Because nano-TiO₂ is not radio-labeled and
8 does not fluoresce, analytical methods based on these two attributes are not relevant. Additionally, the
9 importance of chemical analysis of nanomaterials is acknowledged (such as for identifying their
10 molecular components and for characterizing certain surface properties), but these methods also are not
11 discussed in this section. Some of the chemical analysis methods suitable for nanomaterials are discussed
12 in (Powers et al., 2006; U.S. EPA, 2008c). For detailed comparison of various methods, readers are
13 referred to review articles by Maynard and Aitken (2007), Powers et al. (2006; 2007), and Domingos et
14 al. (2009b).

1.6.1. Methods for Laboratory Research

15 The physicochemical properties of nano-TiO₂ can change over time (Kolář et al., 2006) and in
16 various milieus; therefore, the characteristics of engineered nanomaterials at the point of production could
17 be vastly different after transport, storage, and preparation for testing. Nanomaterials used in
18 toxicological testing ideally would be characterized by analyzing the raw material (as received from the
19 manufacturer or supplier); nanomaterials in the testing media for the duration of the experiment; and
20 nanomaterials (and possibly degraded products or biotransformed products) in the biological samples
21 being tested, such as in urine, organs, and cells.

22 The equipment and methods for measuring nanomaterials in the laboratory are numerous and are
23 evolving. In addition to methods that can be used for characterizing nanomaterials in aerosols and liquids
24 (including biological fluids) (Table 1-3) (Maynard and Aitken, 2007; Nanosafe, 2008b; Powers et al.,
25 2006; Powers et al., 2007) and methods specific for radio-labeled or fluorescent nanomaterials, the
26 following methods have been used on biological samples: transmission electron microscopy (TEM),
27 electron-dispersive X-ray analysis (EDS), and inductively coupled plasma mass spectroscopy (ICP-MS)
28 for presence and location; dynamic light scattering (DLS) in conjunction with TEM for size (both core
29 and shell); high resolution transmission electron microscopy (HRTEM) for crystalline structure;

1 inductively coupled plasma atomic emission spectroscopy (ICP-AES) for elemental composition and
 2 quantitative nanomaterial uptake; video-enhanced differential interference contrast (VEDIC) microscopy
 3 for uptake and localization (Marquis et al., 2009); and scanning probe microscopy (SPM) for size and
 4 three-dimensional images (Gwinn, accepted for publication). ICP, X-ray diffraction (XRD), and nuclear
 5 magnetic resonance (NMR) can be used to determine chemical composition (Gwinn, accepted for
 6 publication). The combination of flow field flow fraction (FIFFF) and ICP-AES has been used to detect
 7 nano-TiO₂ in the tested commercial sunscreen, with information on mass-size distribution and Ti content
 8 of extracted nano-TiO₂ from sunscreen.

Table 1-3. Analytical methods for characterizing nanomaterials in aerosol and in liquid.

Metric	Method	Aerosol	Liquid
Number	Condensation particle counter (CPC)	Yes	-
	Scanning mobility particle sizer (SMPS)	Yes	-
	Electrical low pressure impactor (ELPI)	Yes	-
	Optical particle counter (OPT)	Yes	-
	Electron microscopy (EM)	Yes	-
Surface area	Scanning mobility particle sizer (SMPS)	Yes	-
	Electrical low pressure impactor (ELPI)	Yes	-
	SMPS and ELPI used in parallel	Yes	-
	Diffusion charger	Yes	-
Mass	Size selective personal sampler	Yes	-
	Size selective static sampler	Yes	-
	Tapered element oscillating microbalance (TEOM®)	Yes	-
	Scanning mobility particle sizer (SMPS)	Yes	-
	Electrical low pressure impactor (ELPI)	Yes	-
Size	Dynamic light scattering (DLS)	Maybe	Yes
	Centrifugal sedimentation	No	Yes
	Laser diffraction/static light scattering	Yes	Yes
	Low pressure impactor and electrical low pressure impactor (ELPI)	Yes	No
	Scanning/differential mobility analysis	Yes	No
	Field flow fractionation (FFF)	No	Yes
	Size exclusion chromatography (SEC)	No	Yes
	Acoustic techniques	No	Yes
	Electron microscopy (EM)	No	Possible with cryo-techniques
	Scanning probe microscopy (SPM)		Yes
	Time of flight mass spectroscopy	Yes	No
	Atomic force microscopy (AFM)	No	Maybe
	Specific surface area (Brunauer, Emmett, Teller [BET], titration, diffusion charging)	Yes	Titration techniques only

Source: Modified with permission from Maynard and Aitken (2007), Powers et al. (2006), Powers et al. (2007), and data from Nanosafe (2008a).

1.6.2. Methods and Instrumentation to Assess Environmental Occurrence

1 Detecting nanoparticles in the environment can be difficult because available analytical methods
2 often are not sensitive enough for current environmentally relevant concentrations and cannot distinguish
3 natural materials in the nanoscale size range from manufactured nanomaterials (Domingos et al., 2009b;
4 Englert, 2007; Simonet and Valcárcel, 2009). Also, many analytical methods require sample treatment
5 and extraction (Englert, 2007), which may include solvent evaporation, and consequently could cause
6 nanoparticle aggregation and salt precipitation (Simonet and Valcárcel, 2009). Detecting nanoparticles in
7 water or soil is further complicated by the heterogeneous nature of the samples. Ideally such
8 measurements would be done in situ to avoid changes in nanoparticles (such as agglomeration) due to
9 different conditions in the immediate milieu, but portable equipment sufficiently sensitive to detect
10 nanoparticles at environmentally relevant concentrations has not yet been developed (Simonet and
11 Valcárcel, 2009).

12 Analytical methods that are currently available for nanomaterials in soil, sediment and ground
13 water were summarized in a recent U.S. Environmental Protection Agency (U.S. EPA) State of Science
14 Review (U.S. EPA, 2008c) (Table 1-4). Methods can be coupled to enable detection of more than one
15 parameter at a time. For example, FIFFF can be coupled with ICP-MS for both size and chemical
16 analysis.

17 In a study comparing six analytical methods for determining nanomaterial sizes [TEM, atomic
18 force microscopy (AFM), DLS, fluorescence correlation spectroscopy, nanoparticle tracking analysis, and
19 flow field flow traction], Domingos et al. (2009b) concluded that the two most commonly used
20 techniques reported in the literature [electron microscopy (EM) on air-dried samples and DLS] were also
21 the two techniques that appear to be most prone to artifacts. Using multiple analytical techniques or
22 multiple preparation techniques, or both, has been recommended (Domingos et al., 2009b; Englert, 2007).

Table 1-4. Analytical methods for nanomaterials in soil, sediment, and ground water for size fraction and distribution, surface area, and phase and structure.

Metric	Analytical method	Sample type
Size fractionation	Centrifugation	Aquatic colloids and particles extracted from soil and sediment samples. Nanoparticles must be in solution.
	Ultrafiltration – direct-flow ultrafiltration or tangential-flow ultrafiltration (TFF)	
	Field flow fractionation (FFF)	
	Capillary electrophoresis (CE)	
	Size exclusion chromatography (SEC)	
Size distribution	Transmission electron microscopy (TEM)	
	Scanning electron microscopy (SEM)	
	Scanning probe microscopy (SPM)	
	Dynamic light scattering (DLS)	
	Laser-induced breakdown detection (LIBD)	
	Small- and wide-angle X-ray scattering (SAXS/WAXS)	
Surface area	Brunauer, Emmett, Teller method (BET)	Only nanomaterials with a regular or pseudo-regular geometry and without significant porosity
	Calculation from transmission electron microscopy (TEM) (length and width) and atomic force microscopy (AFM) (height) measurements, and particle nanocrystalline geometrics	
Phase and structure	Electron diffraction	
	X-ray diffraction (XRD)	
	X-ray absorption spectroscopy (XAS)	
	Raman spectroscopy	

Source: Data from U.S. EPA (2008c).

1.6.3. Methods and Instrumentation to Assess Workplace Exposure

1 Workplace exposure thus far has focused on measuring nanoparticles in the air. Instruments that
 2 can be used for aerosol sampling are available, but most instruments for aerosol sampling are designed for
 3 laboratory use (Nanosafe, 2008b) and lack one or more the following desired attributes: portability, ease
 4 of use, capacity to distinguish nanoparticles from non-nanoparticles, different size bins in the 1- to
 5 100-nm range, or ability to sample personal breathing zones (Ostraat, in press).

6 Several governmental and non-governmental organizations have begun addressing the need for
 7 equipment and methods for monitoring nanomaterials, particularly nanoaerosols, in the workplace. For
 8 example, NIOSH recently published a document titled Approach to Safe Nanotechnology – Managing the
 9 Health and Safety Concerns Associated with Engineered Nanomaterials (NIOSH, 2009), in which
 10 sampling and monitoring methods and equipment are discussed. Nanoparticle Occupational Safety and

1 Health Consortium (NOSH), an industry-led consortium of participants from academia and governmental
2 and non-governmental organizations, is helping to define best practices for working safely with
3 engineered nanoparticles (NOSH, 2008; Ostraat et al., 2008). The NOSH Consortium has developed
4 portable air monitoring methods intended for daily monitoring in nanoparticle research and development
5 or in manufacturing settings.

6 Maynard and Aitken (2007) summarized available devices and approaches for evaluating numbers,
7 surface areas, and mass concentrations of nanoparticles for monitoring aerosol exposure. In 2008, the
8 NanoSafe2 project, a European Community-sponsored project for safe production and use of
9 nanomaterials, released a report that highlighted findings in measurement methodologies for nanoparticle
10 detection and measurement with various types of on-line and off-line monitoring instruments (Nanosafe,
11 2008b). The report provided examples of new nanoaerosol measurement equipment that is easy to
12 transport and use. No commercially available equipment, however, is currently available for long term
13 monitoring. The report also recommended that monitoring at workplaces include not only personal
14 sampling and measurements inside the facility, but also measurements of nanomaterials in drains and in
15 the exhausted air to help ensure protection of the environment.

16 Finally, several companies are developing or have developed air monitoring devices for
17 nanoparticle detection. The parameters that each device measures vary (Bennett, 2005; TRS
18 Environmental, 2009; van den Brink, 2008).

Questions about Characterizing Nanoscale Titanium Dioxide

- 1-1. To evaluate nano-TiO₂ (in these or other applications) or to compare products containing nano-TiO₂, is further standardization or refinement of terminology needed? If so, is such an effort underway and/or what terminology is most important to standardize?
- 1-2. Have the properties of nano-TiO₂ in different applications been adequately characterized? If not, is the problem that methods are not generally available or that existing methods have not been widely applied? If new methods are needed, what properties should they measure?
- 1-3. Which coatings, dopings, carriers, dispersants, and emulsion types are most prevalent in different applications of nano-TiO₂?
- 1-4. What are the potential implications (e.g., in terms of physical and chemical properties) of differences in the composition and mineralogy of different forms of nano-TiO₂ (e.g., rutile and anatase)?
- 1-5. How do coatings applied for different purposes (e.g., to disperse particles or to decrease photocatalysis) interact or affect other properties of nano-TiO₂?
- 1-6. What factors determine whether and to what extent aggregation or agglomeration of nano-TiO₂ occurs?
- 1-7. Are data available that indicate the level of agglomeration/aggregation/dispersion of nano-TiO₂ in specific products? If so, what do the data show?
- 1-8. Is there a difference between the opacity of nano-TiO₂ aggregates and conventional TiO₂ particles of nominally similar size (e.g., because of light passing through pores in aggregates)? If so, what are the implications of such a difference?
- 1-9. Regarding the properties of aggregates and agglomerates and proper characterization of particle size, what insight is available from study of other nanoparticles?
- 1-10. What existing or emerging analytical techniques might be relevant or useful for material characterization? For example, could field flow fractionation (FFF) be used for characterization of particle size and elemental composition?
- 1-11. Do surface area measurements in air (e.g., BET analysis) correlate to surface area in an aqueous environment? If so, what is the extent of their accuracy and precision?

Chapter 2. Life Cycle Stages

1 This chapter discusses the life cycle of nanoscale titanium dioxide (nano-TiO₂) as either a water
2 treatment agent or an ingredient in topical sunscreen. Each stage in the life cycles of the respective
3 applications is considered from the standpoint of potential releases to the environment.

2.1. Feedstocks

4 Two ores, ilmenite (FeTiO₃) and rutile (TiO₂), predominate as feedstock materials for TiO₂
5 production (nano and otherwise) (Haridasan et al., 2008). Ilmenite and rutile are often found together, but
6 ilmenite is found and mined in far greater quantities (at a ratio of more than 10:1 by weight) (Gambogi,
7 2008) and supplies ~ 90% of titanium minerals worldwide. For rutile-based manufacturing processes, the
8 most common manufacturing pathway for producing TiO₂ of all kinds is via the chloride route using
9 titanium tetrachloride (TiCl₄), a liquid that accounts for about 60% of current manufacturing (Hext et al.,
10 2005). Creating synthetic rutile from ilmenite is often more economical than eliminating impurities from
11 natural rutile.

12 World ilmenite production in 2007 was around 5.6 million metric tons, and world rutile production
13 was around 0.5 million metric tons. The nations that produce the greatest quantities of ilmenite are
14 Australia, South Africa, Canada, China, Norway, India, the United States, and Ukraine. Significant
15 producers of rutile include Australia, Ukraine, South Africa, India, and the United States (Gambogi,
16 2008). An estimated 1 billion tons of TiO₂ could be produced from existing world ilmenite resources,
17 with another 230 million tons from rutile deposits (Mineral Information Institute, 2009).

18 In the United States, ilmenite and rutile are extracted by surface mining or reprocessing of mine
19 tailings at two sites in Florida and Virginia. Combined ilmenite and rutile production is approximately
20 0.3 million metric tons. Mine and mill employment at these sites was estimated at 229 persons in 2007,
21 down from 344 in 2003 (Gambogi, 2008).

22 Low levels of radioactive materials are present in ilmenite and natural rutile (Collier et al., 2001;
23 Haridasan et al., 2008). A study in India found that those who work with ilmenite could be exposed to an
24 annual dose of 1 millisievert (mSv) of gamma radiation and another 0.7 mSv of radioactivity via particle
25 inhalation, mostly due to thorium. Thorium radioactivity in ilmenite was about 60% of the regulatory
26 exemption limit established in the International Atomic Energy Agency (IAEA) Basic Safety Standards.
27 Levels of radioactivity in natural rutile, ilmenite-derived synthetic rutile, and TiO₂ pigment (produced by

1 the chloride route, particle size not specified) are lower than ilmenite, while levels of radioactivity (from
2 radium as well as thorium) in solid wastes and liquid effluent are elevated compared with ilmenite
3 (Haridasan et al., 2008).

4 Another common feedstock is titanium sulfate solution, which can be hydrolyzed to form TiO_2 .
5 The sulfate method begins with ground ilmenite or titanium slag.

Questions about Feedstocks

2.1-1. Are certain feedstocks more relevant to producing nano- TiO_2 specifically for water treatment or sunscreen applications?

2.1-2. What contaminants, nanoscale and larger, might be released, and in what quantities, in relation to the procurement and processing of feedstocks for nano- TiO_2 ?

2.2. Manufacturing

6 Around 2005, annual global production of nano- TiO_2 was estimated at 2000 metric tons, with an
7 overall market value of \$70 million (Dransfield, 2005; Osterwalder et al., 2006). About 65% of
8 production was thought to have gone to “personal care” applications such as topical sunscreens and
9 cosmetics, with the remainder used in industrial applications such as plastics, catalysts, and ceramics.
10 Commercial production of nano- TiO_2 for years 2006–2010 has been estimated at 5000 metric tons/year,
11 and more than 10,000 metric tons/year for years 2011–2014 (United Nations Environment Programme,
12 2007). Recently, Robichaud et al. (2009) estimated current and future worldwide production levels of
13 nano- TiO_2 at considerably higher levels, with an upper estimate of approximately 2.5 million metric tons
14 by 2025. Thus far, nano- TiO_2 production has represented a small fraction of overall TiO_2 production,
15 which commanded a market of 4.5 million metric tons and \$9 billion (Dransfield, 2005; Osterwalder et
16 al., 2006).

17 Manufacturers and researchers report nano- TiO_2 synthesis by various techniques, including
18 chemical vapor deposition (CVD), flame hydrolysis, sol-gel, calcination, aerosol pyrolysis, and colloidal
19 synthesis (Wahi et al., 2006). CVD, commonly used for production of both conventional and nanoscale
20 TiO_2 , involves the conversion of a volatile compound to a nonvolatile solid that deposits on a substrate
21 (Li et al., 2003; Nagaveni et al., 2004). A variety of techniques are used to generate the vapor and collect

1 the particles, including plasma, high temperatures, pressure, and injection, among others (Aitken et al.,
2 2004).

3 According to one industrial manufacturer of nanoscale titania, flame hydrolysis can generate high-
4 purity nano-TiO₂ using TiCl₄ as a feedstock (Degussa, 2004). Like CVD, flame hydrolysis can be used to
5 deposit a thin film on a surface, a process known as flame hydrolysis deposition (FHD). In FHD, an inert
6 gas carries TiCl₄ into a flame that produces hydrogen chloride and the metal oxide (Tok et al., 2009).
7 Flame hydrolysis is used for manufacturing P25 and yields agglomerated particles with a mean diameter
8 of about 3.6 μm, with the smallest 4% of particles having an average diameter of 160 nm (Klaessig,
9 2006).

10 Anticipated by-products of the chloride method of TiO₂ production include those resulting from
11 chlorine contamination of the TiO₂ (from the TiCl₄ precursor). Warheit et al. (2007b) have suggested that
12 solutions of P25 in water are acidic (pH = 3.28) because of chloride ions on the particle surface. Other
13 information, however, indicates that a steam washing step during the manufacturing process removes
14 hydrochloric acid adsorbed on the surface of P25 (Vormberg, 2004).

15 When photocatalytic or other applications require smaller particles, additional post-manufacturing
16 processes that are sufficiently energetic to break apart the aggregates/agglomerates might be used, with
17 surfactants or solvents used to help keep the particles apart after separation (Hewitt, 1996; Porter et al.,
18 2008). Also, nanoscale particles might be sonicated to increase dispersion (Bihari et al., 2008).

19 Another method of TiO₂ production, which could be the preferred method of nano-TiO₂ production
20 in commercial settings, is the sulfate process (Medley, 2008). Details on this and other processes used in
21 producing nano-TiO₂ can be found in Appendix B.

2.2.1. Water Treatment

22 No information was found on processes specific to preparing or formulating nano-TiO₂ for use in
23 drinking water treatment. P25 is used in a commercial water treatment system (Photo-Cat from Purifics)
24 that can be used for drinking water, ground water, and waste water treatment (NSF International, 2009;
25 Pichat, 2003; Purifics Solutions, 2008);. For this treatment system, P25 is neither specially prepared nor
26 coated (Powell, pers. comm., 2009).

2.2.2. Sunscreen

27 Unlike for water treatment agents, information on the manufacture of topical sunscreens that
28 incorporate nano-TiO₂ is relatively abundant. Although specific details of manufacturing protocols are

1 typically proprietary, general information on manufacturing processes and materials is available. The
2 choice of nano-TiO₂ crystalline form is a key issue in manufacturing sunscreens because forms differ in
3 photostability. In particular, rutile is much more photostable than anatase (Chaudhuri and Majewski,
4 1998; Maynard, 2008). Although less photostable, anatase appears to be in common use: Barker and
5 Branch studied five TiO₂ sunscreens purchased over the counter and found that one was pure rutile, while
6 the other four were anatase/rutile mixes in which anatase predominated (Barker and Branch, 2008).

7 To increase nano-TiO₂ photostability, the particles are commonly given a surface coating such as
8 silica, alumina, simethicone, or a variety of other compounds (see Appendix B for more information on
9 coatings). Another technique for increasing photostability is “doping” nano-TiO₂ particles by embedding
10 within them minute amounts of metals such as manganese, vanadium, chromium, and iron (Park et al.,
11 2006).

12 Another important consideration in the manufacture of most topical sunscreens is the use of a
13 liquid medium, or dispersion, to ensure that nano-TiO₂ will be distributed evenly, thereby reducing
14 aggregation and agglomeration (which could negatively impact ultraviolet (UV) scattering performance
15 and transparency by increasing the effective particle size). Sunscreen manufacturers can purchase nano-
16 TiO₂ powder and formulate their own dispersion, or they can purchase ready-made “predispersions.”

17 Surface coatings influence the interaction of nano-TiO₂ with the dispersion medium, which can be
18 water-based (aqueous), oil-based, or silicone-based. These and many other factors figure into the
19 manufacture of sunscreens, including pH; emulsifiers; emollients; other physical UV blockers (e.g., ZnO,
20 which can also be micronized); chemical UV filters; and various inert ingredients to achieve the desired
21 viscosity/liquidity, spray-ability, color/transparency, water resistance, and spreadability. More detailed
22 information on manufacturing processes is presented in Appendix B.

Questions about Manufacturing

- | |
|---|
| <p>2.2-1. How do various manufacturing processes for nano-TiO₂ affect their physicochemical properties?</p> <p>2.2-2. How are manufacturing processes likely to evolve with increasing demand for nano-TiO₂?</p> <p>2.2-3. Are certain manufacturing processes used specifically for nano-TiO₂ as a water treatment agent or as topical sunscreen?</p> <p>2.2-4. What waste products or other by-products, both nanoscale and larger, might be released, and in what quantities, for nano-TiO₂ manufacturing processes?</p> <p>2.2-5. Where is nano-TiO₂ manufactured? What is the potential for general population exposure to nano-TiO₂ in these areas?</p> |
|---|

2.3. Distribution and Storage

1 Limited information about nano-TiO₂ distribution and storage was located. P25 is shipped as a
2 powder in 10-kilogram (kg) “multilayer ventilated paper bags, equipped with an additional polyethylene
3 lining when required” (Degussa, 2007). Another brand of photocatalytic nano-TiO₂ (KRONOS vlp 7000,
4 7001, and 7500) is also shipped in 10-kg paper bags (KRONOS International, 2006). Nano-TiO₂ powders
5 from Sigma, on the other hand, are shipped in amber glass bottles enclosed in foil or plastic bags, which
6 are shrink-wrapped before being placed in cardboard boxes with shipping cushion peanuts. P25
7 presumably could be stored as a powder in a chemical storage facility in the original 10-kg shipping bags.
8 Degussa recommends storing it in closed containers under dry conditions (Degussa, 2007). Releases
9 could occur if bags were damaged during shipping or storage. Standard good management practices
10 would be expected to reduce the occurrence of accidental releases, but to what extent is unknown.

11 As a dispersion, nano-TiO₂ is shipped in pails, drums, or totes (Klaessig, 2008). Sigma ships its
12 nano-TiO₂ dispersion in essentially the same way nano-TiO₂ powders are shipped. Dispersion-formulated
13 nano-TiO₂ presumably would require protection from freezing. Depending on where accidental releases
14 of such dispersions occurred, nano-TiO₂ could be released into water or soil during shipment or
15 discharged into industrial or municipal waste water treatment systems during storage.

2.3.1. Water Treatment

16 No information pertaining specifically to the distribution and storage of nano-TiO₂ water treatment
17 agents was located.

2.3.2. Sunscreen

18 Topical sunscreen products are generally packaged in retail-sized bottles and shipped in larger
19 containers to wholesalers, retailers, and direct marketers. Little information is available on methods of
20 shipping or storage. Consumers generally handle only retail-sized packages.

21 Industry data from the 1990s, although perhaps out of date, sheds light on the distribution chain of
22 sunscreens. Sales in supermarkets, drugstores, and mass merchandise outlets accounted for about two-
23 thirds of the total U.S. sun-care retail sales in 1992–1993, according to Davis (1993). The remaining one-
24 third was attributed to sales in department stores and other “prestige” stores. Sun-care products are also
25 sold by direct marketers (e.g., Avon, Amway, Mary Kay), discount stores, swimwear stores, and small
26 variety stores (e.g., those near beaches and ski slopes) (Davis, 1993).

27 At any point in the distribution-to-storage chain, accidental releases could occur. For example, a
28 shipping accident, a dropped palette, or crushed retail-size container(s) could lead to releases.

Questions about Distribution and Storage

- 2.3-1. How is nano-TiO₂ shipped (i.e., what are the relative frequencies for shipments in bulk, paper bags, or drums, or by truck or rail)? How far is it shipped? In what quantities?
- 2.3-2. Are data available or can they be collected or estimated for accident rates and routine product releases associated with various modes of shipping and storage? To what degree could best practices reduce such occurrences?
- 2.3-3. How is nano-TiO₂ stored (e.g., in warehouses, sunscreen manufacturing plants, and water treatment facilities)?
- 2.3-4. Does the use of “ventilated paper bags” increase the possibility of accidental spillage during shipment and storage? Are any guidelines available on whether protective packaging (e.g., additional polyethylene lining) is warranted?
- 2.3-5. Could vermin breach storage containers and contribute to environmental releases or become part of an environmental exposure pathway?
- 2.3-6. Would prolonged storage in adverse or less than ideal climates (e.g., cold or humid environments) alter nano-TiO₂ characteristics and behavior?
- 2.3-7. How much nano-TiO₂ could be released under various routine and accidental scenarios of distribution and storage?

2.4. Use

2.4.1. Water Treatment

1 Nano-TiO₂ could be used in various ways to treat drinking water, as discussed in Section 1.5.1.
2 This discussion, however, assumes that nano-TiO₂ would be used in water treatment facilities only for
3 removing arsenic.

4 Roughly 54,000 community water systems in the United States serve more than 95% of the
5 population (U.S. EPA, 2006c). Most of these systems apply some form of treatment to remove or
6 neutralize chemical or microbial contaminants. Those that do not apply treatment serve less than 5% of
7 the U.S. population; these systems are generally small or medium-sized (i.e., serving no more than 10,000
8 people) and rely on ground water (U.S. EPA, 2002). Public water systems are required to keep arsenic
9 concentrations in delivered water at or below a maximum contaminant level (MCL) of 0.01 milligrams
10 per liter (mg/L) (U.S. EPA, 2006a). About 5% of community water systems in the United States (i.e.,
11 about 3,000 systems serving 11 million people) have taken some action to be in compliance with the
12 arsenic MCL (U.S. EPA, 2007a). Likewise, about 5% of 20,000 non-transient non-community water
13 systems that serve at least 25 of the same people more than 6 months of the year, such as schools,

1 churches, nursing homes, and factories (i.e., about 1,100 systems serving 2 million people) have also
2 taken some action to comply with the arsenic MCL (U.S. EPA, 2007a). Altogether, about 13 million
3 people use water that is treated to remove arsenic. Although it is unknown to what extent nano-TiO₂
4 might be used in any of these systems in the future, these numbers provide perspective on its potential
5 usage for drinking water treatment.

6 Depending on the type of water treatment system, nano-TiO₂ might be used as powder (e.g., in a
7 slurry) or fixed on a supporting material. Each approach has its potential advantages and disadvantages.
8 Powdered nano-TiO₂ has a large surface area and offers highly efficient photocatalytic oxidation, but a
9 means to filter or recycle all of the photocatalyst is required (Dionysiou, pers. comm., 2009; Pichat,
10 2003). This suggests the possibility that some amount of nano-TiO₂ suspended in water might pass
11 through filters, including microfilters. Also, if nano-TiO₂ builds up on the filter matrix (i.e., if it is not
12 removed by filter backwashing and hydraulic cleaning of sand), it could saturate the filtration medium,
13 and small quantities might be released with filtered water into subsequent steps of the treatment sequence.
14 Fixed nano-TiO₂ has a smaller surface area and thus is less efficient. Although the attachment to the
15 supporting material should allow no leaching, a fixed photocatalyst might not require filters or recycling
16 systems to remove nano-TiO₂ from the final product (Dionysiou, pers. comm., 2009).

17 Zhang et al. (2008) investigated the removal of nano-TiO₂ in a simulated conventional water
18 treatment procedure, which included coagulation, flocculation, sedimentation, filtration, and disinfection.
19 Two types of nano-TiO₂ (crystal form unspecified, primary particle sizes of 15 and 40 nm, and aggregates
20 200 and 500 nm, respectively) in 2-L jars were subjected to the treatment procedure. Adding magnesium
21 chloride (MgCl₂) or alum (Al₂(SO₄)₃·16H₂O), followed by coagulation, flocculation, and sedimentation,
22 still left more than 20% of an initial 10-mg/L concentration of nano-TiO₂ in the settled water.
23 Furthermore, the removal efficiency was lower in tap water than in buffered nanopure water (pH 5.6) due
24 to the presence of organic matter in the tap water. Membrane filtration with a pore size of 0.45 μm (450
25 nm) after sedimentation removed nano-TiO₂ aggregates larger than 500 nm, leaving only 1-8% of the
26 initial TiO₂ in the treated water. Although most, but not all, of the nano-TiO₂ in the initial water was
27 removed, this level of filtration is not typical in water treatment plants (Flummer, 2008; Kline, 2008), nor
28 is it available in most whole-house filtration systems (Johnson, 2005).

29 At least two commercially available water treatment systems can employ nano-TiO₂, although to
30 date they are not known to be routinely used in this manner. One uses nano-TiO₂ in a fixed membrane
31 and the other uses nano-TiO₂ in a slurry. A system from Matrix Photocatalytic Inc. uses a tube covered
32 with fiberglass mesh in which nano-TiO₂ is embedded; the tube contains water that circulates and
33 ultraviolet (UV) lamps illuminate the outside (Dionysiou, pers. comm., 2009; Pichat, 2003). In the Photo-
34 Cat system by Purifics, nano-TiO₂ (P25) circulates in a slurry inside a narrow annulus surrounded by a
35 UV lamp (Pichat, 2003). A ceramic membrane filters out nano-TiO₂ (Purifics Solutions, 2008). No

1 empirical data are available on the life expectancy of either system or whether they can release nano-TiO₂
2 into treated water.

3 The Purifics system was pilot-tested for two months in a community drinking water treatment
4 facility (Purifics Solutions, 2008). The ceramic membrane used to filter nano-TiO₂ (particles as small as
5 12 nm) from the finished product was reported to require no servicing or cleaning during the 2-month
6 period because the nano-TiO₂ particles collected in the membrane were removed by bursts of high-
7 pressure air (Pichat, 2003; Purifics Solutions, 2008). Although the purpose of this pilot test was not to
8 remove arsenic, several studies have bench-tested nano-TiO₂ in slurry systems for removal of arsenic from
9 water (Dutta et al., 2004; Ferguson et al., 2005; Lee and Choi, 2002; Li et al., 2003; Meridian Institute,
10 2006). Higher arsenic oxidation rates occurred using a slurry that was continuously stirred (compared to
11 immobilized nano-TiO₂) (Li et al., 2003). In actual use, steps likely would be taken to keep nano-TiO₂
12 dispersed during treatment, which could affect solubility and particle agglomeration. Surface
13 modification could affect dispersion and could also improve the material's photocatalytic properties as
14 described (Ryu and Choi, 2004). Additionally, numerous chemicals can be added for drinking water
15 treatment (NSF International, 2007), any or some combination of which could affect the solubility,
16 particle size, and behavior of the nano-TiO₂.

2.4.2. Sunscreen

17 The estimated use of sunscreen can vary greatly among surveys, but it is clear that its use is
18 significant (Kasparian et al., 2009; Keeney et al., 2009). Four U.S. studies that collected data in the years
19 1995–1999, with 1,000 to more than 10,000 participants in each survey, showed that approximately one in
20 three people said they use sunscreen regularly (Cokkinides et al., 2001; Geller et al., 2002; Santmyire et
21 al., 2001; Weinstock et al., 2000). In three studies, 31–45% of survey respondents said they routinely or
22 often use sunscreen (Cokkinides et al., 2001; Geller et al., 2002; Weinstock et al., 2000). In another
23 study, 30% of respondents said they were very likely to use sunscreen when they were outdoors
24 (Santmyire et al., 2001). More recently, data from the 2005 Health Information National Trends Survey
25 in the United States showed that among a total of 496 Latino participants, 15% reported that they always
26 use sunscreen, 9% reported often use of sunscreen, and 20% reported that they sometimes use sunscreen
27 (Andreeva et al., 2009). In a 2007 survey, the Skin Cancer Foundation and iVillage (2007) found that
28 11% of respondents use sunscreen with a sunburn protection factor (SPF) of 15 or higher “every day” and
29 59% of respondents use sunscreen at least occasionally (up from 39% in a 2003 survey), where SPF is
30 defined by FDA (2009) as a “measure of how much solar energy (UV radiation) is required to produce
31 sunburn on protected skin (i.e., in the presence of sunscreen) relative to the amount of solar energy

1 required to produce sunburn on unprotected skin.” Of those who wear sunscreen, 74% reapply it “at least
2 every 4–6 hours or after swimming or sweating,” and 28% reapply it every two hours, the Skin Cancer
3 Foundation’s recommended rate of reapplication (Skin Cancer Foundation, 2007).

4 While the use of sunscreen may be lower in young adults and adolescents than adults (Kasparian et
5 al., 2009), sunscreen use is likely to be higher in young children. Robinson et al. (2000) surveyed 503
6 people in the summer of 1997, and found that 54% of parents reported that their child always or usually
7 used a sunscreen, but only 27% of parents used sunscreen themselves during the previous weekend. This
8 is consistent with a survey of 254 parents in June–July of 1999 by Weinstein et al. (2001) in Chicago, in
9 which parents reported more frequent use of sunscreen on their children than on themselves.

10 The total amount of sunscreen, and more particularly the total amount of nano-TiO₂ in sunscreen,
11 used in the United States is unknown. Furthermore, the available survey data does not differentiate
12 between sunscreen products with or without nano-TiO₂, although the percentage of sunscreen with nano-
13 TiO₂ is thought to be substantial. In 2006, the Australian Therapeutic Goods Administration (TGA)
14 estimated that 70% of sunscreens containing titanium and 30% of sunscreens containing zinc in Australia
15 were formulated with nanoparticles (TGA, 2006).

16 As noted in Section 2.2, annual global production of nano-TiO₂ was estimated at 2000 metric tons
17 around 2005, with about 65%, or 1300 metric tons, used in “personal care” products such as topical
18 sunscreens and cosmetics (Dransfield, 2005; Osterwalder et al., 2006).

19 A recent report by Barker and Branch (2008) has noted that the surface coatings on nano-TiO₂ in
20 many sunscreens might not be stable or effective. The investigators studied the weathering of paint in
21 contact with sunscreen. Of five nano-TiO₂ sunscreens tested, four released photocatalytically generated
22 hydroxyl radicals that accelerated the weathering of the paint. All four of those sunscreens used an
23 anatase/rutile mix. The one nano-TiO₂ sunscreen formulation that showed no appreciable effect on paint
24 weathering used 100% rutile doped with manganese rather than surface coating (Barker and Branch,
25 2008).

Questions about Use

2.4-1. To what extent is nano-TiO₂ used or could be used for either drinking water or waste water treatment? Are data available (e.g., volume of water currently treated in the United States for arsenic, amount of TiO₂ needed to treat a given volume of water) that would permit an estimate of potential use?

2.4-2. Which water treatment processes use or would use nano-TiO₂ and in what quantities? Would the type of process depend on the size of a treatment facility or the size of the population served, or both?

Questions continued on next page.

Questions about Use

- 2.4-3. What percentage of the nano-TiO₂ would settle out in floc or become part of the filter matrix? What percentage would be released into finished water? Are measurement or monitoring methods adequate to detect such particles?
- 2.4-4. Water distribution systems often have substantial biofilm or corrosion development, despite the implementation of control practices. Would the presence of nano-TiO₂ influence the bacterial biofilm community or the occurrence of corrosion?
- 2.4-5. What is the total quantity of nano-TiO₂ used in topical sunscreen products in the United States and worldwide?
- 2.4-6. What is the maximum quantity and frequency of personal sunscreen use in relation to season, geographic location, demographics, and other variables?
- 2.4-7. How much nano-TiO₂ enters the environment under different scenarios and conditions of sunscreen use (e.g., ambient air and water temperature, swimming, bathing)? Under what conditions would nano-TiO₂ be released from the sunscreen matrix?

2.5. Disposal

2.5.1. Water Treatment

1 Most community water treatment filters, with regular backwashing, have an indefinite life span.
2 Slow sand filters are generally cleaned not by backwashing, but by scraping and replacing the top layer of
3 sand. Scraped sand is normally cleaned hydraulically and stockpiled for later reuse (Cleasby and
4 Logsdon, 1999). This process creates waste water, which might be recycled in the treatment train or
5 discharged (e.g., to a municipal sewer). Eventually, the filter sand or other filter materials would need to
6 be disposed of.

7 After nano-TiO₂ is used in water treatment, a sludge material (floc) containing nano-TiO₂ would
8 likely be created. In one scenario, the sludge might be taken to a landfill. Whether TiO₂ could diffuse
9 (and thus be released) from a solid matrix such as sludge is unknown. Nano-TiO₂ and other contaminants
10 such as residual arsenic could become suspended in leachate and enter ground water, or they could pass
11 through a solid waste facility liner into the subsurface.

12 Under a different scenario, the sludge could be used for land application. In this case, the sludge
13 would undergo some treatment, which is generally required for removing pathogenic organisms and
14 regulated contaminants such as lead and arsenic [titanium is not regulated in biosolids under U.S. EPA's
15 Biosolids Rule, Part 503; see (U.S. EPA, 1994)]. Such treatment might include high temperature or high

1 pH processing (U.S. EPA, 1994). The treated sludge then could be applied to land for agricultural use,
2 reclamation sites, golf courses, public parks, and other areas where nutrient-rich organic matter is useful,
3 including forests, parks, roadsides, and in some cases, residences (U.S. EPA, 1994). Roughly half of
4 treated sewage sludge is applied to land, and less than 1% of all U.S. agricultural land uses treated sewage
5 sludge (U.S. EPA, 2006b).

6 If nano-TiO₂ is present in finished drinking water that reaches the tap, it would eventually enter the
7 ambient environment or be captured by a waste water stream, after which it could enter sewage treatment
8 facilities.

2.5.2. Sunscreen

9 Sunscreen containers likely would be disposed of primarily as municipal solid waste and thus end
10 up in landfills or incinerators. The potential for leaching of nano-TiO₂ from landfill disposal of containers
11 would depend on many factors, including the integrity of liners and leachate collection systems, if
12 present. Incineration of sunscreen containers raises the question of whether nano-TiO₂ could enter the
13 stack and be released to air, or become a trace contaminant in fly or bottom ash.

14 Depending on the packaging, sunscreen containers might be recycled, suggesting the possibility
15 that nano-TiO₂ could be incorporated into recycled materials.

Questions about Disposal

- 2.5-1. How much residual nano-TiO₂ is present in packaging of the primary material or derived products? How is such packaging disposed of?
- 2.5-2. If nano-TiO₂ were to become much more widely used and produced at a much higher volume, would packaging and shipping methods of nano-TiO₂ change? If so, how would such change affect the potential release and exposure during transport, storage, and disposal?
- 2.5-3. In water treatment, how are filter materials and associated waste/waste water containing nano-TiO₂ disposed of or recycled?
- 2.5-4. How are large quantities of sunscreen (e.g., sub-par batches rejected during manufacturing) handled?
- 2.5-5. How much nano-TiO₂ is present in sunscreen containers that are discarded? Are there any circumstances where such discarded product could enter a microenvironment at significant levels?

Chapter 3. Fate and Transport

1 Chapter 3 explores what might happen to nanoscale titanium dioxide (nano-TiO₂) after it is
2 released to the environment at various stages of the product life cycles for water treatment agents or
3 topical sunscreens. Nano-TiO₂ could be released to air, water, or soil and then transported or transformed
4 through chemical or biological processes. The lack of data on the fate and transport of nano-TiO₂ by-
5 products and waste produced during the manufacturing process precludes a comprehensive discussion in
6 this chapter. This chapter does, however, summarize what is known about the environmental pathways
7 and transport and transformation processes of nano-TiO₂ related to the various life-cycle stages described
8 in Chapter 2.

9 Although most studies cited in this chapter consider nano-TiO₂ in aggregate or agglomerate form
10 (as discussed in Chapter 1), whether all constituent primary particles remain in clusters if conditions
11 change is unclear. Disaggregation, for example, can occur at certain pH_{pzc} levels. The pH_{pzc} of a
12 nanoparticle is defined as the pH at the “point of zero charge,” which occurs when the net electric charge
13 at the particle surface is zero. At the pH_{pzc} particles fail to electrostatically repel each other. In laboratory
14 studies, the size range of aggregates and the presence of free nano-TiO₂ particles (ranging from 5 to 50
15 nm in size) were found to be pH-dependent: when the solution pH differed from the pH_{pzc} of the
16 particles, the aggregates tended to be smaller (Dunphy Guzman, pers. comm., 2007; Dunphy Guzman et
17 al., 2006). Sampled aggregates ranged up to 150 nm in size, and contained an estimated 8 to 4,000
18 nanoparticles (Dunphy Guzman et al., 2006). The pH_{pzc} also depends at least in part on the crystallinity
19 of the nano-TiO₂ particles: Finnegan et al. (2007) reported pH_{pzc} values of ~5.9 for rutile and ~6.3 for
20 anatase.

21 The pH_{pzc} depends in part on the crystal form of the nano-TiO₂ particles. Finnegan et al. (2007)
22 reported pH_{pzc} values of ~5.9 for rutile and ~6.3 for anatase. The degree of aggregation generally
23 increases with the presence of salt or increases in ionic strength, minerals, and organic matter in water
24 (Domingos et al., 2009a; French et al., 2009).

25 Despite the presence, and sometimes the predominance, of large particles, several researchers
26 investigating laboratory-synthesized and commercial nano-TiO₂ products have found free particles or
27 aggregates with diameters less than 100 nm in varying amounts, depending on synthesis method,
28 temperature, solution pH, and the presence of buffers (Kormann et al., 1988; Li et al., 2003; Nagaveni et
29 al., 2004; Pena et al., 2006; Ryu and Choi, 2006; Sun et al., 2007; Wahi et al., 2006). Moreover, some
30 preparations are specifically designed to generate dispersed particles (e.g., Seok et al., 2006) to increase

1 the efficacy of nano-TiO₂ as a catalyst, increasing the potential for the presence of disaggregated nano-
2 TiO₂ to occur in the environment. However, no studies of nano-TiO₂ aggregation/disaggregation behavior
3 under “real-world” ambient environmental conditions, irrespective of medium, were located.

3.1. Water

4 Although numerous studies characterize nano-TiO₂ particles in aqueous solution under laboratory
5 conditions, the fate and behavior of the particles in the environment have received less attention. One
6 report indicates that nano-TiO₂ was detected in river water in Montana, but the source (natural or
7 engineered) and the concentration of nano-TiO₂ were not determined (Wigginton et al., 2007).

8 Several physicochemical properties of nano-TiO₂ can contribute directly to its environmental fate
9 and transport in water. Long et al. (2006) reported that P25 rapidly aggregated in both Hank’s Basic Salt
10 Solution (HBSS) and Dulbecco’s Modified Eagle’s Medium (DMEM) buffer solutions, both of which are
11 high-osmolarity fluids that contain high concentrations of the monovalent cations Na⁺ and K⁺ [160
12 millimolar (mM)] and the divalent cations Ca²⁺ and Mg²⁺ (2 mM). The ionic strengths of these two
13 solutions are approximately 155 mM and 166 mM, respectively. After 1 minute of sonication,
14 aggregation continued for 20–45 minutes until a steady-state, stable aggregate size formed. The steady-
15 state aggregate sizes ranged from 826 to 2,368 nm and the concentration of P25 ranged from 2.5 to 120
16 parts per million (ppm).

17 Ridley et al. (2006) found that results were reproducible for classical titration procedures (with
18 modification) to characterize the surface charging properties of a commercially available, uncoated
19 anatase nano-TiO₂ product (from Ishihara Techno Corporation, Osaka, Japan) in suspension. These
20 findings demonstrate that water treatment pH can affect the surface charging properties, and thus the
21 aggregation/agglomeration, potential bioavailability, and reactivity of nano-TiO₂.

22 Schmidt and Vogelsberger (2006) studied the solubility of four types of nano-TiO₂ (P25 from
23 Degussa, DT51D and G5 from Millennium Chemicals, and an original formulation – presumably all
24 uncoated particles) in various aqueous solutions, particularly focusing on the kinetics of the dissolution
25 process. At the beginning of the process, solubility increased rapidly over time and then reached a steady-
26 state value. The maximum solubility value (i.e., saturation concentration) was observed to depend on the
27 morphology of the TiO₂, the crystalline form of the nano-TiO₂, and on the size of the nanoparticles
28 exposed to dissolution. The saturation concentrations were higher in hydrolysis-generated nano-TiO₂
29 than in precipitation-generated nano-TiO₂, and higher in smaller particles than larger particles.

30 Sager et al. (2007b) attempted to disperse nano-TiO₂, and other types of nano-sized particles in
31 several suspension media, including phosphate-buffered saline (PBS), rat and mouse bronchoalveolar

1 lavage fluid (BALF), and dipalmitoyl phosphatidylcholine (DPPC). Although PBS was not a satisfactory
2 medium, BALF was an excellent medium for dispersing the particles. The dispersion was also
3 unsatisfactory in saline containing albumin alone or DPPC alone at concentrations found in BALF.
4 Combinations of protein and DPPC were satisfactory, but slightly less effective, substitutes for BALF.
5 These findings demonstrate the importance of the suspension media, but they are not necessarily relevant
6 to natural aquatic conditions.

7 Although many studies have demonstrated the potential to use nano-TiO₂ for waste water treatment
8 (Chen and Ray, 2001; Han et al., 2009; Khataee et al., 2009; Rincon and Pulgarin, 2003; Wang et al.,
9 2008c; Watlington, 2005; Xu et al., 2009b), data on the fate of nano-TiO₂ in waste water treatment are
10 scarce. Westerhoff et al., (2008) however, have reported the occurrence of nano-TiO₂ at full-scale waste
11 water treatment plants (in both raw and finished waters) in a conference proceeding abstract. The authors
12 predicted nominal nanomaterial concentrations on the order of one part per billion in liquid discharges
13 from waste water treatment systems, with higher concentrations in waste water biosolids (which may
14 subsequently be applied to land, landfilled, or incinerated).

15 Other types of nanoparticles also have been studied in waste water treatment plants. Limbach et al.
16 (2008) studied the fate of cerium oxide nanoparticles (20–50 nm diameter) in a model waste water
17 treatment plant under a variety of conditions (e.g., with different surfactants to stabilize dispersions, and
18 in media with different ionic strengths and pH values). They found that surfactants stabilized dispersions
19 under a wide range of test pH values even at high ionic strength. The model sewage treatment plant
20 consistently reduced the cerium oxide nanoparticle concentration in the waste water from 100 ppm to 2–5
21 ppm. Most nanoparticles were removed via agglomeration with microorganisms in the sedimentation
22 sludge. Comparing the physical properties and behavior of various oxides, the investigators speculated
23 that TiO₂ and other insoluble oxides would behave similarly to cerium oxide, while more soluble or
24 reactive oxides like zinc oxide (ZnO) would be even more likely to aggregate and be more amenable to
25 removal by sedimentation. The investigators cautioned, however, that the high nanoparticle concentration
26 (100 ppm) used in the study favors aggregation, and that at more realistic initial concentrations, a greater
27 percentage of nanoparticles are likely to break through.

28 Although no field studies on the behavior of nano-TiO₂ in the environment were identified, that
29 conventional TiO₂ can photogenerate fairly long-lived reactive oxygen species such as hydrogen peroxide
30 in aqueous environments has long been recognized (Harbour et al., 1985). Similar behavior would be
31 anticipated for nano-TiO₂.

32 The interaction between nano-TiO₂ and natural organic matter, which is ubiquitous in the
33 environment, has been investigated in controlled conditions in the laboratory. Yang et al. (2009) found
34 that humic acid, a common type of natural organic matter, is easily adsorbed onto nano-TiO₂ in aqueous

1 media (Yang et al., 2009). Because humic acid adsorption decreased the ξ (Chi) potential (i.e., increased
2 electrostatic repulsion) of nano-TiO₂ particles, humic acid-coated nano-TiO₂ could be more easily
3 dispersed and suspended and thus more stable in an aqueous medium than uncoated nano-TiO₂ (Yang et
4 al., 2009).

3.1.1. Drinking Water Treatment-specific

5 Although the processes for using nano-TiO₂ for commercial water treatment are not yet well
6 established and therefore a definitive understanding of nano-TiO₂ fate is not possible, nano-TiO₂ is not
7 expected to be destroyed. One might anticipate that, given the size of nano-TiO₂, it would remain
8 suspended in solution; alternatively, it could adsorb to other particles and become part of the
9 sedimentation (floc). Some evidence suggests that nano-TiO₂ suspended in water could pass through
10 various stages of conventional treatment and filtration, perhaps even microfiltration (Zhang et al., 2007).

11 Various fate pathway scenarios could be anticipated for nano-TiO₂ post-treatment. For example,
12 nano-TiO₂ might remain in solution as colloidal particles in the water and enter water tanks or reservoirs.
13 If some water were lost from the distribution system via leaks or spills, nano-TiO₂ could end up in surface
14 waters or the subsurface environment. If nano-TiO₂ were to enter ground water aquifers, nano-TiO₂
15 would presumably persist as a particle, given that other inorganic compounds are not readily broken down
16 in that environment; however, particle size and other characteristics could change. Conceivably, nano-
17 TiO₂ could release, or modify the bioavailability of, other water contaminants of concern.

18 In another scenario, nano-TiO₂ might settle with floc in the sedimentation step, where it
19 presumably could become part of the sediment sludge and be partially removed from the water with the
20 sludge (AWWA, 2003). The discarded sediment could be transported off-site for disposal or reuse. For
21 example, sludge could be used as cover in municipal solid waste landfills or applied to agricultural or
22 recreational land.

23 Alternatively, nano-TiO₂ might become part of the filter matrix. Conventional water treatment
24 processes apply filtration following flocculation and sedimentation. U.S. EPA's Filter Backwash
25 Recycling Rule (U.S. EPA, 2001) requires that, when the filter is backwashed, the water be recycled back
26 into the coagulation process. This could reintroduce nano-TiO₂ into the treatment process, but the
27 implications for levels of nano-TiO₂ in finished water are not clear.

28 If nano-TiO₂ is present in the final drinking water product that reaches the tap, it eventually might
29 enter the ambient environment or be captured by a waste water stream, after which it could reach a waste
30 water treatment plant. If the particular waste water treatment method employed does not remove nano-
31 TiO₂, it is likely to enter downstream water sources.

3.1.2. Sunscreen-Specific

1 The environmental fate of nano-TiO₂ in topical sunscreens could be affected by the surface
2 treatments and doping applied to nano-TiO₂ particles, by the sunscreen vehicle, or by any number of other
3 constituents in such products (see Appendix B). Nano-TiO₂ in emulsion, dispersion, and possibly
4 powdered form could be present in waste water (e.g., from equipment and site cleaning) and solid waste
5 from sunscreen manufacturing facilities, depending on the trapping and filtration processes the facility
6 uses. In the powdered form, nano-TiO₂ could escape the facility through air venting and filtration
7 systems.

8 Nano-TiO₂ also could be released to natural water bodies or waste water through bathing or laundry
9 following sunscreen use. Swimming in artificial pools could result in an accumulation of sunscreen
10 material in the water and potential release into the environment as untreated waste water. If nano-TiO₂
11 remains mobile in water, it could enter downstream water sources in a manner similar to that of the nano-
12 TiO₂ used for drinking water treatment.

13 Parallels are suggested by recent studies that have detected topical sunscreen constituents in
14 untreated waste water, treated waste water, surface water (lakes and rivers), fish from lakes and rivers,
15 and biosolids (Balmer et al., 2005; Fent et al., 2008; Rodil and Moeder, 2008). The organic compounds
16 detected in these studies were UV filter compounds such as 4-MBC (4-methylbenzylidene camphor) and
17 OC (octocrylene), which generally biodegrade slowly and can bioaccumulate. Some evidence also
18 indicates that nano-TiO₂ can bioaccumulate (Zhang et al., 2006). Although nano-TiO₂ is unlikely to
19 behave exactly the same way as other components of sunscreen, the observed nano-TiO₂ bioaccumulation
20 in fish (Zhang et al., 2006) suggests the possibility of persistent presence of nano-TiO₂. However, no
21 studies to date have documented the occurrence of nano-TiO₂ specifically from sunscreens in waste water
22 or natural water bodies.

3.2. Soil

23 Three studies were located that address the fate and transport of nano-TiO₂ in soil. Dunphy
24 Guzman et al. (2006) studied the effect of pH on nano-TiO₂ mobility in a model soil column. They found
25 that both surface potential and aggregate size influence transport. In the pH region where electrostatic
26 forces between nano-TiO₂ aggregates and the experimental Pyrex surface should have been strong (pH
27 2.5 to 5.9), nano-TiO₂ was highly mobile. The calculated interaction energy was expected to be greatest
28 for the largest aggregates at pH 12, but these were the particles that most strongly attached to
29 microchannel surfaces. At pH 3, where conditions were predicted to be favorable for negative/positive

1 interaction, 84% of the particles were transported. The authors concluded that current transport theory
2 does not adequately predict nanoparticle and aggregated nanoparticle transport. The results suggest that
3 nano-TiO₂ particles and aggregates of nanoparticles in a stable dispersion might be highly mobile in the
4 subsurface over a wide range of conditions. This also raises the possibility that colloid transport
5 mechanisms might be more relevant than particle transport.

6 Lecoanet et al. (2004) showed that the mobility of aqueous anatase nano-TiO₂ particles in a porous
7 medium was comparable to that of other types of nanoparticles when compared on the basis of particle
8 size. Primary particles of 40-nm diameter were found to be aggregated to a diameter of 198 nm. About
9 55% was recovered after three pore volumes passed through the column, roughly twice the quantity of
10 ferroxane particles with mean diameter of 303 nm and just more than half the quantity of silica particles
11 with a diameter of 57 nm. After three pore volumes, approximately 95% of the 57-nm silica particles
12 were recovered, compared with 60% of the 135-nm silica particles. Although the results are specific to
13 the experimental protocol, they suggest that particle size affects mobility of nanoparticles and that anatase
14 might be mobile in ground water (Lecoanet et al., 2004).

15 A recent study using soil samples from 11 sites found that nano-TiO₂ could remain suspended in
16 soil suspensions for 10 days (Fang et al., 2009). Furthermore, the calculated maximum travel distance for
17 some soil samples was more than 30 cm, which suggested that nano-TiO₂ might be transferred to deep
18 soil layers or even to ground water. In general, large soil particles and low ionic strength conditions favor
19 nano-TiO₂ movement, while high clay content, dissolved organic carbon, and salinity conditions favor
20 soil retention of nano-TiO₂.

21 If nano-TiO₂ enters municipal sewage systems, the plants would separate liquid waste from solid
22 waste and nano-TiO₂ would likely be present in both waste streams. The solid waste, or sludge, could
23 present a route by which nano-TiO₂ could enter soil media, and could be dealt with in a number of ways.
24 In one scenario, the sludge might be sent for land disposal. The ability of TiO₂ to diffuse (and thus be
25 released) from a solid matrix such as sludge is unknown. Nano-TiO₂ and other contaminants such as
26 residual arsenic could become suspended in leachate and enter ground water, or they could pass through a
27 solid waste facility liner into the subsurface.

28 Under a different scenario, the sludge could be used for land application. In this case, the sludge
29 would undergo some type of treatment, generally to remove pathogenic organisms and regulated
30 contaminants such as lead and arsenic [titanium is not regulated under U.S. EPA's Biosolids Rule, Part
31 503; see (U.S. EPA, 1994)]. The treatment might include high temperature or high pH processing, or both
32 (U.S. EPA, 1994). The treated sludge could then be applied to land for agricultural use, reclamation sites,
33 golf courses, public parks, and other areas where nutrient-rich organic matter is useful, including forests,
34 parks, roadsides, and in some cases, residences (U.S. EPA, 1994). Roughly 50% of treated sludge is

1 applied to land, and less than 1% of all U.S. agricultural land uses treated sewage sludge (U.S. EPA,
2 2006b).

3 Nano-TiO₂ in sludge could be broadly distributed to land used for crops or grazing, where it could
4 enter the food chain, or to high-use areas such as parks, where people and pets could contact nano-TiO₂ in
5 soil or inhale wind-blown material. The nanomaterial could enter runoff and storm water during wet
6 weather events, returning to the aquatic medium. Ecological receptors also could also be exposed to
7 nano-TiO₂ in soil by direct contact with soils or via the food web, including uptake by plants. Because it
8 is an inorganic compound, nano-TiO₂ in soil could be expected to persist, in the same way that
9 conventional TiO₂ is very thermodynamically stable and is unlikely to undergo significant transformation
10 in the environment. Reactivity of nano-sized TiO₂, however, might differ (and is largely unknown at this
11 time) due to its greater surface area-to-volume ratio.

3.2.1. Drinking Water Treatment-specific

12 One scenario by which nano-TiO₂ specifically used in drinking water treatment could enter soils
13 would be through land application of sludge. In addition to the sludge produced in waste water treatment
14 described above, a sludge material (floc) containing nano-TiO₂ would likely be created in the process of
15 using nano-TiO₂ to treat drinking water. If nano-TiO₂ settles with floc in the sedimentation step, it would
16 likely become part of the sediment sludge. Similarly, as described above, if nano-TiO₂ is present in
17 finished drinking water, it will eventually enter sewage treatment facilities where any residual nano-TiO₂
18 could also enter the sediment sludge. The discarded sediment would be transported off-site and could be
19 used as cover in a municipal solid waste landfill or used for land application. Either use would result in
20 direct application of nano-TiO₂-contaminated waste to soils. Alternatively, nano-TiO₂ could enter soils if
21 treated water were used to irrigate residential or agricultural plants. These scenarios could have
22 implications for soil microbes and could also be noteworthy in relation to uptake by edible vegetation.

3.2.2. Sunscreen-specific

23 As described above, nano-TiO₂ in topical sunscreens could end up in the sludge produced at a
24 waste water treatment plant. The disposal of this sludge on land seems likely to represent the primary
25 pathway by which nano-TiO₂ in sunscreen could enter soil.

3.3. Air

1 Nano-TiO₂ manufacturing facilities could emit such particles to the ambient atmosphere. An
2 occupational exposure study by Berges et al. found that “outside the plant,” the airborne TiO₂ particle
3 concentration was approximately 13,000 particles per cubic meter, with nearly 94% of particles 100 nm or
4 less in size, and approximately 52% at 40–60 nm (Berges, 2007, 2008).

5 Some potential for environmental or occupational atmospheric emissions and releases of nano-TiO₂
6 presumably exists if the transport or storage containers were to be compromised (e.g., due to a forklift
7 error, train derailment, or truck accident). Also, land application of sludge from either drinking-water or
8 waste-water treatment might also contribute nano-TiO₂ to the atmosphere if dried material were to be re-
9 entrained.

10 The large surface area of nano-TiO₂ presents an opportunity for other co-occurring contaminants to
11 adsorb onto the surface, potentially changing the physicochemistry of the particle and the behavior and
12 effects of the other contaminant(s). Such interactions have been well documented for particulate matter
13 and gasses (U.S. EPA, 2004).

Questions about Fate and Transport

- 3-1. What are the relative contributions of different stages of the life cycles of water treatment and sunscreen products to environmental levels of nano-TiO₂ and associated contaminants in air, water, and soil?
- 3-2. How do specific physicochemical properties, including particle surface treatments and aggregation/agglomeration, affect the fate and transport of nano-TiO₂ in various environmental media?
- 3-3. Are available fate and transport models applicable to nano-TiO₂? If not, can they be adapted, or are new models required?
- 3-4. Is information on environmental fate and transport of other substances available that might provide insights applicable to nano-TiO₂?
- 3-5. If nano-TiO₂ production were to increase greatly, the packing and transport methods are likely to be changed as well. How would this affect the fate and transport of nano-TiO₂?
- 3-6. How might nano-TiO₂ affect the fate and transport of metals and other potentially toxic substances in water or other environmental media?
- 3-7. What is the bioavailability of nano-TiO₂ in land-applied sludge to both terrestrial and aquatic organisms? Is bioavailability likely to change when nano-TiO₂ is incorporated into sludge and is allowed to “age” (in-situ weathering)?
- 3-8. What effect, if any, do coatings, dopings, carriers, dispersants, and emulsion types have on biopersistence and bioaccumulation?
- 3-9. Can the photocatalytic properties of nano-TiO₂ cause other unintended substances to form, for example, degradation products, in various environmental media?
- 3-10. Will nano-TiO₂ affect the efficacy of other major elements of water treatment processes (e.g., chemical disinfection, the coagulant concentration necessary for effective organics removal)?
- 3-11. What influence could other drinking water contaminants, including arsenic, have on the chemical properties or behavior of nano-TiO₂?
- 3-12. Irradiated photocatalytic nano-TiO₂ is potentially biocidal and antimicrobial. What is the potential for interactions of nano-TiO₂ with microbes needed in water treatment systems?
- 3-13. What are the key environmental factors (e.g., pH, natural organic matter type and concentration, temperature) that facilitate or hinder nano-TiO₂ stability in the aqueous environment? Would humic acids or other common constituents or contaminants in water undergoing treatment affect the fate, including agglomeration/aggregation properties, of TiO₂?
- 3-14. What is the impact to nutrient and metals cycling and microbial diversity when sludge with nano-TiO₂ is applied to soils?
- 3-15. How do sunscreen ingredients affect nano-TiO₂ fate and transport?

Questions continued on next page.

Questions about Fate and Transport

- 3-16. Can agglomeration/disagglomeration in the environment be predicted on the basis of physical properties of the particle, for example, size, shape, or coating?
- 3-17. What is the likelihood that nano-TiO₂ in biosolids will become part of the food web and ground water contamination?
- 3-18. What is the potential for plant uptake of nano-TiO₂ from contaminated soil and irrigation water?

Chapter 4. Exposure–Dose Characterization

1 This chapter examines the potential for biota and humans to be exposed to nanoscale titanium
2 dioxide (nano-TiO₂) and associated pollutants through various environmental pathways tracing back to
3 the life cycle of two types of applications of nano-TiO₂, water treatment agents and topical sunscreens.
4 Exposure is more than the occurrence of a substance in the environment; actual contact between the
5 substance and an organism must occur. Exposure characterization entails much more than simply
6 identifying the concentration of a substance in the environment. It also involves, for example, various
7 temporal and spatial dimensions, including activity patterns and other complex variables. For nano-TiO₂,
8 even characterizing the primary material of interest, as discussed in Chapter 1, is not a simple matter.
9 Further complications arise when considering the potential for aggregate exposure across multiple routes
10 (e.g., inhalation, ingestion, dermal absorption) and for cumulative exposure to multiple contaminants that
11 derive, either directly or indirectly, from the life cycle of the products in question.

12 Dose⁴ refers to the amount of a substance that enters an organism by crossing a biological barrier
13 such as the skin, the respiratory tract, the gastrointestinal tract, or the eyes. Dose can vary for individuals
14 exposed to the same concentration of a substance. For example, an adult and a child in a room breathing
15 the same air containing a contaminant would both inhale the same contaminant concentration, but the
16 inhaled contaminant quantity and absorbed dose would differ due to differences in physiology (e.g.,
17 respiration rates), morphology (e.g., lung volume and surface area), and other variables such as clearance.
18 Dose can also reflect the integration of aggregate exposures across different routes of uptake.

19 Organisms might be exposed to nano-TiO₂ in the environment at any stage of the product life cycle.
20 In the feedstock and manufacturing process, nano-TiO₂ could be present in the air exhaust, waste-water
21 effluent, and solid waste, if appropriate control technologies are not in use. Nano-TiO₂ in the air can lead
22 to inhalation exposure to organisms in the area. The material could agglomerate or attach to other
23 pollutants and deposit on soil and water surfaces, as well as on animals, whose grooming habits could
24 then result in ingestion of nanomaterials. Nano-TiO₂ in soil could become airborne when the soil is dry
25 and windblown, or leak into water bodies when the soil is saturated with water.

⁴ The distinction between *exposure* and *dose* in this document is consistent with risk assessment usage. In toxicology, however, the term *dose* is often used to refer to the amount of a substance given to test subjects, as well as the amount that enters the subjects. Applied, external, and potential dose (e.g., on the skin, in the lung or digestive tract) in toxicology roughly equate to exposure in risk assessment; absorbed dose (amount entering the circulation) and target organ dose (amount taken up by a specific organ) in toxicology roughly equate to dose in risk assessment.

1 During distribution and storage, nano-TiO₂ could be released accidentally into the environment,
2 and cleaning the contaminated site with water could lead to nano-TiO₂ exposure to both aquatic and
3 terrestrial organisms. The use of nano-TiO₂ in drinking water treatment could result in some level of
4 nano-TiO₂ in water, as described in Chapter 3, and thus potential exposure to human populations as well
5 as biota. The use of sunscreens containing nano-TiO₂ is expected to lead to nano-TiO₂ in waste water
6 after users bathe or shower to remove residual sunscreen on the skin and launder clothes containing traces
7 of sunscreen. Because typical waste water treatment plants currently do not monitor for or specifically
8 target nanomaterials, nano-TiO₂ might not be completely removed by treatment. Therefore, nano-TiO₂
9 might be present in the effluent and lead to exposure to aquatic species. In the disposal stage, wastes
10 from factories and research facilities containing nanomaterials are often incinerated, possibly releasing
11 nano-TiO₂ into the air. Household wastes containing consumer products with nano-TiO₂ might be
12 incinerated or landfilled; landfilling might lead to nano-TiO₂ leaching into ground water.

13 Occupational exposure to nano-TiO₂ and associated contaminants (e.g., waste by-products) could
14 occur even with appropriate safety and protective practices. (See Appendix C for a more thorough
15 discussion of occupational exposure control measures.) Such occupational exposures could differ from
16 those of the general public in various ways. For example, workers might more likely be exposed to free
17 nano-TiO₂, whereas the public might more commonly encounter nano-TiO₂ embedded in a product.
18 Exposure durations and concentrations are also likely to be different in occupational settings. Likewise,
19 dose levels could differ between workers and the general population or even between workers in different
20 occupations, depending on factors such as respiration rates in relation to sedentary or strenuous activity in
21 the presence of airborne nano-TiO₂.

4.1. Aggregate Exposure to Nano-TiO₂ from Multiple Sources and Pathways

22 Nano-TiO₂ is used in various products, raising the possibility that biota and humans could be
23 exposed to nano-TiO₂ from more than one source. Such sources might include water treatment agents,
24 topical sunscreens, cosmeceuticals (traditional cosmetics such as moisturizers and color cosmetics that
25 incorporate active sunscreen ingredients with nano-TiO₂), sun-protective clothing, cleaning agents, air
26 purifiers, coatings, and food packaging, among many others (Woodrow Wilson International Center for
27 Scholars, 2006). Kaegi et al. (2008), for example, reported nano-TiO₂ in water runoff from both new and
28 naturally aged building façades painted with paint containing nano-TiO₂. Hsu and Chein (2007) found
29 that nano-TiO₂ powder-coated materials (wood, polymer, and tiles) under various conditions emitted
30 nanoparticles to the air. Of course, merely the presence of nano-TiO₂ in a product does not mean that

1 exposure will occur. For example, if nano-TiO₂ is firmly embedded in a product and the product remains
2 intact, little or no exposure to nano-TiO₂ might actually occur.

3 A hypothetical scenario for aggregate exposure to nano-TiO₂ in both water and sunscreen could
4 involve a person's ingesting the water (oral route), bathing (dermal) or showering (dermal and inhalation)
5 in it, applying sunscreen lotion to the skin (dermal), ingestion of sunscreen through hand-to-mouth
6 contact (oral), or uptake from hand-to-eye (ocular) contact. The latter two exposures pathways are
7 particularly relevant for young children. Biota also could be subject to aggregate exposures. A fish, for
8 example, could take up nano-TiO₂ that originated from a waste water treatment facility and could also
9 ingest prey whose contamination originated from ambient water, sediment, or other biota containing
10 sunscreen constituents. The seemingly widespread occurrence of nanoparticles of various types in aquatic
11 media reported by Wigginton et al. (2007) lends plausibility to these scenarios.

4.2. Cumulative Exposure to Nano-TiO₂ and Other Contaminants

12 Nano-TiO₂ is not the only substance relative to the life cycle of products containing nano-TiO₂ to
13 which biota and humans could be exposed. As noted in Chapter 2, releases of other contaminants might
14 also occur during various stages of the product life cycle, particularly waste materials during feedstock
15 processing and during manufacturing of the primary product. Such waste materials are not necessarily
16 nanoscale in size. As described in Chapter 3, if wastes are released into the environment, they could
17 undergo transformation, potentially resulting in even more types of contaminants; they might also be
18 transported to other locations, e.g., downstream or downwind.

19 The creation of secondary contaminants through transformation processes in various environmental
20 media also raises the possibility of exposure to substances indirectly related to nano-TiO₂. Many
21 nanoparticles, including nano-TiO₂, tend to bind transitional metals and organic chemical pollutants
22 (Nagaveni et al., 2004; Pena et al., 2006). With a tendency to adsorb pollutants and an ability to penetrate
23 the body and cells (see sections 4.6.1 Inhalation, 4.6.3 Ingestion, and 4.6.4 Blood-Brain Barrier and
24 Placental Transfer), nano-TiO₂ could carry toxic pollutants to sites where the pollutants would not
25 normally go (Moore, 2006). This type of "Trojan horse" effect could result in increased uptake of other
26 pollutants or interactive effects that would otherwise not occur if these substances were only present
27 individually.

28 Increased uptake of other pollutants in the presence of nano-TiO₂ has been reported by Sun et al.
29 (2007) and Zhang et al. (2007; 2006) (see Table 4-1). Sun et al. (2007) demonstrated that arsenic as
30 arsenate [As(V)] strongly binds to AEROXIDE[®] P25 (P25) in water and that carp exposed to water
31 containing 10 milligrams per liter (mg/L) of this photocatalytic nano-TiO₂ and 200 micrograms per liter

1 ($\mu\text{g/L}$) arsenate accumulated more arsenic than fish exposed to either nano-TiO₂ or arsenic alone. The
 2 bioconcentration factor of arsenic⁵ was more than twice as high when nano-TiO₂ was present than when it
 3 was not (Sun et al., 2007). The tested arsenate concentration, 200 $\mu\text{g/L}$, is environmentally relevant,
 4 given that higher total arsenic concentrations (mainly inorganic arsenic in the forms of arsenite and
 5 arsenate) in drinking water have been reported in many countries, including Bangladesh, China, Chile,
 6 and India (Basu et al., 2004; Feng et al., 2001; Moore et al., 1997; Tian et al., 2001). The presence of
 7 nano-TiO₂ did not alter the distribution of arsenic within fish tissues. Over various time intervals, arsenic
 8 and TiO₂ accumulated significantly in the intestine, stomach, and gills, and to a lesser degree in liver,
 9 skin, and scales; the least accumulation occurred in muscle. Because the accumulation of arsenic was
 10 much greater in the presence of nano-TiO₂, Sun et al. (2007) concluded that adsorption to nano-TiO₂
 11 facilitated arsenic transport and uptake.

Table 4-1. Tissue concentrations of various pollutants in fish after exposures to nano-TiO₂ in water.

Test Species	Material	Protocol (no UV illumination, unless specified)	Study Outcome	Reference
Fish (carp, <i>Cyprinus carpio</i>)	21-nm primary particle, 50- to 200-nm aggregates in water (P25) (photocatalytic)	Up to 25-day exposure to 3 and 10 mg/L nano-TiO ₂ (water changed daily, TiO ₂ concentrations in water ~2 and ~7 mg/L, respectively, after the first few hours)	TiO ₂ accumulated in internal organs > gills > skin and scales > muscle Bioconcentration factors were higher at 3 mg/L than at 10 mg/L	Zhang et al. (2006)
Fish (carp, <i>Cyprinus carpio</i>)	21-nm primary particle, 40- to 500-nm aggregates in water (P25) (photocatalytic)	Up to 25-day exposure to 10 mg/L nano-TiO ₂ with and without 200 $\mu\text{g/L}$ arsenate	Arsenate adsorbed onto nano-TiO ₂ Higher arsenic concentrations in tissues (skin and scales; muscle; gills; liver; stomach; intestine) with arsenate plus nano-TiO ₂ exposure, compared to arsenate exposure alone	Sun et al. (2007)
Fish (carp, <i>Cyprinus carpio</i>)	21-nm primary particle, BET 50 m ² /g (P25) (photocatalytic)	Up to 25-day exposure to ~97 $\mu\text{g/L}$ cadmium alone, cadmium with 10 mg/L nano-TiO ₂ , or cadmium with 10 mg/L natural sediment particles	Cadmium adsorbed onto nano-TiO ₂ Higher cadmium concentrations in tissues (skin and scale; muscle; gills; viscera; whole body) with cadmium plus nano-TiO ₂ exposure, compared to cadmium exposure alone, or cadmium plus natural sediment particles	Zhang et al. (2007)
Fish (rainbow trout, <i>Oncorhynchus mykiss</i>)	21-nm, 75% rutile: 25% anatase, sonicated (P25) (photocatalytic)	0-, 7-, or 14-day exposure to 0, 0.1, 0.5, or 1.0 mg/L nano-TiO ₂	No clear treatment or time-dependent effects on Ti levels in gill, liver, or muscle. In brain, a transient but statistically significant decrease in Ti concentrations compared to initial fish, but no exposure concentration-effect. Respiratory distress, organ pathologies, and oxidative stress at concentrations as low as 0.1 mg/L.	Federici et al. (2007)

BET – Brunauer, Emmett, Teller method of calculating surface area
 P25 – AEROXIDE® P25

⁵ The bioconcentration factor of arsenic = 1000 x arsenic concentration in fish ($\mu\text{g/g}$ dry weight) / arsenic concentration in water ($\mu\text{g/L}$).

1 Zhang et al. (2007) showed that nano-TiO₂ (21 nm) also enhanced cadmium uptake in carp. After
2 20 days of exposure, the bioconcentration factor for whole-body cadmium was 64.4 in carp exposed to
3 cadmium alone, but reached 606 in carp exposed to both cadmium and nano-TiO₂. Natural sediment
4 particles (19 μm) did not increase cadmium uptake. Both nano-TiO₂ and sediment particles adsorb
5 cadmium and reach equilibrium within 30 minutes, but nano-TiO₂ adsorbed more than 5 times as much
6 cadmium as the sediment particles. Based on the facts that nano-TiO₂ can adsorb cadmium and that
7 concentrations of cadmium and nano-TiO₂ are positively correlated, the authors suggested that increased
8 cadmium uptake in the presence of nano-TiO₂ may have been due to accumulation of cadmium adsorbed
9 on nano-TiO₂ (i.e., facilitated transport).

10 Zhang et al. (2007) also found that carp exposed to cadmium in water (at approximately 97 μg/L)
11 along with 10 mg/L photocatalytic nano-TiO₂ accumulated more cadmium than fish exposed to either
12 nano-TiO₂ or cadmium alone (Table 4-1). After 25 days of exposure, cadmium concentration in the whole
13 fish was 9.07 μg/g in the cadmium-only group and 22.3 μg/g in the cadmium-plus-nano-TiO₂ group,
14 indicating a 146% increase in the cadmium bioconcentration factor in the presence of nano-TiO₂. When
15 carp were analyzed after 20 days of exposure, cadmium concentrations in all groups were higher in
16 internal organs than in gills, muscle, and skin and scale (Zhang et al., 2007). Unlike nano-TiO₂, natural
17 sediment particles (at equivalent concentrations) did not affect cadmium bioaccumulation. The authors
18 also reported a positive correlation between nano-TiO₂ concentration and cadmium concentration in the
19 carp, and found high nano-TiO₂ concentrations in the gills. The increases in cadmium bioaccumulation
20 could be due to increased transport of cadmium into carp via adsorption to nano-TiO₂. The transport
21 routes could be from water onto the gill surfaces or from consumed food into internal organs. Toxicity
22 was not measured in this study.

23 The fact that organic disinfection by-products can be formed by the photocatalytic oxidation of
24 conventional TiO₂ in treating drinking water (Richardson et al., 1996) suggests the possibility that nano-
25 TiO₂ could have the same effect. Richardson et al. (1996) compared the organic disinfection by-products
26 detected after using (1) chlorine as the sole disinfectant and (2) TiO₂/ultraviolet (UV) light treatment
27 followed by chlorination. The authors reported detecting an additional by-product (tentatively identified
28 as dihydro-4,5-dichloro-2(3H)furanone) after the combined TiO₂/UV and chlorine treatment compared to
29 chlorine treatment alone. Overall, however, the numbers and concentrations of chlorinated disinfection
30 by-products were lower after combined TiO₂/UV and chlorine treatment than after chlorination alone.

31 Cumulative exposure to nanomaterials could also occur. Some consumer products contain more
32 than one type of nanomaterials, e.g., nano-TiO₂ and nano-silver have been used together in multiple
33 products (The Project on Emerging Nanotechnologies, 2009).

4.3. Models to Estimate Exposure

1 The U.S. Environmental Protection Agency (U.S. EPA) uses various models to estimate exposures
2 for chemical assessments, some of which are described on the Web sites for the Council for Regulatory
3 Environmental Modeling (U.S. EPA, 2009b) and the Center for Exposure Assessment Modeling (U.S.
4 EPA, 2009a). For example, the Exposure and Fate Assessment Screening Tool Version 2.0 (E-FAST
5 V2.0) is a publicly available program EPA uses for screening-level assessments of conventional industrial
6 chemicals. The tool provides estimates of aquatic exposure, general population exposure, and consumer
7 exposure based on release data (U.S. EPA, 2007b). Other fate and transport models also might be
8 relevant, for example, the Particle Tracking Model (PTM) the Army Corps of Engineers developed
9 (Demirbilek, 2005). However, these models were not developed for nanomaterials and have not been
10 tested for their ability to estimate nanomaterial exposures, although they perhaps could be used or adapted
11 for qualitative exposure estimation in lieu of quantitative release data.

12 Although empirical data on nano-TiO₂ concentrations in the environment are currently lacking, a
13 recent study used computer modeling to predict nano-TiO₂ concentrations in different environmental
14 media. Using limited data from published literature and various assumptions, researchers in Switzerland
15 developed models to estimate predicted environmental concentrations (PEC) and predicted no-effect
16 concentrations (PNEC). PEC values were calculated for “realistic exposure scenarios” (based on nano-
17 TiO₂ use, estimated as 25 tons per year in Switzerland) and for “high exposure scenarios” (based on
18 500 tons per year). The authors estimated that more than 60% of nano-TiO₂ is used in cosmetics,
19 including sunscreen, and that most of it is discharged into wastewater. To estimate PNEC, the lowest no-
20 observed-effect concentration [based on a published study on acute toxicity to *Daphnia* by Hund-Rinke
21 and Simon (2006)] was divided by an assessment factor of 1000, in accordance with the Technical
22 Guidance Document on Risk Assessment published by European Chemicals Bureau, because, as the
23 authors noted, the “accuracy of the data was low” (European Chemicals Bureau, 2003; Mueller and
24 Nowack, 2008). The PEC of nano-TiO₂ in water was 0.7 µg/L (“realistic scenario”) or 16 µg/L (“high
25 scenario”), compared to a PNEC of <1 µg/L (for daphnia). The authors (Mueller and Nowack, 2008)
26 stated that, given that the PEC is close to or greater than the PNEC, European Union authorities would
27 consider the substance “of concern” and call for more data to validate the result (Umwelt Bundes Amt,
28 2009).

4.4. Biota

29 Various scenarios and ways in which nano-TiO₂ from water treatment agents and topical sunscreens
30 could enter different environmental media were described in Chapters 2 and 3. In this section, some of

1 these scenarios are explored further in relation to exposure of aquatic and terrestrial organisms to nano-
2 TiO₂ under various conditions. Also discussed are the potential for bioaccumulation and entry of nano-
3 TiO₂ into the food web.

4.4.1. Aquatic

4 Data on sediment concentrations of nano-TiO₂, whether in a laboratory or a natural environment,
5 are limited. Nano-TiO₂ concentrations could be higher at the sediment surface than in the water (Handy
6 et al., 2008b). Settling of nano-TiO₂ aggregates (with nano-TiO₂ or with organic matter) would increase
7 nano-TiO₂ exposure to benthic and benthopelagic species, such as mussels, sea cucumbers, marine
8 worms, flatfish, and other species that sometimes feed at the bottom of water bodies. At the same time,
9 settling decreases nano-TiO₂ concentrations in the water column and would be expected to decrease
10 exposure to suspension feeders (such as *Daphnia*) and animals that live in or drink the water.

11 Nanoparticles can also deposit or aggregate on the surfaces of aquatic organisms. Surface
12 aggregation can be caused by the slower flow near the interface between liquids and solids or by the
13 viscous properties of the surface of an organism (Handy et al., 2008b). Surface deposition or aggregation
14 can result in a higher concentration of nano-TiO₂ on the organism's surface than in the water, and might
15 cause toxicity even if the nano-TiO₂ does not enter the cells. Surface-acting metal toxicity of nano-TiO₂
16 has been suggested as a cause of gill damage in rainbow trout where the titanium concentration in gill
17 tissue was not increased (Federici et al., 2007).

18 Because water flow is also slower near the interface with air, higher concentrations of nanoparticles
19 are also expected at the air-water interface. Consequently, organisms living at the water surface, such as
20 zooplankton (microscopic invertebrates that float or swim in water), phytoplankton (primarily single-
21 celled algae), and eggs of aquatic and amphibian species at the water surface, could be exposed to higher
22 nanoparticle concentrations than organisms living throughout the water column.

4.4.1.1. Bioaccumulation

23 Zhang et al. (2006) found that nano-TiO₂ can accumulate internally in carp (Table 4-1). The
24 authors exposed carp to photocatalytic nano-TiO₂, or P25 for up to 25 days. Before dissection and TiO₂
25 analysis, carp were rinsed and wiped. The nominal concentrations of nano-TiO₂ in the water were 3 and
26 10 mg/L (based on the amount of stock nano-TiO₂ suspension added to the fish tank), and the authors
27 reported that nano-TiO₂ concentrations were 2 and 7 mg/L after 24 hours, with most of the decreases
28 occurring within 4 hours after the addition of stock solution. The TiO₂ concentration in carp tissue
29 increased rapidly over the first 10 days and then more gradually between day 10 and day 25. TiO₂
30 concentrations were highest in visceral organs, distantly followed by gills, and then closely followed by

1 skin and scales (one sample), and muscle. The bioconcentration factors in the visceral organs were
2 approximately 2100 at 3 mg/L, and approximately 1400 at 10 mg/L.

3 In contrast to the finding of bioaccumulation of nano-TiO₂ in carp that Zhang et al. (2006)
4 reported, Federici et al. (2007) detected no accumulation in trout exposed to up to 1 mg/L nano-TiO₂ for
5 14 days. Although the findings appear contradictory, each study might simply reflect the results of the
6 specific test conditions. For instance, the rainbow trout were exposed to lower concentrations of nano-
7 TiO₂ than were the carp. The Federici et al. (2007) study used photocatalytic nano-TiO₂ (P25), and 80%
8 of the water in the fish tank was changed every 12 hours. Similar to Zhang et al. (2006), Federici et al.
9 (2007) reported that more than 85% of the initial nano-TiO₂ concentrations in the tank water remained
10 after 12 hours. Other environmental factors, such as water temperature at 14 °C for trout and at 23 °C for
11 carp, could influence the behavior or effects of nano-TiO₂ and contribute to the difference between these
12 two studies. Furthermore, carp feed mainly by grubbing in sediments, and therefore also could be
13 exposed to settled nano-TiO₂ aggregates, to which rainbow trout might not be exposed.

14 Although nano-TiO₂ can bioaccumulate in fish, the uptake mechanism is not clear. Substances in
15 water can enter fish through waterborne exposure (through gills and then into blood through absorption),
16 dietary uptake, or cutaneous absorption. Handy et al. (2008a) suggested that the absorption of nano-TiO₂
17 on the gill surface into the blood might be slow or uncertain, but that nano-TiO₂ on the gut surface might
18 be taken into cells by endocytosis. Although intact fish skin is unlikely to be permeable to nano-TiO₂,
19 these authors proposed that cutaneous uptake of nano-TiO₂ might be possible if the skin is infected or
20 inflamed (Handy et al., 2008a). Handy et al. (2008a) did not provide experimental data to support nano-
21 TiO₂ uptake through endocytosis, but a recent in vitro study indicated that an endocytosis inhibitor,
22 Nystatin, decreased the mutation frequencies induced by exposures to 5-nm and 40-nm nano-TiO₂, but
23 not 325-nm TiO₂, in mouse embryo fibroblasts, implying that endocytosis is involved in modulating cellular
24 response to nano-TiO₂ exposure (Xu et al., 2009a). The concentration of nano-TiO₂ or Ti in cells was not
25 measured (Xu et al., 2009a).

4.4.2. Terrestrial

26 Terrestrial organisms could be exposed to nano-TiO₂ under various scenarios. For example,
27 spillage during shipping or storage, including breaching of containers by vermin, could result in contact
28 by microbial, invertebrate, and vertebrate species. Plants could be exposed by taking up water containing
29 nano-TiO₂ or by growing in soil that contains nano-TiO₂, for example, as a result of application of sludge
30 from water treatment facilities. No empirical data on the potential for such exposures to terrestrial
31 organisms have been located.

4.4.3. Food Web

1 Nano-TiO₂ could enter the food web at various levels, depending on the point and extent of its
2 release to the environment. If nano-TiO₂ were dispersed in water, for example, it could be taken up by
3 algae, which are primary producers in ecosystems. Many invertebrates, which are primary consumers in
4 aquatic systems, eat algae and in turn are consumed by larger animals such as fish. A common aquatic
5 invertebrate is the water flea (genus *Daphnia*), which is a small crustacean filter feeder (also known as
6 suspension feeder). Daphnids use their legs to generate water flow and use the comb-like setae on their
7 thoracic limbs to strain or catch smaller organisms (such as algae) for consumption. Because daphnids
8 have been reported to filter up to 120–160 mL each per day (Vanoverbeke, 2008), they could be exposed
9 to quite high numbers of nanoparticles in water (Griffitt et al., 2008). Even if nano-TiO₂ is not absorbed
10 into tissues, nano-TiO₂ in the digestive tract of daphnids could still contribute to bioaccumulation in the
11 food web.

4.5. Humans

12 As noted at the beginning of this chapter, exposure is a complex function of not only the amount of
13 a substance in the environment but also various temporal and spatial dimensions of contact with the
14 substance. At this early stage of investigation and understanding of human exposure to nano-TiO₂,
15 however, even basic information on the potential for and amount of human contact with this material is
16 limited. Moreover, exposure characterization encompasses not just the primary material but the
17 secondary waste and transformation products related to the entire life cycle of nano-TiO₂ in various
18 applications. These indirect and secondary aspects of exposure are even less well understood and
19 therefore not discussed here. Their potential significance, however, should not be discounted.

20 The potential for human exposure to nano-TiO₂ depends first on the production and use of this
21 material in the applications under consideration here. Generally, exposure related to life-cycle stages
22 leading up to actual use appears more likely to occur in occupational situations, whereas exposure related
23 to the use and disposal stages of the life cycle could occur in either occupational or non-occupational
24 settings. Although not absolute, this distinction provides a basis for discussing exposure with reference to
25 either the general population or the occupational population, both of which are essential in examining the
26 broad implications of nano-TiO₂ in water treatment and topical sunscreens.

4.5.1. General Population

4.5.1.1. Water Treatment-specific

1 Although the actual use of nano-TiO₂ in water treatment facilities appears to be limited at present
2 to pilot testing (see Section 2.4), the potential for general population exposure to nano-TiO₂ *if it were to*
3 *be used widely* could involve sizeable numbers of people, given the number of U.S. community water
4 suppliers that currently treat drinking water to reduce arsenic levels. As discussed in Section 2.4.1, such
5 water suppliers serve roughly 13 million people in the United States alone.

6 If nano-TiO₂ were present in potable water, exposure could involve more than just ingesting the
7 water. Such water could be used for bathing, including showering, which could imply exposure not only
8 by dermal contact but by inhalation of water droplets and even contact through the eyes. Also, the general
9 population includes infants and other individuals who could have relatively greater exposure to water and
10 thus possible vulnerability if the water were contaminated. For example, on a body weight basis, 1- to 3-
11 month-old infants consume far more water directly and indirectly than 18- to 21-year olds. The 90th
12 percentile consumption rate is 151 milliliters per kilogram per day (mL/kg/day) for these infants versus
13 17 mL/kg/day for the older age group [see Table 3-9 in (U.S. EPA, 2008a)]. Children also have a greater
14 water intake while swimming, so they may be more vulnerable to contaminated water in that respect as
15 well (U.S. EPA, 2008a).

4.5.1.2. Sunscreen-specific

16 As discussed in Section 2.4.2, survey data from 2007 suggest that sunscreen might be used on a
17 daily basis by 33 million people in the United States and on an occasional basis by another 177 million.
18 Moreover, sunscreen use appears to be increasing. According to the Skin Cancer Foundation (2007), the
19 percentage of people who use sunscreen at least occasionally rose from 39% to 59% between 2003 and
20 2007. Sunscreen use is presumably greatest during the warmer months of the year, in warmer climates, or
21 during outdoor recreational activities at various times during the year. No information was found
22 regarding the proportion of use associated with water recreation and other specific venues or activities.

23 Topical sunscreens are available as traditional lotions, in spray-on form, and as wipes (Jeffries,
24 2007). Nano-TiO₂ sunscreen powders are also available, according to the Woodrow Wilson Center's
25 nanotechnology consumer product inventory (Woodrow Wilson International Center for Scholars, 2006).
26 Another sun protection option available to consumers is "cosmeceuticals," cosmetics that incorporate
27 active sunscreen ingredients (Davis, 1994). In the mid-1990s, up to 30% of lipsticks and 20% of makeup
28 were estimated to have sunburn protection factor (SPF) ratings, sunscreen claims, or both (Davis, 1994).
29 Other products with active sunscreen ingredients include hair care products (e.g., hair spray, gel, mousse,

1 and conditioner), alpha-hydroxy skin treatments, nail polish, and bath products. Sun-protective clothing
2 is also available (Davis, 1994).

3 For the general population, the principal exposure route to nano-TiO₂ in sunscreen is through the
4 skin. When sunscreen is applied by spray, inhalation presents another route, although it is not clear that
5 the primary nanoparticles as such would be inhaled. Ingestion is also conceivable through hand-to-mouth
6 contact and mucociliary clearance of inhaled nano-TiO₂.

Dermal Exposure

7 Potential nano-TiO₂ dermal exposure from sunscreen use can be estimated by the amount of
8 applied sunscreen. Although the recommended sunscreen application rate is 2 milligrams per square
9 centimeter (mg/cm²) of skin (roughly 1.5 ounces or 3 tablespoons for the entire body of an average adult),
10 most consumers use 0.5 to 1.5 mg/cm² skin (Srinivas et al., 2006). Assuming sunscreen is applied to all
11 areas of skin exposed to sun on a day at the beach or exposed to water while swimming, an adult would
12 use an estimated 10–46 g sunscreen per application, and a 3-year old would use an estimated 3–15 g
13 sunscreen per application (Table 4-2). Assuming that a sunscreen contains 5% nano-TiO₂ (the mass
14 percent concentrations of nano-TiO₂ in sunscreens range from 2% to 15%, see Table A-1 in Appendix A),
15 the amounts of nano-TiO₂ applied on the skin could range from 0.5 to 2.3 g per person per application for
16 an adult, and 0.17 to 0.76 g per person per application for a 3-year old (Table 4-2). Sunscreens, including
17 the water-resistant or water-proof types, should be reapplied every 2 hours, regardless of the SPF values.
18 Exposure to nano-TiO₂ from sunscreen could range from 1.0 to 4.6 g for an adult and 0.33 to 1.5 g for a
19 3-year old for a half day at the beach (2 applications in 4 hours). As shown in Table 4-2, the ranges of
20 applied nano-TiO₂ would be 12–55 mg per kg of body weight per application for a 3-year old and 8.0–37
21 mg per kg of body weight per application for an adult. This relatively higher exposure in young children
22 could be noteworthy in relation to indications that the skin of infants and young children might have less
23 barrier function than matured skin (Hostynek, 2003), although this contrasts with another report
24 indicating that human skin is mature both structurally and functionally at 2–3 weeks of age (Makri et al.,
25 2004). Although not everyone applies sunscreen at the recommended dose and frequency in real life,
26 parents reported greater use of sunscreen on their children than on themselves (Weinstein et al., 2001).

Table 4-2. Estimated dermal exposure to nano-TiO₂ from sunscreen containing 5% nano-TiO₂ for adults and 3-year-old children. ^a

Subject	Surface area of skin ^b (cm ²)	Applied sunscreen surface density (mg/cm ²)	Applied sunscreen amount (mg/person/application)	Applied nano-TiO ₂ (mg/person/application)	Applied nano-TiO ₂ (mg/ kg BW ^c / application)
3-year-old child, total body surface (50 th percentile)	6,640	0.5	3,320	166	12.0
		1.5	9,960	498	35.9
		2	13,280	664	47.9
3-year-old child, total body surface (95 th percentile)	7,640	0.5	3,820	191	13.8
		1.5	11,460	573	41.3
		2	15,280	764	55.1
Adult, body surface area subjected to water contact in swimming (50 th percentile)	20,000	0.5	10,000	500	8.0
		1.5	30,000	1,500	24.0
		2	40,000	2,000	32.1
Adult, body surface area subjected to water contact in swimming (95 th percentile)	23,000	0.5	11,500	575	9.2
		1.5	34,500	1,725	27.6
		2	46,000	2,300	36.9

BW – Body weight

^a Actual concentrations of nano-TiO₂ in commercial sunscreen on the market vary, with the high at nearly 15%. (See Table A-1 in Appendix A.)

^b Body surface area values are based on Tables 6-6 and 6-16 of U.S. EPA (1997).

^c The body weights used in the calculation were 14 kg, the median for 36-month old females (CDC, 2000), and 62 kg, the median for adults 18–74 years old [Table 7.5 of U.S. EPA (1997)].

Inhalation Exposure

1 Consumers could inhale water aerosol while showering or from nebulizing room humidifiers.
 2 Spray sunscreen products also present an inhalation exposure scenario. For such products and for water
 3 containing nano-TiO₂, the characteristics of the resulting aerosol have not been documented in the
 4 published literature. Section 4.5.2 discusses inhalation exposure to nano-TiO₂ for several occupational
 5 scenarios.

Oral Exposure

6 Nano-TiO₂ from sunscreen could be ingested by accident or as a result of routine hand-to-mouth
 7 contact (from residual sunscreen on hands), particularly for young children. If nano-TiO₂ were inhaled,
 8 mucociliary clearance could lead to uptake through the gastrointestinal tract. Although no estimates of
 9 this type of nano-TiO₂ exposure are available, dietary intake of all sizes of TiO₂ from all sources (food,
 10 pharmaceuticals, etc.) has been estimated. The estimation was based on 7-day food diaries and records of
 11 pharmaceutical, dietary supplement, and toothpaste use of 182 people in the United Kingdom. The
 12 amounts of TiO₂ were calculated or estimated from product labels (the listing of food-additive TiO₂ is
 13 required by British law in most foods), manufacturer reports, and laboratory testing. The total median

1 dietary intake of nano-TiO₂ and micro-TiO₂ (0.1–3 μm) was estimated to be 2.5 mg per individual per day
2 (Lomer et al., 2004). Food was the main source of dietary TiO₂, followed by pharmaceuticals, dietary
3 supplements, and toothpaste. Individual TiO₂ intake varied widely (0–112 mg per individual per day),
4 and no particle size information was provided.

4.5.2. Occupational

5 Nearly every stage of the life cycle for the applications considered here presents some potential for
6 occupational exposure to nano-TiO₂. Moreover, no exposure route can be ruled irrelevant to these
7 workers. Thus, assessing occupational exposure is essential to completing a CEA of nano-TiO₂ in either
8 water treatment agents or topical sunscreens. As a frame of reference, NIOSH (2005) proposed a draft
9 occupational exposure limit of 1.5 milligrams per cubic meter (mg/m³) for fine TiO₂ (less than 2.5 μm in
10 size) and 0.1 mg/m³ for ultrafine TiO₂ (less than 0.1 μm [100 nm]).

11 Most information on workplace TiO₂ exposure relates to the production of conventional TiO₂, not
12 nano-TiO₂ specifically. Additionally, given that nano-TiO₂ tends to agglomerate or aggregate,
13 occupational exposure conditions for nano-TiO₂ could involve both nanoscale and larger than nanoscale
14 TiO₂ particles. The manufacturing stage of the life cycle comprises multiple processes that might vary in
15 exposure characteristics. An epidemiologic study conducted in four U.S. TiO₂ manufacturing factories
16 indicated that occupational exposure to TiO₂ is greatest during bagging, milling/micronizing, and internal
17 recycling (shoveling spilled material from the floor into the processing bins) stages (Fryzek et al., 2003).

18 The manufacturer of P25 has stated on its Web site that workplace inhalation exposures to TiO₂ are
19 typically less than 0.5 mg/m³ (Degussa, 2007). The Web site also indicated that photocatalytic P25
20 production occurs in a closed reactor, which presumably limits exposure. The highest exposures the
21 manufacturer reported were less than 0.5 mg/m³ and occurred during the packaging step, which is also
22 enclosed. This manufacturer is said to require the use of personal protective equipment during any repair
23 work that could lead to dust exposure (Maier, 2007). Such information suggests limited potential for
24 inhalation exposure during P25 manufacturing, but it does not address other routes such as dermal
25 exposure or incidental ingestion from hand-to-mouth contact.

26 Another manufacturer of nano-TiO₂ products reported that air concentrations in production areas
27 for DuPont™ Light Stabilizer 210 and 220 (which protects plastic from UV damage) were less than
28 2 mg/m³, and in most cases were lower than the detection limit of 0.3 mg/m³ (size not specified) (DuPont,
29 2007). No exposure data were available for the material incorporation, packing, and product fabrication
30 areas. Although occupational exposure was stated to be low (DuPont, 2007), the detection limit
31 (0.3 mg/m³) is above the draft NIOSH recommended limit for ultrafine or nano-TiO₂, 0.1 mg/m³ (NIOSH,
32 2005).

1 Preliminary estimates of workplace exposure in a factory that produces rutile nano-TiO₂ for
2 sunscreen and cosmetics were reported by Berges (2007, 2008). Measurements were made in 2006, and
3 then in 2007, when improvements to local exhaust systems were in operation (Berges, 2007, 2008). In
4 2007, the TiO₂ in the “inhalable” dust mass concentration at the bin filling station was 0.014 mg/m³, and
5 the TiO₂ in the “respirable” dust mass concentration was 0.004 mg/m³. [*Inhalable* refers to all particles
6 that can enter the respiratory tract through the nose or mouth (e.g., up to about 100 μm); *respirable* refers
7 to particles that penetrate to the alveolar (pulmonary) region with a mass median aerosol diameter
8 (MMAD) of about 4 μm (European Committee for Standardization, 1993).] In the bag filling area in
9 2007, the TiO₂ inhalable fraction was 0.028 mg/m³, and the respirable fraction was 0.022–0.042 mg/m³.
10 Personal sampling in 2007 over a 4.87-hour period measured 0.010 mg/m³ TiO₂ in the respirable fraction.

11 Liao et al. (2009) further reported and analyzed the Berges (2007, 2008) data, as well as data from
12 several other sources to model the occupational exposure and characterize risk. In the bin filling area of
13 the facility studied by Berges (2007, 2008), the total airborne TiO₂ particle number concentrations ranged
14 from 15,000 to 156,000 particles/cm³, with a measured size range of 14–673 nm. More than 97% of the
15 particles were 100 nm or less in size, and 60% were 20–30 nm. After a leak was sealed, the high-end
16 concentration decreased to less than 29,000 particles/cm³. Near the leak, the particle surface area
17 concentrations reached 200 square micrometers per cubic centimeters (μm²/cm³) for “alveolar deposited”
18 particles and 50 μm²/cm³ for “tracheobronchial deposited” particles. Under normal operating conditions,
19 the particle surface area concentrations were 50 μm²/cm³ for the alveolar deposited particles and
20 13 μm²/cm³ for the tracheobronchial deposited particles. Outside the plant, the airborne TiO₂ particle
21 concentration was approximately 13,000 particles/cm³. Among other things, their model indicated that
22 the highest TiO₂ burdens in terms of lung surface area of packers were 0.174 m² (anatase) and 0.122 m²
23 (rutile) for particles sized 10–20 nm. For particle sizes 80–300 nm, the burdens were 0.002 m² (anatase)
24 and 0.0017 m² (rutile). So-called surface treatment workers (involved in drying, packing, and blending
25 operations) had a higher TiO₂ burden in the lung surface area. For particles 10–20 nm, the burdens were
26 0.40 m² (anatase) and 0.28 m² (rutile).

27 Using exposure data specific to particle size in the workplace from the Berges (2007, 2008) reports
28 as well as conventional TiO₂ studies (Boffetta et al., 2004; Fryzek et al., 2003), Liao et al. (2009) used
29 computer modeling to calculate that exposures to nano-TiO₂ (expressed as particle surface area
30 concentrations) were 0.1685 m² TiO₂ per 300 m³ air (working space volume) for packers and 0.387 m²
31 TiO₂ per 300 m³ air for surface treatment workers. For nano-TiO₂ in the 10- to 50-nm size range, the
32 airborne concentrations (expressed as particle surface area concentrations) were higher in anatase nano-
33 TiO₂ than in rutile nano-TiO₂ for both packers and surface treatment workers. The highest airborne
34 concentration was anatase for surface treatment workers, followed in order by rutile for surface treatment
35 workers, anatase for packers, and rutile for packers.

1 Liao et al. (2009) also modeled the dose-response relationships from in vitro cytotoxicity studies of
2 human dermal fibroblasts and inflammatory responses of human lung epithelial cells. They then
3 compared exposure levels to the dose-response functions and concluded that packers and surface
4 treatment workers at the studied location were “unlikely to [be at] substantial risk [of] lung inflammatory
5 response, [but they] have significant risk [of] cytotoxicity response at relatively high airborne TiO₂
6 anatase NP [nanoparticle] concentrations at size 10-30 nm” (Liao et al., 2009).

7 In a presentation at a professional conference, Li et al. (2008b) displayed photographs of a factory
8 that mixed, but did not manufacture, nano-TiO₂. The photographs appeared to show that nano-TiO₂ was
9 stored in shipping bags piled on pallets. White powder was visible on the facility floor, but its
10 composition is unclear as the factory also handled conventional “pigmentary grade” and “food grade”
11 TiO₂ (Ichihara, 2009). Li et al. (2008b) reported that workers had been given masks and shirt-like
12 protective clothing but that the masks were not always worn. The authors also noted that shirt-like
13 protective clothing provided no protection for the forearms and legs of the workers, many of whom wore
14 short-sleeved tops and shorts. Although this factory may not be representative, it illustrates how
15 inhalation and dermal exposure might occur during the manufacturing or mixing process.

16 As noted in Section 2.3, nano-TiO₂ is routinely shipped in paper bags, which could be a source of
17 exposure if they were to be ruptured, punctured, or otherwise compromised during distribution or storage.
18 Nano-TiO₂ in dispersion form shipped in pails, drums or totes (Klaessig, 2008) could be subject to
19 accidents resulting from forklift errors, train derailments, and truck accidents, but no empirical data on
20 such incidents specifically related to nano-TiO₂ are available.

21 The above information suggests that inhalation and dermal exposure could occur during
22 manufacturing, packaging, shipping, and storage of nano-TiO₂. Without incidence and concentration
23 data, however, the potential for and nature of worker exposure cannot be characterized.

4.6. Dose

24 Dose is defined as the amount of a substance that actually enters an organism by crossing a
25 biological barrier. Uptake of nano-TiO₂ by different routes has been investigated in various species.
26 Table 4-1 in Section 4.2 summarizes several studies that measured tissue concentrations in fish that had
27 been exposed to nano-TiO₂ in water. The exposures included, but were not necessarily limited to, nano-
28 TiO₂, appropriately reflecting the multiple substances to which fish can be exposed in the natural
29 environment. For terrestrial organisms, including laboratory animals used for toxicological studies and as
30 models for human health effects, the route of exposure is important in determining the dose that actually
31 enters the body, hence information on uptake of nano-TiO₂ is presented here according to the route of

1 uptake, i.e., inhalation, ingestion, or dermal. Additionally, this section discusses special biological
2 barriers (blood brain barrier and placenta), and issues related to dose-metrics for nano-TiO₂.

4.6.1. Respiratory (Inhalation and Instillation)

3 Animal studies have shown that inhaled or instilled nano-TiO₂ can translocate into the interstitium
4 of the lung, lymph nodes (Ma-Hock et al., 2009; Oberdörster et al., 1992; Oberdörster et al., 1994), blood
5 (Geiser et al., 2005), and the brain (Wang et al., 2005; Wang et al., 2008b; Wang et al., 2007a).

6 Particles in the nasal cavity may enter the brain through: (1) the olfactory nerve (Elder et al., 2006;
7 Oberdörster et al., 2004) [upper particle size limit: 200 nm (Elder et al., 2006)]; (2) the circulating blood
8 and then crossing the blood-brain barrier (Oberdörster et al., 2004); and (3) the olfactory mucosa and
9 through the ethmoid bone into cerebrospinal fluid (Illum, 2000). One of the most visually convincing
10 demonstrations of olfactory nerve transport, as mentioned in (Oberdörster et al., 2004), is a study by De
11 Lorenzo (1970). De Lorenzo showed sequential transmission electron microscopy (TEM) images of
12 intranasally instilled gold nanoparticles in the olfactory mucosa, uptake into the olfactory rods, retrograde
13 translocation within the olfactory dendrites, anterograde translocation in the axons of the olfactory nerve,
14 and appearance in the olfactory bulbs. For more discussion of nanoparticle translocation from the nasal
15 cavity to the brain, see (Oberdörster et al., 2004).

16 Intranasal instillation of three sizes of nano-TiO₂ particles (approximately 20, 70, and 155 nm)
17 resulted in increased titanium concentrations in the olfactory bulb of mice (Wang et al., 2005, 2007a).
18 Also, two forms of nano-TiO₂ particles (80-nm rutile and 155-nm anatase) were found to increase Ti
19 concentrations in the hippocampus, central cortex, and cerebrum, in addition to olfactory bulb, in mice
20 after repeated intranasal instillation (Wang et al., 2008b). The authors noted that the fact that brain tissue
21 Ti concentrations were higher than lung tissue concentrations suggested that the olfactory nerve was the
22 path of transport in this study.

23 For respiratory exposure, the deposition pattern and concentration of particles in the respiratory
24 tract influence the health effects of these particles. Particles of various sizes can have different
25 mechanisms of deposition (Gebhart, 1992; Heyder et al., 1985; Oberdörster et al., 2005a). For
26 nanoparticles, diffusive deposition, also known as thermodynamic deposition or diffusion (due to
27 Brownian motion), predominates, whereas for particles larger than 1 µm, aerodynamic deposition
28 predominates. Between 0.1 and 1 µm, the combined effects of aerodynamic and diffusive deposition are
29 important.

30 Oberdörster et al. (2005a) summarized the principles and models of respiratory tract nanoparticle
31 deposition and retention in the lung. Modeling of humans who are resting and breathing through the nose
32 indicated that for 1-nm particles, about 90% will be deposited in the nasal, pharyngeal, and laryngeal

1 region; about 10% in the tracheobronchial region; and almost none in the alveolar region. These results
 2 contrast with a 5-nm particle, which is deposited roughly equally in the three regions. About 50% of
 3 larger, 20-nm particles are deposited in the alveolar region, with about 15% deposition in each of the
 4 other two regions.

5 In contrast, a model that incorporates convective flow and axial diffusion predicted that very few
 6 small nanoparticles would deposit in the alveolar area (Asgharian and Price, 2007). Nanoparticles less
 7 than 10 nm in diameter were predicted to deposit mainly in the tracheobronchial airway, and very few
 8 nanoparticles smaller than 5 nm would reach the alveolar region (Asgharian and Price, 2007). Depending
 9 on particle size, consideration of axial diffusion and dispersion can result in increased predicted
 10 deposition in the alveolar region of up to 10%.

11 Inhaled nano-TiO₂ persisted in the lung longer than fine TiO₂ in rats (Oberdörster et al., 1994).
 12 After 12 weeks of inhalation (6 hours/day, 5 days/week) of approximately equivalent mass concentrations
 13 of fine TiO₂ (22.3 ± 4.2 mg/m³) and nano-TiO₂ (23.5 ± 2.9 mg/m³), the total retained lung burdens were
 14 6.62 ± 1.22 mg for fine TiO₂ and 5.22 ± 0.75 mg for nano-TiO₂. The estimated retention half-times were
 15 174 days for fine TiO₂ and 501 days for nano-TiO₂ (Oberdörster et al., 1994).

16 In animal studies of nano-TiO₂ disposition (Table 4-3), 13 weeks of inhalation exposure to nano-
 17 TiO₂ increased TiO₂ burden in lymph nodes in rats (2 and 10 mg/m³), mice (10 mg/m³), but not in
 18 hamsters (at up to 10 mg/m³) (Bermudez et al., 2004).

Table 4-3. Nano-TiO₂ disposition in animals after inhalation or intratracheal instillation of nano-TiO₂.

Species/strain	Aerosol	Study Protocol	Observations	Reference
Fischer 344 rats, females (6 wks) B3C3F1 mice, females (6 wks) Hamsters, females (6 wks)	TiO ₂ : 1.29–1.44 μm MMAD (σ _g = 2.46–3.65), 21-nm primary particles	Animals exposed via inhalation 6 hours per day, 5 days per week, for 13 weeks to 0.5, 2, and 10 mg/m ³ . Control animals exposed to filtered air. Animals sacrificed at 0, 4, 13, 26, and 56 days (49 for hamsters) post exposure. Groups of 25 animals per species and time point.	TiO ₂ pulmonary retention half-times for the low-, mid-, and high-exposure groups, respectively: 63, 132, and 365 days in rats; 48, 40, and 319 days in mice; and 33, 37, and 39 days in hamsters. Burden of TiO ₂ in lymph nodes increase with time post exposure in mid- and high-dosed rats, and in high-dosed mice, but was unaffected in hamsters at any time or in any dosage group. In high-exposure groups of mice, epithelial permeability remained elevated (~2 x control groups) out to 52 weeks without signs of recovery. Epithelial permeability was 3 to 4 x control in high exposure group rats through 4 weeks post exposure, but approached control by 13 weeks. Epithelial permeability was unaffected in all groups of hamsters.	Bermudez et al. (2004)

Table 4-3. Nano-TiO₂ disposition in animals after inhalation or intratracheal instillation of nano-TiO₂ (continued).^a

Species/strain	Aerosol	Study Protocol	Observations	Reference
Wistar rats, 20 adult males, 250±10 g	TiO ₂ (22-nm CMD, $\sigma_g = 1.7$) Spark generated 0.11 mg/m ³ 7.3×10^6 particles/cm ³	Rats exposed 1 hour via endotracheal tube while anesthetized and ventilated at constant rate Lungs fixed at 1- or 24-hours post exposure	Distributions of particles among lung compartments followed the volume distribution of compartments and did not differ significantly between 1- and 24-hours post-exposure. On average, 79.3±7.6% of particles were on the luminal side of the airway surfaces, 4.6±2.6% in epithelial or endothelial cells, 4.8±4.5% in connective tissues, and 11.3±3.9% within capillaries. Particles within cells were not membrane-bound.	Geiser et al. (2005)
WKY/NCrl (Charles River) rats, 5 young adult males, 250±10 g	TiO ₂ (22-nm CMD, $\sigma_g = 1.7$) Spark generated	Rats exposed 1 hour via endotracheal tube while anesthetized and ventilated at constant rate Lungs fixed immediately post exposure	Of particles in tissues, 72% were aggregates of 2 or more particles; 93% of aggregates were round or oval; 7% were needle-like. The size distribution of particles in lung tissues (29 nm CMD, $\sigma_g = 1.7$) was remarkably similar to the aerosol; the small discrepancy could have been due to differences in sizing techniques. A large 350-nm aggregate was found in a type II pneumocyte, a 37-nm particle in a capillary close to the endothelial cells, and a 106-nm particle within the surface-lining layer close to the alveolar epithelium	Kapp et al. (2004)

^a CMD – Count median diameter; MMAD – Mass median aerosol diameter; σ_g – Geometric standard deviation

Source: U.S. EPA (2008b).

4.6.2. Dermal

1 Because sunscreen is used on the skin, human skin penetration of nano-TiO₂ (as particles in
2 vehicles or in sunscreens) has been discussed in several reports and reviews (NANODERM, 2007;
3 Nohynek et al., 2007; TGA, 2006). Most dermal exposure studies reviewed used human skin and pig
4 skin; several were in vivo studies in humans. Compared to other routes of exposure, dermal exposure
5 may be more directly relevant in assessing potential health effects associated with its use in sunscreens, at
6 least for unflexed skin from healthy adults.

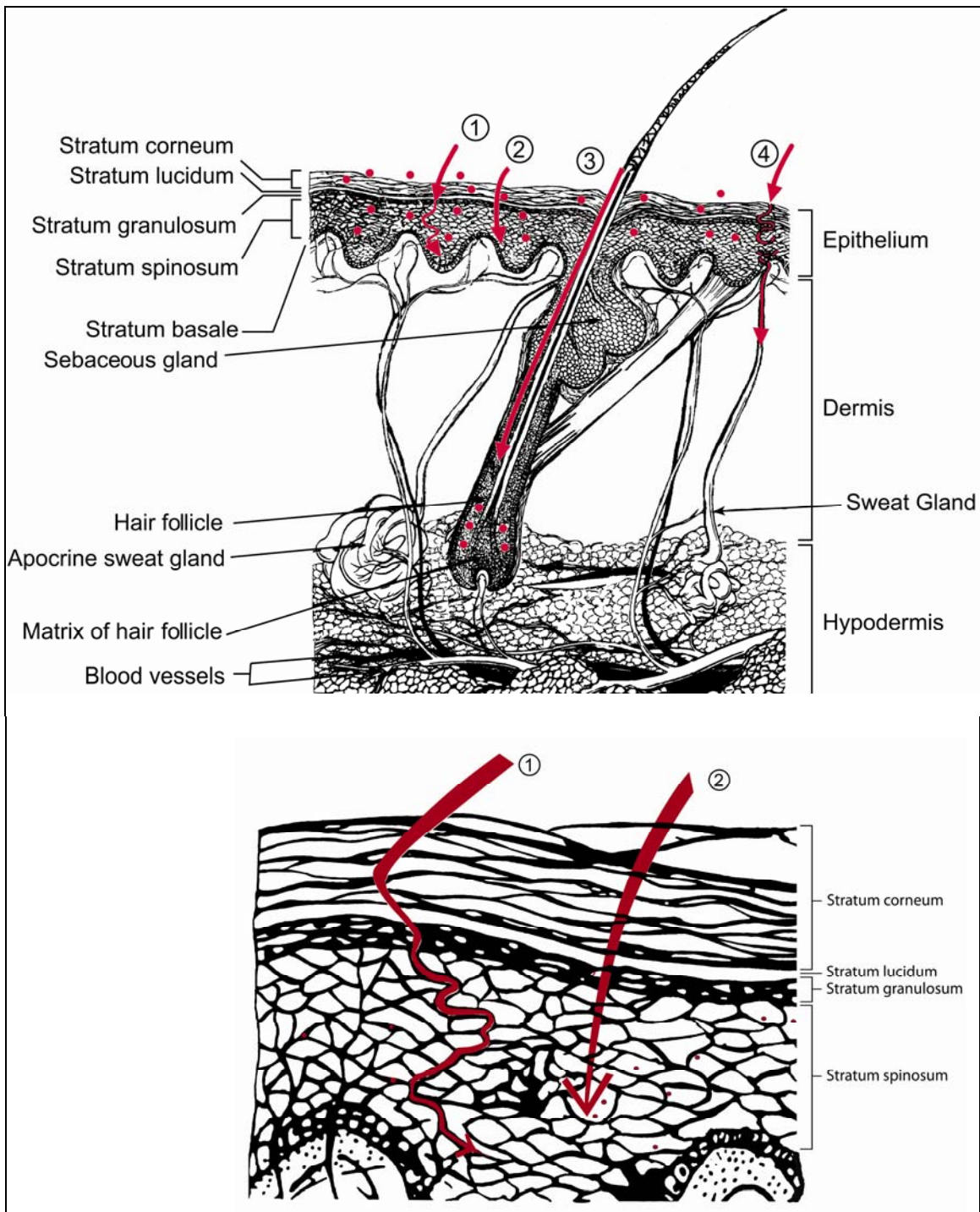
7 Because of the relatively noninvasive nature of skin penetration testing, several laboratory studies
8 have focused on skin absorption in humans, rather than animals. Human skin regulates the penetration of
9 contaminants primarily through the stratum corneum layer, which contains keratinized cells and has no
10 blood vessels. The thickness of the layer varies, ranging from hundreds of micrometers to several
11 micrometers in different parts of the body. Published studies indicate the stratum corneum of full-term
12 infants and babies is comparable to that of adults (Fairley and Rasmussen, 1983); such is not the case with
13 pre-term infants (Kalia et al., 1998). Skin studies include a range of experimental conditions, including in
14 vivo and ex vivo / in vitro. With few exceptions discussed below (Kertész et al., 2005; Menzel et al.,
15 2004; Sadrieh et al., 2008), most of these studies (Table 4-4) found clear evidence that nano-TiO₂ does not

1 penetrate beyond the stratum corneum or hair follicles, and does not penetrate into living cells of healthy
2 skin (Figure 4-1).

3 In healthy human skin, topically applied nano-TiO₂ penetrates only into the upper layers of the
4 stratum corneum (Table 4-4). The pathways of skin penetration can include intracellular penetration,
5 intercellular penetration, and penetration through hair follicles (Figure 4-1) (Nohynek et al., 2007).
6 Penetration through sweat glands has not been reported, according to one source (page 29 of
7 (NANODERM, 2007)). Although increased skin penetration of other nanomaterials has been reported in
8 flexed skin (Zhang and Monteiro-Riviere, 2008) and in UV-exposed skin (Mortensen et al., 2008), studies
9 of skin penetration in healthy flexed skin or damaged skin are still underway for nano-TiO₂.

10 Nano-TiO₂ was observed in some hair follicles (Lekki et al., 2007), but did not reach the living
11 follicle cells. The presence of nano-TiO₂ in hair follicles is most likely due to mechanical force, such as
12 the movement of the hair during sunscreen application. Nano-TiO₂ in hair follicles might contribute to
13 increased Ti levels in the dermis (Sadrieh et al., 2008) because parts of hair follicles are in the dermis.
14 Nanoparticle loss from hair follicles is expected to be slow because the elimination occurs only by its
15 flowing out with sebum or by its being pushed out with sebum. In a study using a hydrogel formulation
16 containing fluorescence-labeled nanoparticles (Resomer RG 50.50 H, poly(lactide-co-glycolide) on
17 human skin (Mittal and Ravi Kumar, 2009), approximately 15% of total nanoparticles detected in hair
18 follicles 30 minutes after application remained in the hair follicle for 10 days, which is at least 10 times
19 longer than particles remain in the stratum corneum (Lademann et al., 2006).

20 In human skin that is diseased, nano-TiO₂ might penetrate more deeply. The only available study
21 of nano-TiO₂ on skin with dermal lesions was completed on psoriatic skin. Psoriatic skin is a symptom of
22 a chronic, and possibly immune-mediated or genetic, disease called psoriasis. Unlike normal skin cells,
23 which mature and are shed in 28 to 30 days, psoriatic skin cells mature in 3 to 4 days, accumulate on the
24 skin surface (instead of shedding, because new skin develops faster than dead skin sheds), and develop
25 into patches of dead skin (National Psoriasis Foundation, 2006; Pinheiro et al., 2007). Psoriatic skin has a
26 looser corneocyte organization than healthy skin due to the loss of stratum corneum cohesion (Pinheiro et
27 al., 2007). In the Pinheiro et al. (2007) study, nano-TiO₂ in a sunscreen formulation penetrated into
28 deeper areas of the stratum corneum in psoriatic skin than in healthy skin, but not into living cells in
29 either psoriatic or healthy skin (Table 4-4).



Adapted from: Monteiro-Reviere (1991 ; 2004) and Nohynek et al. (2007).

Figure 4-1. Possible pathways of nano-TiO₂ skin penetration.

TOP GRAPHIC – Nanoparticles may penetrate into skin by passing through the (1) intercellular space between cells, (2) skin cells, (3) opening of hair follicles, or (4) opening of sweat glands. Nano-TiO₂ has been seen in the stratum corneum and inside hair follicles, but not in sweat glands.

BOTTOM GRAPHIC – Skin surface (from stratum corneum to stratum granulosum) at a high magnification showing simplified paths of nanoparticles passing through (1) intercellular space and (2) skin cells.

Nanoparticles are not drawn to scale in either graphic.

Table 4-4. Overview of TiO₂ skin absorption/penetration studies.^a

Test Material	Skin Model ^b (Sampling Technique)	Results	Reference	
Sunscreen Formulations Containing Nano-TiO₂				
Nano-TiO ₂ in a sunscreen formulation	Primary particle 17 nm (Kemira, 2000), rutile, Al ₂ O ₃ /stearic acid coated, aggregates 150 to 170 nm (UV-Titan M 160) in an oil-in-water emulsion, provided by L'Oréal (Clichy, France)	Human forearm, repeated application for 4 days (tape stripping, biopsy)	Most particles on and in the upper layers of stratum corneum. In the lower half of the horny layer, only in the openings of hair follicles and sebaceous glands. In deeper tissue, exclusively in the follicle channels. No penetration into living skin.	Lademann et al. (1999)
Sunscreen that contains nano-TiO ₂	Not specified	Human skin (healthy and psoriatic), in vivo, 2 hr (biopsy)	Deeper nano-TiO ₂ penetration in psoriatic skin than in healthy skin. No penetration beyond stratum corneum in both psoriatic and healthy skin.	Pinheiro et al. (2007)
Nano-TiO ₂ in a sunscreen formulation	20-nm nano-TiO ₂ , coated with silicone	Human skin, in vitro, and human skin, in vivo (skin stripping)	Penetration limited to upper layers of stratum corneum. Nanoparticles in skin furrows or follicular opening could be mistaken to be in the epidermal compartment.	Mavon et al. (2007)
Sunscreen that contains nano-TiO ₂	A commercially available sunscreen, hydrophobic emulsion containing nano-TiO ₂ (Anthelios XL SPF 60, La Roche Posay, France)	Human foreskin grafts transplanted onto SCID mice; TiO ₂ emulsion on the graft in occlusion for 1, 24, or 48 hr	TiO ₂ in the corneocyte layers of stratum corneum. In two cases, penetration through the stratum corneum, to the stratum granulosum was observed.	Kertész et al. (2005)
Sunscreen that contains nano-TiO ₂	A commercially available sunscreen, hydrophobic emulsion containing nano-TiO ₂ (Anthelios XL SPF 60, La Roche Posay, France)	Human foreskin grafts transplanted onto SCID mice; TiO ₂ emulsion on the graft at 2 mg/cm ² in occlusion for 24 hours	TiO ₂ in stratum corneum, not in deeper layers of the skin.	Kiss et al. (2008)
Nano-TiO ₂ in sunscreen formulation / Sunscreen that contains nano-TiO ₂	50 to 100 nm, mixture of anatase and rutile, no coating information	Human abdominal skin, in vitro	Penetration limited to upper layers of stratum corneum.	Dussert and Gooris (1997)
Various TiO ₂ in sunscreen formulations	Sunscreen base formulation containing no TiO ₂ or 5% of one of three types TiO ₂ : Micro-sized TiO ₂ Nano-TiO ₂ , uncoated Nano-TiO ₂ , coated with aluminum hydroxide and dimethicone/methicone copolymer	Female Yucatan minipigs (in vivo), 2-mg emulsion/cm ² skin, 5 days per week for 6 weeks (necropsy)	Increased Ti levels in epidermis in all TiO ₂ -treated groups. Increased Ti levels in dermis in some TiO ₂ -treated groups (not specified). No increases in Ti levels in lymph nodes or liver of any treated animals.	Sadrieh et al. (2008)

Table 4-4. Overview of TiO₂ skin absorption/penetration studies (continued).^a

Test Material	Skin Model ^b (Sampling Technique)	Results	Reference
Photostable nano-TiO ₂ in various formulations	Photostable nano-TiO ₂ , needle-like shape, 45–150 nm x 17–35 nm, coated with alumina and silica (Lodén et al., 2006), in the following formulations: (1) Eucerin® Micropigment Crème 15: commercial sunscreen, 5% TiO ₂ concentration (Beiersdorf company) (2) a liposome dispersion: 18% TiO ₂ , containing Phospholipon 90 G and Tioveil AQ-N (Tioxide Specialties Ltd., Billingham, UK) (3) formula SG110: 4.5% TiO ₂ , containing Tioveil AQ-N (4) pure predispersion Tioveil AQ-N: 40% TiO ₂	Pig skin, in vitro Particles on/in the stratum corneum; minimal penetration into stratum granulosum. No penetration into living skin.	Menzel et al. (2004)
Photostable nano-TiO ₂ in sunscreen formulations	(1) T-Lite SF-S: rutile, coated with SiO ₂ and methicone (2) T-Lite SF: rutile, coated with methicone Both primary particles are needle-like: 30–60 nm x 10 nm. Aggregates and agglomerates in water phase, mostly up to 200 nm Both are oil/water emulsions containing 10% TiO ₂	Pig skin, in vitro, up to 24 hours (tape stripping) No penetration beyond stratum corneum. Receptor solution recoveries of 0.8–1.4% of applied dose.	Gamer et al. (2006)
Other Nano-TiO₂ Formulations			
UV-Titan M160®	"Microcrystalline," coated	Human, in vivo Most TiO ₂ in the superficial part of the stratum corneum. Some TiO ₂ in follicles (in the deeper layers of the stratum corneum).	Ref 62, 70 in SCCNFP (2000)
Various nano-TiO ₂ in oil-in-water emulsions	Emulsions contained 4% nano-TiO ₂ , only differed in nano-TiO ₂ types: (1) 20-nm cubic primary particle, coated with trimethyl octylsilane, hydrophobic surface (T805, Degussa) (2) 10–15 nm primary particle, aggregated into ~100-nm needles, coated with Al ₂ O ₃ and SiO ₂ , amphiphilic surface (Eusolex T-2000, Merck) (3) 100-nm needles, coated with alumina and silica, hydrophilic surface (Tioveil AQ-10P, in dispersion, Solaveil)	Human forearm, in vivo, 6 hours (biopsy) Penetration of particles into the upper layers of stratum corneum. No penetration into living skin.	Pflücker et al. (2001) and Schulz et al. (2002)

Table 4-4. Overview of TiO₂ skin absorption/penetration studies (continued).^a

	Test Material	Skin Model ^b (Sampling Technique)	Results	Reference
Anatase (Tioveil AQ-N)	"Microcrystalline," coated with alumina and silica	Human, repeated application (tape stripping)	No penetration beyond the stratum corneum. Some TiO ₂ at the opening of follicles.	Ref. 63 in SCCNFP (2000)
Nano-TiO ₂	10–100 nm, coated with SiO ₂ -, Al ₂ O ₃ -, Al ₂ O ₃ /SiO ₂	Human, in vivo (biopsy)	Particles on or in the outmost surface of the stratum corneum. No penetration into living skin.	Schulz et al. (2002)
Various TiO ₂ and nano-TiO ₂	14-nm to 200-µm, anatase and rutile, coated and uncoated materials	Pig and human skin, in vivo and in vitro (skin stripping or biopsy)	No penetration beyond the stratum corneum in any study.	SCCNFP (2000)
Degussa T805	21 nm, coated with SiO ₂	Human, in vitro	No penetration beyond the stratum corneum.	Ref. 24 in SCCNFP (2000)
Eusolex TA and Eusolex TC	"Microcrystalline," coated	Human, in vitro	No penetration beyond the stratum corneum.	Ref. 25 in SCCNFP (2000)
Eusolex TA and Eusolex TC	"Microcrystalline," coated	Human	No penetration beyond the stratum corneum.	Ref. 26 in SCCNFP (2000)
Hombifine S35	"Microcrystalline," coated	Human, in vitro, and mouse, in vitro	No penetration beyond the stratum corneum.	Ref. 27 in SCCNFP (2000)
Tioveil AQG, Tioveil TG, and Tioveil OP	"Microcrystalline" (though SCCNFP not 100% certain)	Human	No penetration beyond the stratum corneum.	Ref. 29 in SCCNFP (2000)

Table 4-4. Overview of TiO₂ skin absorption/penetration studies (continued).^a

Test Material	Skin Model ^b (Sampling Technique)	Results	Reference	
Degussa T805	21-nm, coated with SiO ₂	Human, in vitro	No penetration beyond the stratum corneum.	Ref. 112 in SCCNFP (2000)
TiO ₂	Mixed particle sizes, mostly less than 10 µm in aqueous solution (range from <2 µm to >20 µm), no coating information, 20% TiO ₂ in water, castor oil, or polyethylene glycol	Rabbit skin, in vivo, 4 hours for 1 day or 2 hours daily for 3 day	Penetration of particles into stratum corneum and outer hair follicles. No penetration into living skin. Uptake of TiO ₂ affected by the vehicle: in castor oil > in water > in polyethylene glycol.	Lansdown and Taylor (1997)
Nano-TiO ₂ in various gels	For ion microscopy study: 20-nm x 100-nm primary particles, coated (photostable UV-filter) (Eusolex® T-2000, Merck). Four formulations: hydrophobic basis gel, isopropyl myristate gel, microemulsion gel, and polyacrylate gel, each containing 5%-weight nano-TiO ₂ particles For autoradiography study: proton-irradiated 20-nm TiO ₂ , rutile (R-HD2, Huntsman), coated with alumina (Huntsman, 2008)	Porcine and human skins, for 30 minutes to 48 hours (biopsy)	After wash with water, nano-TiO ₂ remains on skin, with most in stratum corneum and some in hair follicles. Nano-TiO ₂ observed seen in hair follicles as deep as 400 µm, but not in living cells surrounding the follicles.	Lekki et al. (2007)
TiO₂/Nano-TiO₂ Particles of Unknown Size				
Sunscreen that contains TiO ₂	Not specified	Human (tape stripping)	Particles on or in the outmost layers of the stratum corneum. No penetration into living skin.	Gottbath and Mueller-Goymann (2004)
TiO ₂	Not specified	Mouse, pig, and human skin, in vitro	TiO ₂ detected in the intercellular spaced between corneocytes of the outermost layers of the stratum corneum. No penetration into living skin.	Gontier et al. (2004)
Sunscreen that contains TiO ₂	Sunscreen containing 8% microfine TiO ₂ (size, crystal form, and coating were not specified)	Human skin (13 patients, 59–82 years old), in vivo, applied TiO ₂ sunscreen daily for 9–31 days until 2 days prior to surgical removal of the skin (tape stripping)	Ti concentration in the dermis of patients exposed to sunscreen overlaps with concentration in cadavers (controls). If the highest Ti concentration in cadavers is excluded, sunscreen increased skin Ti concentration. No correlation between the duration of sunscreen application and Ti concentration.	Tan et al. (1996)

^a SCID – Severe combined immunodeficiency; SCCNFP – Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers

^b Topical application unless specified.

1 Mortensen et al. (2008), working with quantum dots rather than TiO₂, reported greater skin
2 penetration following UV exposure and suggested that even mildly sunburned skin might be more
3 susceptible to penetration by nanoparticles of similar size and chemistry to the quantum dots used in their
4 study.

5 Using “microfine” TiO₂, Tan et al. (1996) compared uptake in skin samples from 13 elderly persons
6 (age 59–82 years) with samples from 6 control cadavers (used to determine background exposure). The
7 authors reported some dermal uptake, although they suggested caution when interpreting their results,
8 citing the advanced age of their participants, the fact that skin samples were taken from different
9 locations, and the fact that TiO₂ concentrations were close to analytical detection limits. Kertész et al.
10 (2005) reported penetration of nano-TiO₂ into the stratum granulosum of grafted human foreskin in two
11 samples (of an unknown total number).

12 Penetration of nano-TiO₂ into dermis of minipigs was suggested in a meeting abstract, but the
13 abstract contained insufficient information to determine whether Ti was detected inside the hair follicles
14 or in the living cells of the dermis (Sadrieh et al., 2008). Several other studies that evaluated absorption
15 using pig skin suggest little or no absorption beyond the stratum corneum. In a study using nano-TiO₂ in
16 four formulations on pig skin (Menzel et al., 2004), the authors stated that nano-TiO₂ penetrated through
17 the stratum corneum into the underlying stratum granulosum (but not into stratum spinosum) via
18 intercellular space. The presence of Ti in the dermis, however, was deemed to be an artifact of the
19 preparation process. Other studies using pig skin did not find nano-TiO₂ penetration beyond the stratum
20 corneum (Gamer et al., 2006; Lekki et al., 2007; Pflücker et al., 2001).

21 Some nanomaterials have been shown to penetrate deeper in damaged skin than in intact skin
22 [quantum dots in human skin (Mortensen et al., 2008); nano-silver in murine skin (Larese et al., 2009)],
23 but no experimental data on nano-TiO₂ dermal penetration in damaged skin were found. Preliminary (not
24 yet peer reviewed) data showed that two types of coated nano-TiO₂ topically applied on either
25 dermabraded or intact skin of SKH-1 hairless mice did not increase Ti concentrations in blood, lymph
26 nodes, liver, spleen, or kidney (Gopee et al., 2009). The depth of nano-TiO₂ penetration in either
27 damaged or intact skin was not reported. Hairless mice data, however, do not exclude the possibility that
28 nano-TiO₂ might penetrate deeper into damaged human skin than intact human skin because relative
29 penetration of chemicals between hairless mice and humans varies and could be chemical specific
30 (Benavides et al., 2009; Simon and Maibach, 1998).

4.6.3. Ingestion

31 Currently only three toxicological studies of nano-TiO₂ through oral exposure have been reported
32 (see Section 5.3.1.2.2), and of these, only one (Wang et al., 2007a) reported tissue concentrations of nano-

1 TiO₂. In the Wang et al. (2007a) study, male and female mice received a single oral gavage of 5 g/kg TiO₂
 2 as 25-nm rutile spindles, 80-nm rutile spindles, or 155-nm anatase octahedrons (10 male and 10 female
 3 mice for each type of TiO₂, and negative controls) (Table 4-5). The organs with elevated TiO₂
 4 concentrations (measured only in female mice) were liver, spleen, kidney, lung, and brain. Although the
 5 liver is expected to receive most of the TiO₂ absorbed from the gastrointestinal tract through the portal
 6 vein, elevated TiO₂ levels in the liver were observed only in the 80-nm group. The reason for this size-
 7 specific elevation in hepatic TiO₂ concentration is unknown.

4.6.4. Blood Brain Barrier and Placental Transfer

8 The potential of nanoparticles in general to cross the blood brain barrier (BBB) has been
 9 investigated and developed primarily in relation to drug delivery systems (Beduneau et al., 2007; Emerich
 10 and Thanos, 2007). In addition to size (Sonavane et al., 2008), the surface properties of nanoparticles
 11 influence the potential for a nanomaterial to penetrate the BBB (Singh and Lillard, 2009). Nanoparticles
 12 developed for drug delivery often have ligands conjugated on the surface or other surface modifications to
 13 facilitate cellular uptake (Beduneau et al., 2007).

Table 4-5. Animal studies that measured Ti concentrations in brain after nano-TiO₂ exposures through injection or oral gavage.^a

Nano-TiO ₂	Study design	Findings in the brain	Reference
Nano-TiO ₂ , 25 nm and 80 nm, rutile, uncoated (from Hangzhou Dayang Nanotechnology Co. Ltd., 杭州大洋纳米技术有限公司) Fine TiO ₂ , 155±33 nm TiO ₂ , anatase, uncoated, > 10 wt% at <100 nm (from Zhonglina Chemical Medicine Co., 中联化学制药有限公司) (Chen, 2008)	Single oral gavage at 5 g/kg to male and female CD-1(ICR) mice Ti content was measured 2 weeks after gavage by ICP-MS with a detection limit of 0.074 ng/mL	Ti concentrations in brain were increased in all three TiO ₂ treatment groups compared to negative controls. The increase was smaller in the 25-nm group than the 155-nm group, while the 80-nm group had the same increase as the 155-nm group. Vacuoles in the neuron of hippocampus, suggesting fatty degeneration, observed in the 80-nm (but not typical) and 155-nm (frequently) groups, but not in the 25-nm group.	Wang et al. (2007a)
Nano-TiO ₂ , 20-30 nm, 17% anatase, 30% rutile, uncoated, BET surface area 48.6 m ² /g	Single i.v. injection at 5 mg/kg BW through the tail vein of male Wistar rats TiO ₂ concentrations in the brain were measured on days 1, 14, and 28 by ICP-AES with a Thermo Jarrell Ash "IRIS 1" spectrometer with a detection limit of 0.5 µg/organ	TiO ₂ was not detected in the brain at any tested time points.	Fabian et al. (2008)
Nano-TiO ₂ , 15 nm, rutile, coated with silica (27.5 wt%)	Single i.v. injection at approximately 60 mg/kg BW through the tail vein of male ddY mice Ti concentrations in brain were measured at 5 minutes, 72 hours, and 1 month after injection by ICP-MS with an unspecified detection limit	No increase of Ti in the brain of treated mice was observed compared to negative controls at any tested time points.	Sugibayashi et al. (2008)

Table 4-5. Animal studies that measured Ti concentrations in brain after nano-TiO₂ exposures through injection or oral gavage (continued).^a

Nano-TiO ₂	Study design	Findings in the brain	Reference
Nano-TiO ₂ , 5 nm, anatase Conventional TiO ₂ Both types of TiO ₂ were made from controlled hydrolysis of titanium tetranitoxide.	Multiple i.p. injection to female CD-1 (ICR) mice once per day for 14 days with nano-TiO ₂ at 5, 10, 50, 100, and 150 mg/kg BW or conventional TiO ₂ at 150 mg/kg BW Ti concentration was measured 14 days after the treatment began by ICP-MS with a detection limit of 0.076 ng/mL	Ti concentrations in the brain increased with increasing nano-TiO ₂ doses. All TiO ₂ treatments increased Ti concentration in the brain, as compared to negative controls. At 150 mg/kg, brain Ti concentration was higher in the nano-TiO ₂ group than in the conventional TiO ₂ group.	Liu et al. (2009)
Nano-TiO ₂ , 25-70 nm, anatase, surface area 20-25 m ² /g, purity 99.9% (from Sigma-Aldrich)	Subcutaneous (s.c.) injections of 100 µL of 1 mg/mL nano-TiO ₂ (i.e., 0.1 mg nano-TiO ₂) each time per pregnant Slc:ICP mice once per day at 3, 7, 10 and 14 days post-mating. Presence of nano-TiO ₂ in the brain was assessed in the male offspring at age of 4 days and 6 weeks by FE-SEM/ EDS	Nano-TiO ₂ particles were seen in the brain (olfactory bulb and the cerebral cortex – frontal and temporal lobes) of the 6-week-old mice from nano-TiO ₂ -exposed dams. (Results from 4-day-old mice were not reported.) Markers of apoptosis (activation of caspase-3 and crescent-shaped cells), occlusion of small vessels, and perivascular edema observed in the brain of 6-week-old mice from nano-TiO ₂ -exposed dams.	Takeda et al. (2009)

^a BET – Brunauer, Emmett, Teller method of calculating surface area

BW – Body weight

FE-SEM/EDS – Field emission-type scanning electron microscopy/energy dispersive X-ray spectrometry

ICP-AES – Inductively coupled plasma atomic emission spectrometry

ICP-MS – Inductively coupled plasma-mass spectrometry

i.p. – Intraperitoneal

i.v. – Intravenous

s.c. – Subcutaneous

1 Increased Ti concentrations in the brain were observed in mice 2 weeks after they were exposed to
2 fine and nano-TiO₂ through a single oral gavage (Wang et al., 2007a), and in mice at the end of exposure
3 to nano-TiO₂ through once-daily intravenous injections for 14 days (Liu et al., 2009) (Table 4-5). No
4 increase in Ti concentration in the brain was observed in rats or mice exposed to nano-TiO₂ through a
5 single intravenous injection (Fabian et al., 2008; Sugibayashi et al., 2008). Due to the variations in tested
6 nano-TiO₂, treatment regimen, and other experimental design elements, no specific characteristic of nano-
7 TiO₂ or its administration has been identified as determining factors for BBB penetration.

8 A recent study showed TiO₂ particles and pathological changes in the brain of 6-week-old mice
9 from nano-TiO₂ exposed dams (Takeda et al., 2009) (Table 4-5), suggesting that nano-TiO₂ might be
10 passed through undeveloped or developing BBB in embryos or young mice. Because the dams were
11 exposed to nano-TiO₂ during pregnancy and the offspring were tested at 4 days and 6 weeks of age, the
12 nano-TiO₂ exposure to the offspring could have been in utero (i.e., nano-TiO₂ could penetrate the
13 placental barrier) or through milk, which was not tested in this study. In addition to the brain, nano-TiO₂
14 particles and pathological changes were also observed in the reproductive system of male offspring of
15 nano-TiO₂-exposed dams (female offspring were not studied) (Takeda et al., 2009). Although no data on
16 humans for nano-TiO₂ and placental barrier were located, an ex vivo study using perfused human
17 placentas showed that nano-gold (PEGylated gold nanoparticles at 15 and 30 nm) did not cross the
18 placenta into the fetal circulation at the tested condition (Myllynen et al., 2008). Nano-gold might behave

1 differently from nano-TiO₂, given that uncoated nano-gold does not penetrate either the BBB or placental
2 barrier in mice (Sadauskas et al., 2007), whereas nano-TiO₂ does pass to BBB in mice (Liu et al., 2009;
3 Wang et al., 2007a).

4.6.5. Dose-Metrics

4 Quantitative risk assessment requires dose-response relationships. Selecting a measure of dose that
5 is appropriate for nanoparticle toxicity has drawn attention from both researchers and risk assessors. No
6 one metric is recommended in this case study, but supporting evidence for various selections of a dose
7 metric is noted. The criterion for selecting a “good” dose metric is often based on generating a consistent
8 dose-response relationship. However, an appropriate dose metric need not constitute measurement of
9 only one physicochemical property (such as surface area, mass, or number of particles). Although dose
10 metrics based on one property, such as mass concentration, have been used successfully in toxicology, a
11 combination of measurements of two or more physicochemical properties also might be appropriate for
12 use in assessing nanomaterial toxicity.

13 Total particle surface area, which is closely related to primary particle size, has been suggested as a
14 suitable dose metric for inhalation and instillation studies (Faux et al., 2003; Liao et al., 2008;
15 Oberdörster et al., 2005). Although two distinctive dose-response curves for fine TiO₂ and nano-TiO₂ can
16 be drawn based on mass concentration, certain observed respiratory effects of fine TiO₂ and nano-TiO₂
17 have been shown to fit well with a single linear dose-response curve based on primary particle surface
18 area, even where both types of particles agglomerated to about 0.7 μm in diameter (Oberdörster et al.,
19 1994). Hext et al. (2005) found that, compared to gravimetric lung burden (particle mass per lung mass),
20 administered primary particle surface area correlated better with lung burdens, clearance half-lives, and
21 certain biological responses in rats, mice, and hamsters. However, the evidence on this issue is somewhat
22 mixed. For instance, biological responses after exposure to similarly-sized agglomerates of fine TiO₂ and
23 nano-TiO₂ were similar in severity according to Warheit et al. (2007c; 2006); by contrast, Sager and
24 Castranova (2009) found that well-dispersed nano-TiO₂ yielded greater effects than well-dispersed fine
25 TiO₂

26 As mentioned previously, any one or more of various characteristics, including particle number,
27 size (including agglomerations or aggregations), shape, crystalline form, mass, surface area, and surface
28 modifications, could play a role in nano-TiO₂ toxicity. Including one or more of these factors in the dose
29 metric could be a better choice than surface area alone. For instance, based on administered primary
30 particle surface area, the data used in the Hext et al. study (2005) – the increases in the numbers of
31 pulmonary polymorphonuclear neutrophil (PMN) due to exposure to anatase fine and nano-TiO₂
32 (Oberdörster et al., 1994) and rutile fine TiO₂ (Tran et al., 1999) – would better fit two dose-response

1 curves (one each for anatase TiO₂ and rutile TiO₂), instead of one dose-response curve. Similarly, a recent
2 study of pulmonary effects of intra-tracheal instilled rutile fine TiO₂ and 80% anatase/20% rutile nano-
3 TiO₂ (Sager et al., 2008) showed that when dose was normalized to surface area of the particles
4 administered, the dose-response curves for inflammogenic responses were not statistically different
5 between fine and nano-TiO₂, but the anatase-rutile nano-TiO₂ always yielded greater (1.3- to 2-fold)
6 responses than the rutile fine TiO₂.

7 Due to limited toxicological data from oral or dermal exposure to nano-TiO₂, the choice of dose
8 metric for these exposure routes has not been widely discussed. For in vitro studies, nanoparticle
9 concentration (mass or surface area) is often used to express dose. In vitro cytotoxicity, however, has
10 been reported to be affected by both the concentration and the total mass (or total number or total surface
11 area, since these three are closely related) of nanoparticles (Lison et al., 2008). In the Lison et al. study
12 (2008), when cells were cultured in various volumes of a medium containing the same amount of nano-
13 silica (same mass/number/surface area), higher toxicity occurred in a lower volume of medium, that is, in
14 higher nano-silica concentrations. When the medium contained the same concentrations of nano-silica,
15 higher toxicity occurred in cells cultured with a higher volume of medium than lower volume of medium.

16

Questions about Exposure–Dose Characterization

- 4-1. Which sources, pathways, and routes pose the greatest exposure potential to nano-TiO₂ for biota? ...for humans?
- 4-2. What is the potential for biota and human (both occupational and general population) exposure to secondary contaminants (e.g., waste or transformation products) associated with the entire life cycle of water treatment or sunscreen applications of nano-TiO₂?
- 4-3. Do particular species of biota and populations of humans have greater exposure potential (e.g., high-end exposures due to unusual conditions or atypical consumption)? In particular, do children get a higher exposure and/or dose?
- 4-4. What is the total population that could be exposed to nano-TiO₂ via drinking water? ...via topical sunscreens?
- 4-5. Approximately how many workers are involved in nano-TiO₂ production, distribution, and use?
- 4-6. What concentrations, routes, frequencies, and durations characterize worker exposures to nano-TiO₂ across the life cycle and within certain stages (e.g., manufacturing)?
- 4-7. What management practices exist to control occupational exposures to nano-TiO₂?
- 4-8. What personal protective equipment do workers use at the various life cycle stages of nano-TiO₂ applications? How effective is such equipment in controlling exposures by all routes?
- 4-9. Are occupational monitoring methods available or in place for all relevant stages of the life cycle for nano-TiO₂ applications?
- 4-10. Are available methods adequate to characterize nano-TiO₂ exposure via air, water, and food? What properties of nano-TiO₂ should be included in such exposure characterizations?
- 4-11. Given the potential for greater uptake of certain substances in the presence of nano-TiO₂, should monitoring and exposure studies include a suite of substances that might interact with nano-TiO₂?
- 4-12. What happens when nano-TiO₂ is trapped in the stratum corneum and the dead skin flakes off? Is there a potential for dead-skin nano-TiO₂ to settle around households, or be inhaled? How much might accumulate after a day (or a few days) in the sun (and numerous reapplications)?
- 4-13. Since nano-TiO₂ may increase the uptake of other pollutants, such as arsenic, would nano-TiO₂ be a greater concern for exposure and ecological effects in areas with high concentrations of certain pollutants than in other areas? If so, how do we predict or identify such “hot spots?”
- 4-14. Which, if any, exposure models have been evaluated for applicability to nano-TiO₂?
- 4-15. Which physiologically-based pharmacokinetic models are optimal for understanding absorption, distribution, and elimination of nano-TiO₂ in humans?

Questions continued on next page.

Questions about Exposure–Dose Characterization

- 4-16. Are exposure-dose models available (and adequate) to quantitatively extrapolate the exposure used in animal toxicology studies (by inhalation, instillation, oral, dermal, and in vitro) to the human exposure that would result in an equivalent dose to the target of interest?
- 4-17. What is the potential for nano-TiO₂ to transfer to or accumulate in the food web and cause adverse effects on ecological receptors?
- 4-18. Nano-TiO₂ has been shown to attach to the surfaces of algae and fish as well as bioaccumulate in fish. Does nano-TiO₂ biomagnify?

Chapter 5. Characterization of Effects

1 The preceding chapters have laid a foundation for the present chapter by providing an exposure
2 context for characterizing the effects of nanoscale titanium dioxide (nano-TiO₂) used for water treatment
3 and in topical sunscreens. This chapter provides information on the factors that influence nano-TiO₂
4 ecological and health effects (Section 5.1), the ecological effects of nano-TiO₂ (Section 5.2), and the
5 toxicological and human health effects of nano-TiO₂ (Section 5.3). Whether there are specific by-
6 products (e.g., waste and transformation products) or interactions with other substances that should or can
7 be evaluated has not yet been determined. For this reason, the focus of this chapter is on nano-TiO₂.

8 Although literature exists on the effects of conventional TiO₂ on humans and laboratory animals
9 [for a review, see NIOSH (2005)], comparatively less information is available on the effects of nano-TiO₂.
10 Consistent with studies of other nanomaterials (Ostrowski et al., 2009), most nano-TiO₂ studies have
11 investigated the ecological or health effects of nano-TiO₂ itself, and relatively few have investigated the
12 ecological or health effects of end-use products containing nano-TiO₂ or their life-cycle by-products.

13 The physicochemical characteristics of nano-TiO₂ could be important to the biological effects of
14 these materials (Section 5.1), yet those characteristics frequently are not evaluated or reported as part of
15 studies of such effects. This observation should serve as a caveat in examining and interpreting the
16 results described throughout this chapter.

17 The following sections are not meant to be an exhaustive review of the ecological and human
18 health effects literature for nano-TiO₂. Instead, this chapter is intended to highlight recent work on the
19 effects of nano-TiO₂ and to identify current knowledge status and gaps in information needed for
20 assessing potential risks of nano-TiO₂ in water treatment and sunscreen.

5.1. Factors that Influence Ecological and Health Effects of Nano-TiO₂

21 The large number of variables associated with nano-TiO₂ material itself and its ecological and
22 health effects makes it extremely difficult to identify the primary characteristic(s) of nano-TiO₂
23 contributing to an effect or to compare the importance of different characteristics to such effects. A
24 common statement from early studies is the announcement of size effects (or the lack of size effects) from
25 nano-TiO₂ of different crystalline forms or anatase/rutile ratios. That size alone does not account for the
26 effects of nano-TiO₂, however, is now generally accepted; other factors, such as shape, surface chemistry,

1 photoreactivity, and other characteristics, could also play a role in these effects (Gonzalez et al., 2008;
2 Hassellöv et al., 2008; Powers et al., 2006). With the advance of nanoparticle synthesis, the influence of
3 different physicochemical characteristics of nano-TiO₂ has been investigated using well-characterized
4 nano-TiO₂ and better control of variables in recent studies (Jiang et al., 2008).

5 Three categories of factors (nano-TiO₂ physicochemical characteristics, experimental conditions,
6 and environmental conditions) that could influence the ecological and toxicological or health effects of
7 nano-TiO₂ are discussed here in Section 5.1. These are not the only factors of potential importance. As
8 noted previously, exposure route can play a major role in the effects of nano-TiO₂, and the importance of
9 this is reflected in the fact that much of the information in this chapter is organized around environmental
10 media and routes of exposure. Host effects, particularly species differences, can also play an important
11 role in the effects of nano-TiO₂. For example, skin penetration is greatest in rabbits, followed by rats,
12 pigs, monkeys, and humans (Nohynek et al., 2007). However, little information is available on these
13 species differences or on differences in susceptibility of different cell types to nano-TiO₂ effects (Kiss et
14 al., 2008). The phenomenon of pulmonary particle clearance “overload” and subsequent effects in rats
15 and mice are much more understood and are discussed in Section 5.3.1.2.3. In the following sections, the
16 order in which factors are presented does not imply relative importance. This section focuses on factors
17 that have been shown to be important for nano-TiO₂, but findings related to other types of nanomaterials
18 are noted where relevant.

5.1.1. Nano-TiO₂ Physicochemical Characteristics

19 Size, crystal structure, and surface chemistry (such as coating) are among the factors that influence
20 nano-TiO₂ effects. Other physicochemical properties, such as shape (Warheit et al., 2006; Yamamoto et
21 al., 2004), manufacturing process, doping, and purity (or impurities) could also play a role in nano-TiO₂
22 toxicity, but such information is usually not reported in ecological and toxicological studies. Contributing
23 to this lack of reported characteristics are limitations in the availability of analytic methods for
24 characterizing such nanomaterials. Databases describing detailed nanoparticle properties and health
25 effects are being developed (Miller et al., 2007a).

26 The need for characterization of nanomaterials used in toxicity studies has been noted in reports
27 and journal articles, with possible attributes for minimal characterization including chemical composition,
28 size and size distribution (for primary particles and agglomerates), shape, specific surface area, and
29 number of particles per unit mass (Department for Environment Food and Rural Affairs, 2007; Powers et
30 al., 2006; Powers et al., 2007; Warheit et al., 2007a). For more information on nanomaterial
31 physiochemical characteristics that could affect ecological and toxicological effects, readers are referred

1 to reports listing recommended information to be included in nanomaterial studies (OECD, 2008;
2 Attachment 5 of Taylor 2008; Warheit et al., 2007c).

5.1.1.1. Size

3 Size is a main determining factor for the distribution of (inhaled or instilled) nano-TiO₂ in and
4 outside of the respiratory tract (Oberdörster et al., 2004). For particles with a diameter less than 100 nm,
5 the smaller the particles are, the more total particle deposition in the respiratory tract and deposition in
6 nasopharyngolaryngeal regions (Oberdörster, 2000). Smaller sizes, however, do not always result in more
7 deposition in other regions of the respiratory tract. For example, the highest percentages of alveolar
8 deposition have been observed in nanoparticles of about 20 nm in size, and the highest percentages of
9 tracheobronchial deposition were observed in nanoparticles 1–10 nm in size (Oberdörster, 2000).
10 Furthermore, particles less than 200 nm in size can be transported from olfactory mucosa to the olfactory
11 bulb of the brain via the olfactory nerve (Elder et al., 2006). Exposures to nano-TiO₂ (with mean
12 diameters of 21.05 ± 5.08 nm, 71.43 ± 23.53 nm, and 154.98 ± 32.98 nm) through intranasal instillation
13 increased titanium concentrations in the olfactory bulb in mice (Wang et al., 2005; Wang et al., 2007a),
14 and two types of nano-TiO₂ particles (80-nm rutile and 155-nm anatase) were found to increase Ti
15 concentrations in hippocampus, central cortex, and cerebrum, in addition to olfactory bulb, in mice after
16 repeated intranasal instillation (Wang et al., 2008b).

17 Jiang et al. (2008) investigated the size effects of nano-TiO₂ on reactive oxygen species (ROS)
18 generation per unit of particle surface area. Using nine different sizes (4–195 nm) of anatase nano-TiO₂,
19 the investigators found that the highest levels of ROS generation per unit surface area were generated by
20 30-nm and larger particles. For nano-TiO₂ less than 30 nm, the ROS generation per surface area
21 decreased with decreasing particle diameter down to 10 nm, below which it was constant (Jiang et al.,
22 2008).

5.1.1.2. Crystallinity

23 TiO₂ crystalline forms also influence TiO₂ and nano-TiO₂ photoreactivity, reactive species
24 generation, and toxicity. Nano-TiO₂ containing more anatase tends to generate more free radicals and
25 induce more toxicity (e.g., cytotoxicity, inflammatory response) than nano-TiO₂ containing more rutile
26 (Hidaka et al., 2005; Sayes et al., 2006; Uchino et al., 2002). The influence of crystal forms of nano-TiO₂
27 on ROS generation was investigated using a fixed total surface area by Jiang et al. (2008), who tested 13
28 nano-TiO₂ particles of varying crystallinity, all within the size range of 42–102 nm. Size was found not to
29 affect ROS generation per unit surface area in this study. The researchers found that the ROS generation

1 per unit surface area was highest in amorphous nano-TiO₂, followed by anatase and then nano-TiO₂
2 containing both anatase and rutile, and was lowest in rutile nano-TiO₂ (Jiang et al., 2008). This finding is
3 consistent with those of a study investigating unusually fast weathering (loss of gloss) or degradation of
4 surface coating on steel roofing, associated with sunscreens left by workers during installation (Barker
5 and Branch, 2008). Nano-TiO₂ in the coating-damaging sunscreens was an anatase/rutile mixture,
6 whereas nano-TiO₂ in the one sunscreen that did not accelerate loss of gloss was pure rutile (Barker and
7 Branch, 2008).

8 The cytotoxicity of anatase and anatase-mixtures was further increased by UV illumination.
9 Anatase nano-TiO₂ can be 100 times more cytotoxic under UV than rutile of similar size (Sayes et al.,
10 2006). The hydroxyl (·OH) radical production by nano-TiO₂ in cultured cells was found to depend on the
11 crystalline form and size, but differences in OH radical production were not explained by the differences
12 in UV-A absorption between anatase and rutile (Uchino et al., 2002). Smaller particles that contain more
13 anatase, however, are not always more toxic either in vitro (Sayes et al., 2006) or in vivo (Warheit et al.,
14 2006) than larger particles containing more rutile.

5.1.1.3. Surface Chemistry

15 Although coatings have been used to decrease the photoreactivity of nano-TiO₂ intended for
16 sunscreen (see Section 2.2.2), coatings affect more than photoreactivity. Coatings for nano-TiO₂ particles
17 can be designed to reduce agglomeration/aggregation, which in turn affects the behavior of the particles in
18 various media, including sedimentation. This also affects the exposure to organisms living in different
19 parts of water bodies or feeding on different sized particles. Particle surface modifications can also
20 change the effects of nano-TiO₂ on living cells, tissues, or organisms. Using in vitro methods, Serpone et
21 al. (2006) reported that a “thermally assisted” modification of the TiO₂ particle surface reduced
22 photocatalytic activity, which in turn decreased (if not eliminated) toxicity to DNA plasmid, human cells,
23 and yeast. In rats intra-tracheally instilled with two types of nano-TiO₂ having the same core material, the
24 nano-TiO₂ with a hydrophobic surface (Aeroxide[®] T805, silanized) caused a slightly lower bioactivity
25 than hydrophilic P25, although the authors concluded that silanization⁶ did not “lead to remarkable
26 differences in lung reaction” (Rehn et al., 2003).

⁶ Silanization is the covering of a surface that has hydroxyl (OH) with molecules that contain only silicon and hydrogen (silane), such as SiH₄. Silanization is one type of surface modification applicable to particles, such as metal oxides, and can render the particle surface chemically inert.

5.1.1.4. Recommended Characterization of Nanomaterial for Ecological and Toxicological Studies

1 As noted in Chapter 1, nanomaterials, and nano-TiO₂ in particular, can be characterized in several
 2 ways in terms of physicochemical properties (see Table 1-1). Given that the relationship between such
 3 properties and the behavior and effects of nanomaterials, including nano-TiO₂, remains to be fully
 4 understood, it might seem desirable for researchers to characterize every possible property of the material
 5 they are investigating. In practice, this is not feasible. Consequently, recommendations for
 6 characterization of nanomaterials have come forth from time to time.

7 For in vitro studies, Murdock et al. (2008) recommended characterizing nanomaterial dispersion in
 8 solution for (in no specific order) particle size and size distribution; particle morphology; particle
 9 composition; surface area; surface chemistry; particle reactivity; agglomeration; zeta potential; and
 10 impact of sonication. For “hazard studies with nanoparticle-types”, Warheit (2008a) prioritized the
 11 characterization needs as (highest priority first): (1) particle size and size distribution (wet state) and
 12 surface area (dry state) in the relevant media in the relevant media; (2) crystal structure/crystallinity; (3)
 13 aggregation status in the relevant media; (4) composition and surface coatings; (5) surface reactivity; (6)
 14 method of nanomaterial synthesis and/or preparation; and (7) purity of sample.

15 An expert working group convened by the International Life Sciences Institute (ILSI) Research
 16 Foundation/Risk Science Institute recommended measuring mass, size distribution, surface area, and
 17 number for exposure characterization in inhalation studies (Table 5-1), and 17 measurements/aspects for
 18 off-line nanomaterial characterization for toxicological studies (Table 5-2) (Oberdörster et al., 2005b).

Table 5-1. Recommendations for measuring exposure during inhalation studies.

Metric Measurement	Recommendation	
	Off-line	On-line
Mass	E (coupled with on-line)	E
Size distribution	E	E/D
Surface area	O	O
Number	N	E

E – These measurements are considered to be essential.

D – These measurements are considered to provide valuable information, but are not recommended as essential due to constraints associated with complexity, cost and availability.

O – These measurements are considered to provide valuable but nonessential exposure information.

N – These measurements are not considered to be of significant value to inhalation studies.

Source: Modified from Oberdörster et al. (2005b)

Table 5-2. Recommendations for off-line nanomaterial characterization for toxicological studies.

Characterization	Human Exposure	Toxicity Screening Studies		
		Supplied Material	Administered Material	Material in vivo / in vitro
Size distribution (primary particles)	E (combine with agglomeration state)	E	D	D
Shape	E	E	O	O
Surface area	D	E	D	O
Composition	E	E	O	O
Surface chemistry	D	E	D	D/O
Surface contamination	D	N	D	N
Surface charge – suspension/solution	O	E	E	O
Surface charge – powder (use bio fluid surrogate)	O	E	N	O
Crystal structure	O	E	O	O
Particle physicochemical structure	E	E	D	D
Agglomeration state ^a	E	N	E	D
Porosity	D	D	N	N
Method of production	E	E	--	--
Preparation process	--	--	E	--
Heterogeneity ^b	D	E	E	D
Prior storage ^c of material	E	E	E	--
Concentration	E	--	E	D

E – These characterizations are considered to be essential.

D – These characterizations are considered to provide valuable information, but are not recommended as essential due to constraints associated with complexity, cost and availability.

O – These characterizations are considered to provide valuable but non-essential information.

N – These characterizations are not considered to be of significant value to screening studies.

^a As primary particle, secondary particle (primary particle agglomerates and self-assembled structures) and tertiary structure (assemblies of secondary structures). When possible, material agglomeration or de-agglomeration in different liquid media should also be characterized.

^b Time and conditions, including temperature, humidity, exposure to light and atmosphere composition

^c Ratios of different components

Source: Reprinted from Oberdörster et al. (2005b).

- 1
- 2 Three factors figured into these recommendations: “the context within which a material is being
- 3 evaluated, the importance of measuring a specific parameter within that context, and the feasibility of
- 4 measuring the parameter within a specific context” (Oberdörster et al., 2005b).

5.1.2. Experimental Conditions

- 5 Experimental conditions, particularly the choice of media/vehicle in which to disperse nano-TiO₂,
- 6 preparation of testing solutions or suspensions, and the formation of aggregates, can influence the
- 7 behavior and effects of nano-TiO₂ and other nanomaterials. The advantages and disadvantages of various

1 dispersion preparation methods are compared in a recent publication of nanomaterial ecotoxicity test
2 methods (Crane et al., 2008).

5.1.2.1. Media/Vehicle

3 Nano-TiO₂ in an oily dispersion penetrates deeper into skin than nano-TiO₂ in an aqueous
4 dispersion, as shown in an ex vivo study using healthy adult skin (intact samples of tissue removed from
5 the body, and manipulated in vitro) (Bennat and Muller-Goymann, 2000). Furthermore, when the
6 dispersal of nano-TiO₂ was made in the aqueous phase of an oil-in-water emulsion, nano-TiO₂ did not
7 penetrate into skin, but the emulsion was not stable (Bennat and Muller-Goymann, 2000). Although the
8 stability could be improved by encapsulating the nano-TiO₂ into liposomes, liposome formulation increases
9 nano-TiO₂ skin penetration. An in vivo study by Lansdown and Taylor (1997) in rabbits also
10 demonstrated that uptake of TiO₂ particles in sizes ranging from 2 to 20 μm was affected by the vehicle:
11 uptake was greatest in castor oil, followed by water, and then polyethylene glycol. According to Bennat
12 and Muller-Goymann (2000), the ideal sunscreen formulation, which is stable and does not allow nano-
13 TiO₂ penetration into skin, has yet to be developed.

14 Different levels of radical production in cultured cells were observed in similar nano-TiO₂ within
15 different formulae of suspensions (Uchino et al., 2002). Although nano-TiO₂ F-1R (a formula containing
16 nano-TiO₂ that is 3% anatase and 97% rutile, with an average size of 93 nm and a surface area of 17 m²/g)
17 produced OH radicals after UV-A exposure, no OH radical production was detected after UV-A exposure
18 in nano-TiO₂ in a different formula, St-C n (sunscreen standard C from the Japan Cosmetic Industry
19 Association containing nano-TiO₂ that is 2% anatase, 98% rutile, with an average size of 85 nm and a
20 surface area of 19 m²/g). Most rutile nano-TiO₂ is relatively inefficient in radical production, and the
21 F-1R used in this study produced more OH radicals than all four other, mainly rutile nano-TiO₂ forms and
22 one of the anatase forms tested (Uchino et al., 2002). Although nano-TiO₂ has been reported to generate
23 ROS in cell-free conditions but not in cells (a murine macrophage cell line, RAW 264.7) (Xia et al.,
24 2006), whether nano-TiO₂ in different formulae also causes different levels of ROS production in cells
25 has not been verified.

26 The purity of water affects the degree of aggregation, which in turn may affect exposure-dose and
27 toxicity. The degree of aggregation generally increases with the presence of salt or with an increase in
28 ionic strength, minerals, and organic matter in water (i.e., decreased purity as compared to pure water)
29 (Domingos et al., 2009a; French et al., 2009). Aggregation was more severe in tap water than in nanopure
30 water (Zhang et al., 2008), and is likely to be more severe in fish tank water or pond water than in tap
31 water. Because nano-TiO₂ in the environment is more likely to be present in aggregated form, results
32 from nano-TiO₂ suspensions with aggregates can be informative, and as noted earlier, might even be more

1 relevant than results from a perfectly dispersed suspension with nano-TiO₂ in primary particle form. The
2 lack of accurate measurement of nano-TiO₂ (e.g., size distribution, mass concentrations, numbers, and
3 surface area) and a generally-agreed-upon choice of dose metrics, however, impede the establishment of a
4 reliable dose-response relationship.

5 In respiratory exposure studies, intra-tracheal instillation exposure typically uses saline as a vehicle
6 for TiO₂ delivery while inhalation exposure uses air. The behavior of nano-TiO₂ (such as agglomeration)
7 is expected to be different in air than in solution. Furthermore, the vehicle alone can affect respiratory
8 system responses, at least for a short time. Transient inflammation in the respiratory tract occurs in rats
9 given saline alone through instillation (Driscoll et al., 1990; Henderson et al., 1995). Sager et al. (2007a)
10 tried to disperse several types of nano-sized particles, including TiO₂, in several suspension media,
11 including: phosphate buffered saline (PBS); rat and mouse BAL fluid; and PBS containing dipalmitoyl
12 phosphatidylcholine (DPPC) or mouse serum albumin or both. Although the dispersion in PBS was not
13 satisfactory, BAL fluid was an excellent vehicle for dispersing the particles. The dispersion was also
14 unsatisfactory in saline containing albumin alone or DPPC alone, in concentrations found in BAL fluid.
15 Adding protein plus DPPC in PBS, however, produced satisfactory, albeit slightly less effective,
16 substitutes for BAL fluid. The Sager et al. (2007a) experiment demonstrates the importance of the
17 suspension medium and suggests that the immediate milieu (such as the BAL fluid and protein and DPPC
18 in lung) affects not only the agglomeration of nano-TiO₂, but also the consequent effects on nano-TiO₂
19 behavior and effects.

5.1.2.2. Dispersion Preparation

20 The potential importance of dispersion preparation for nanomaterial ecotoxicity is illustrated by
21 fullerene (C₆₀) studies. C₆₀ toxicity in daphnids and fishes was higher when the C₆₀ suspension was
22 prepared with the organic solvent tetrahydrofuran (THF) than when the suspension was prepared by
23 stirring and sonication (Henry et al., 2007; Zhu et al., 2006). Entrapped or residual THF in the C₆₀ and
24 THF degradation products were suspected to have contributed to toxicity (Henry et al., 2007).
25 Nevertheless, no difference in toxicity to daphnids was observed between nano-TiO₂ suspensions
26 prepared with and without THF (Klaper, 2008; Lovern and Klaper, 2006). Regardless of dispersion
27 method, aggregation of nano-TiO₂ might be unavoidable. Several studies reported that nano-TiO₂ formed
28 aggregates in water, and that these aggregates could not be disaggregated into primary particles by
29 ultrasound or chemical dispersants (Griffitt et al., 2008; Jeng and Swanson, 2006; Zhang et al., 2008).
30 Furthermore, an unfiltered nano-TiO₂ suspension with aggregates has been reported to be less toxic to
31 daphnia than a filtered nano-TiO₂ suspension, which has a much smaller mean secondary particle size
32 than filtered suspension (Lovern and Klaper, 2006). In contrast to the reported difficulty of

1 disaggregating secondary particles by sonication or chemical dispersants, Federici et al. (2007) reported
2 good dispersion of P25 by sonication in ultrapure water at final working concentrations up to 1 mg/L,
3 although they did not evaluate potential aggregation in test tank water at these concentrations.

4 In addition to the medium itself, the dispersion method can affect not only the nanoparticle
5 agglomeration or aggregation (such as the degree and size of agglomerates) but also the effects of
6 nanoparticles (Bihari et al., 2008). For example, sonication with ultrasound has been used to decrease
7 nano-TiO₂ agglomeration (Bihari et al., 2008) and has been shown to generate particles or agglomerates
8 in the nanoparticle range (Maier et al., 2006). However, sonication alone could increase the size of nano-
9 TiO₂ agglomerates, as reported by Porter et al. (2008) who found that the mean agglomerate size of P25
10 in PBS increased from 1930 nm before sonication to 2849 nm immediately after sonication, while the
11 same sonication procedure decreased the sizes of agglomerates of P25 dispersed in BAL fluid and in a
12 mimic BAL fluid that contained Ca²⁺ - and Mg²⁺ - free PBS, serum albumin, and DPPC. No explanation
13 was provided. Furthermore, ultrasound sonication has been reported to increase nano-TiO₂ catalytic
14 activity in breaking down an organic dye (acid red B) (Wang et al., 2009b), but also to decrease changes
15 in enzyme activity caused by ingested nano-TiO₂ in isopods (Jemec et al., 2008). Post-preparation
16 analysis of particle size is important when comparing laboratory studies and formulations with sunscreen
17 preparations. Although studies of nano-TiO₂ particle and agglomerate sizes are available (Delrieu et al.,
18 unknown), very few health effects studies have characterized nano-TiO₂ in sunscreen formulations and
19 only a few studies characterized nano-TiO₂ in other experimental media. Most health effects studies have
20 reported characteristics of only dry nano-TiO₂ primary particles, which are important but not
21 representative of all exposure scenarios.

22 Finally, without a special hydrophilic coating, nano-TiO₂ forms a suspension in water (rather than a
23 solution). Standard ecotoxicological test methods are intended for soluble or poorly soluble substances,
24 and not designed for testing suspensions (German Federal Institute for Occupational Safety and Health
25 (BAuA) et al., 2007).

5.1.3. Environmental Conditions

26 Once nano-TiO₂ is released into the environment, its fate depends on abiotic and biotic conditions,
27 which are likely to be more complex and diverse than standard ecological testing conditions. Of the many
28 environmental factors that might be relevant to nano-TiO₂ ecotoxicity, ultraviolet (UV) exposure, purity
29 of water (Zhang et al., 2008), and presence of organic matter (Domingos et al., 2009a) have been
30 investigated. Factors that affect nano-TiO₂ aggregation, such as pH value, ionic strength, and cation
31 valence (Domingos et al., 2009a; Dunphy Guzman et al., 2006; French et al., 2009), would influence not

1 only nano-TiO₂ fate and transport (see Chapter 3), but also potential exposure and possibly ecological
2 effects. Only environmental factors that have been shown to affect toxicity in organisms used for
3 ecological effects testing are discussed here.

4 UV is well known to increase the cytotoxicity of nano-TiO₂, particularly photocatalytic nano-TiO₂
5 such as anatase or anatase/rutile mix, to cultured mammalian cells (Sayes et al., 2006) and fish cells
6 (Reeves et al., 2008; Vevers and Jha, 2008) as well as microorganisms (Adams et al., 2006). Genotoxicity
7 (Nakagawa et al., 1997) and clastogenicity (Nakagawa et al., 1997; Theogaraj et al., 2007) of nano-TiO₂
8 to cultured mammalian cells were also increased by UV. This UV-increased toxicity is at least partially
9 due to the greater number of hydroxyl radicals ($\cdot\text{OH}$) generated by anatase than by rutile under UV
10 exposure (Sayes et al., 2006; Uchino et al., 2002). UV exposure may influence the effects of nano-TiO₂
11 in sunscreen indirectly by causing sunburn, which can make skin more permeable (Mortensen et al.,
12 2008). In addition to UV, visible light was shown to increase the cytotoxicity of nano-TiO₂ (carbon-doped
13 TiO₂ and TiO₂ modified with platinum [IV] chloride complexes) in bacteria and fungi (Mitoraj et al.,
14 2007).

15 Nano-TiO₂ was found to aggregate more in pond water than in pure water (Milli-Q water),
16 although no nano-TiO₂ toxicity to soil bacteria, green algae, or water fleas was detected in either pond
17 water or pure water at up to 100 mg/L (Velzeboer et al., 2008). The adsorption of nano-TiO₂ onto
18 certified reference material sediment did not increase the toxicity of the sediment (Blaise et al., 2008).

19 Additional environmental factors that might indirectly influence the effects of TiO₂ nanoparticles in
20 sunscreen include moisture; pH and water chemistry; and temperature. High humidity in the environment
21 could increase the hydration level of the stratum corneum, and could lead to increases in skin
22 permeability and penetration of both hydrophilic and lipophilic components (Benson, 2005; Zimmerer et
23 al., 1986). For example, the level of penetration of nano-TiO₂ on soaked skin, which is likely to occur
24 after swimming or other water activities, has not been investigated. Similar to media and vehicle effects
25 on nano-TiO₂, the pH and chemistry of water with which sunscreen may be mixed might also modulate
26 nano-TiO₂ effects, e.g., in a pool versus a lake or an ocean. Finally, sunscreen is often used at much
27 higher temperatures than typical ambient laboratory temperatures. Although nano-TiO₂ itself is not
28 expected to change in the temperature range tolerable for human beings, increased body temperature and
29 sweat may affect nano-TiO₂ dermal penetration and thus its effects (Lu et al., 2008).

30 The influence of the immediate milieu on nano-TiO₂ behavior and effects is also evident when
31 nano-TiO₂ is inside an organism. For instance, *in vitro* studies showed that in rat BAL, nano-TiO₂ formed
32 smaller aggregates and the aggregates remained small longer than nano-TiO₂ in PBS (Porter et al., 2008;
33 Sager et al., 2007a, b). Because pH affects the charge of nano-TiO₂, it is plausible that nano-TiO₂ would

1 behave differently in tissues and cellular organelles with different pH values, such as very low pH values
2 in the stomach and in lysosomes.

5.1.4. Summary

3 Nano-TiO₂ physicochemical properties, experimental conditions, and the immediate environment
4 or milieu, all can influence nano-TiO₂ ecological and health effects. For example, nano-TiO₂ size,
5 crystalline form, and surface characteristics all influence nano-TiO₂ behavior, including distribution,
6 exposure potential, and effects. Although the influences of media and vehicle and dispersion methods on
7 particle aggregation and distribution have been reported, information on these influences on health effects
8 is very scarce (Jemec et al., 2008). The presence of UV and visible light often increase photocatalytic
9 nano-TiO₂ activity and toxicity; other environmental factors, such as pH, ionic strength, and presence of
10 organic matter of the aquatic environment, could also affect nano-TiO₂ behavior and effects.

5.2. Ecological Effects

11 The ecological effects of nanomaterials have been gaining attention from the research and
12 regulatory communities, and several review articles (Baun et al., 2008; Boxall et al., 2007; Christian et
13 al., 2008; Hassellöv et al., 2008; Navarro et al., 2008; Nowack and Bucheli, 2007; Oberdörster et al.,
14 2006) and conferences (such as the annual International Conference Environmental Effects of
15 Nanoparticles and Nanomaterial) have addressed this topic. Although new information on nanomaterial
16 ecotoxicity seems to emerge almost daily, available data thus far have been insufficient for a quantitative
17 risk assessment of any particular nanomaterial. A thorough discussion of methods for ecotoxicity testing
18 and characterization of nanomaterials (including in environmental media) is beyond the scope of these
19 case studies, and has been reviewed elsewhere (Christian et al., 2008; Crane et al., 2008; Handy et al.,
20 2008b; Hassellöv et al., 2008). Nonetheless, a brief review of ecological effects testing and the
21 importance of the tests are presented at the beginning of each of the following section for the readers'
22 reference.

23 Section 5.2.1 features a review of the ecological effects of nano-TiO₂ exposure. Effects on bacteria
24 and fungi are discussed in Section 5.2.1.1, effects on aquatic organisms are discussed in Section 5.2.1.2,
25 effects on terrestrial organisms are discussed in Section 5.2.1.3, and indirect and interactive toxicity are
26 discussed in Section 5.2.2.4. Section 5.2.1.5 summarizes the available ecological toxicity information.

5.2.1. Ecological Effects of Nano-TiO₂ Exposure

1 Most of the nano-TiO₂ ecological effect studies surveyed in this report (Table 5-3) used
2 photocatalytic nano-TiO₂, some of which could be suitable for water treatment purposes. Two of the
3 studies used photostable nano-TiO₂ intended for topical sunscreen (Wiench et al., 2007) or for protecting
4 plastic from UV degradation (Warheit et al., 2007a). Current FDA regulation of TiO₂ in topical sunscreen
5 does not specify crystalline form and does not require proof of photostability (or lack of photoreactivity).
6 Pure anatase nano-TiO₂ is much more photoreactive than pure rutile nano-TiO₂, but it is possible to have
7 photostable anatase or an anatase/rutile mix of nano-TiO₂ by using doping or surface treatments, such as
8 coating with silica. The coating of photostable nano-TiO₂ is designed to endure the manufacturing
9 process and consumer use (Lademann et al., 2000), but the long-term stability of coated TiO₂ in sunscreen
10 remains unclear. Once nano-TiO₂ is released into the environment, various environmental factors, such as
11 high ionic strength in sea water and high acidity in landfill leachate, could compromise some nano-TiO₂
12 coatings. Therefore, the ecological effects of photocatalytic nano-TiO₂ might be relevant not only for
13 nano-TiO₂ used in drinking water treatment but also for nano-TiO₂ in sunscreen, because photoreactive
14 nano-TiO₂ can be used as the core material of photostable nano-TiO₂ in sunscreen. For example, the core
15 of Aeroxide T805 is P25, a photocatalyst, and has been used as a UV filter in some sunscreens (Barker
16 and Branch, 2008; Evonik, 2007).

17 Because mass concentration is reported for all studies reviewed, this dose metric is presented in
18 Table 5-3 and in all subsequent discussion referring to the literature. Whenever information on surface
19 area of the particles (to calculate particle surface area concentration) or the measured nano-TiO₂
20 concentration (versus calculated based on added mass) in the final test suspension is available, it is also
21 provided in Table 5-3. It should be noted that several studies reported visible turbidity in nano-TiO₂ stock
22 suspension (Velzeboer et al., 2008; Zhang et al., 2006; Zhang et al., 2008). Because turbidity is likely
23 caused by large aggregates of nano-TiO₂, which can settle out of the liquid phase by gravity, actual
24 concentrations of nano-TiO₂ in the liquid phase might be lower than concentrations calculated based on
25 mass of nano-TiO₂ added.

Table 5-3. Summary of nano-TiO₂ ecological effects. ^a

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Microorganisms			
Bacteria (<i>Escherichia coli</i> and <i>Bacillus subtilis</i>) (Adams et al., 2006)	66-nm powder, ~35% rutile:65% anatase, average 330-nm in water (Sigma product 634662) (Lyon, 2008)	6-hr exposure to (1) 50, 100, 500, 1000, 2000, 500 ppm in medium ^b , in direct sunlight, or (2) 1000 ppm in medium ^b , in dark	In dark, similar growth inhibition for both bacteria In light, <i>B. subtilis</i> : 0% and 75% growth inhibition at 500 and 1000 ppm, ^b respectively <i>E. coli</i> : 0%, 15% and 44% inhibition at 100, 500, 1000 ppm, respectively
Bacterium (<i>Vibrio fischeri</i>) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, Canada or France)	15-min exposure, measure the reduction of light output from bioluminescent marine bacterium, <i>Vibrio fischeri</i> (Microtox [®] toxicity test) as an indicator of growth inhibition, tested concentrations not specified	IC ₂₅ >100 mg/L
		Mix in a 1:1 ratio with certified reference material sediment, measure light output (Microtox [®] toxicity test) (indirect toxicity/interaction)	Nano-TiO ₂ did not affect the toxicity of certified reference material sediment
Bacterium (<i>Vibrio fischeri</i>) (Heinlaan et al., 2008)	25- to 70-nm powder mixture of anatase and rutile, ratio not disclosed (Sigma product 13463-67-7, Estonia) (Heinlaan, 2008)	30 min exposure for up to 20000 mg/L nano-TiO ₂ and conventional TiO ₂ , 8 hr exposure to 20000 mg/L conventional TiO ₂	The highest concentration tested: 20000 mg/L nano-TiO ₂ (30 min exposure) did not decrease bacterial growth
	Conventional TiO ₂ : size and crystal form not disclosed (Sigma product 14027, Estonia; a former Riedel-de Haën product) (Heinlaan, 2008)	Measure the reduction of light output from <i>Vibrio fischeri</i> (Flash assay) as an indicator of growth inhibition	The highest concentration tested: 20 g/L conventional TiO ₂ (30 min and 8 hr exposure) did not decrease bacterial growth
Bacterium (<i>Vibrio fischeri</i>) (Velzeboer et al., 2008)	<75-nm (primary particle) nano-TiO ₂ in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer, 2008)	15 min, 1, 10, 100 mg/L, measure light output from bioluminescent bacteria (Microtox [®] method, which could be affected by turbidity of 100 mg/L TiO ₂ suspension) ^c	EC ₅₀ >100 mg/L ^c
Bacteria (from a soil sample, species not identified) (Velzeboer et al., 2008)	<75-nm (primary particle) nano-TiO ₂ in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer, 2008)	7 day (Biolog [®] test, gram positive) ^c , 100 mg/L	EC ₅₀ >100 mg/L ^c
Bacteria and yeast (proprietary information) (Blaise et al., 2008; Dando, 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	18 hr, growth inhibition of 10 bacteria and 1 baking yeast (microbial array for risk assessment [MARA] assay), tested concentrations not specified	MTC >100 mg/L
		18-hr exposure to the filtered elutriate from certified reference material sediment with and without nano-TiO ₂ mixed in a 1:1 ratio (MARA assay) (indirect toxicity/interaction), tested concentrations not specified	Nano-TiO ₂ did not affect the toxicity of the elutriate of certified reference material sediment

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms			
Alga (green alga, <i>Desmodesmus subspicatus</i>) (Hund-Rinke and Simon, 2006)	25-nm primary particle, 20% rutile:80% anatase (Degussa P25) (Baun et al., 2008) (photocatalytic)	72 hr, growth inhibition, following the guidelines for EU standard algal assay (OECD 201, DIN 38412-33, and ISO 8692) with modifications to include pre-illumination of nano-TiO ₂ dispersion with simulated sunlight (wavelength 300–800 nm) at 250 watts for 30 min; illumination alone did not affect <i>D. subspicatus</i> growth Algal growth (without preillumination): 0, 3.1, 6.2, 12.5, 25, 50 mg/L (products 1 and 2) Shading effect: 0, 12.5, 25, 50 mg/L Algal growth (with preillumination): 12.5, 25, 50 mg/L (product 1)	EC ₅₀ and effects of additional particle cleaning: Product 1: EC ₅₀ was not different between nano-TiO ₂ washed once as manufacturer recommendation (32 mg/L) and nano-TiO ₂ with an additional wash (44 mg/L), suggesting toxicity was not from contaminants Product 2: EC ₅₀ >50 mg/L, both nano-TiO ₂ with and without the additional wash (at up to 50 mg/L) caused less than 40% decrease in growth No shading effect: when nano-TiO ₂ dispersion (at up to 50 mg/L) was above algae for 72 hrs, no effects on algal growth, suggesting nano-TiO ₂ effects was not due to lowered light intensity, but due to a toxicity of nano-TiO ₂ Pre-illumination of nano-TiO ₂ (Product 1) did affect nano-TiO ₂ effects on algal growth
	100-nm primary particle, 100% anatase; (Hombikat UV100) (Baun et al., 2008); photocatalytic (Mehrvan et al., 2002)		
Alga (green alga, <i>Pseudokirchneriella subcapitata</i>) (Velzeboer et al., 2008)	<75-nm (primary particle) nano-TiO ₂ in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer, 2008)	4.5 hr, in light, 100 mg/L Photosynthesis efficiency was measured as a pulse amplitude modulation (PAM) fluorescence test, which could be affected by turbidity of 100-mg/L TiO ₂ suspension ^c	EC ₅₀ >100 mg/L ^c
Alga (green alga, <i>Pseudokirchneriella subcapitata</i>) (Warheit et al., 2007a)	140-nm in water, 79% rutile: 21% anatase, coated (90-wt % TiO ₂ , 7% alumina, and 1% amorphous silica) (DuPont uf-C TiO ₂) (photo-passivative/ photo-stable) (Warheit, pers. comm., 2008b)	OECD 201 (72-hr growth), with light ^b 0.01, 0.1, 1, 10, and 100 mg/L (uf-C TiO ₂ and fine TiO ₂)	EC ₅₀ 21 mg/L (based on decreases in cell number) EC ₅₀ 87 mg/L (based on inhibition of growth rate)
	Fine TiO ₂ : 380-nm in water, rutile, coated (~99% TiO ₂ and ~1% alumina)		EC ₅₀ 16 mg/L (based on decreases in cell number) EC ₅₀ 61 mg/L (based on inhibition of growth rate)
Alga (green alga, <i>Pseudokirchneriella subcapitata</i>) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	72-hr growth inhibition, tested concentrations not specified	IC ₂₅ >100 mg/L

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (water flea, <i>Daphnia magna</i>) (Hund-Rinke and Simon, 2006)	25-nm primary particle, 20% rutile:80% anatase (Degussa P25) (Baun et al., 2008) (photocatalytic); ultrasonic dispersion	ISO 63421, OECD 202 and DIN 38412-30 (48-hr immobility), exposure to up to 3 mg/L, 16:8 hr light:dark cycles, compare the effects of pre-illuminated and non-illuminated nano-TiO ₂ 0, 1, 1.5, 2, 2.5, 3 mg/L	Pre-illumination increased toxicity compared to the same concentration No dose-response relationship with either pre-illuminated or non-illuminated nano-TiO ₂
	100-nm primary particle, 100% anatase; (Hombikat UV100) (Baun et al., 2008); photocatalytic (Mehrvan et al., 2002); ultrasonic dispersion		Pre-illumination showed a trend of increasing toxicity No dose-response relationship with either pre-illuminated or non-illuminated nano-TiO ₂
Invertebrate (water flea, <i>Daphnia magna</i>) (Lovern and Klaper, 2006)	Primary particle <25-nm (smallest 5-nm), anatase, uncoated (photocatalytic) (Klaper, 2008); filtered through a 0.22-µm nylon filter, secondary particle 20–30 nm in deionized water	EPA 48-hr tox test (U.S. EPA standard operating procedure 2024) (mortality) Filtered nano-TiO ₂ : 0.2, 1, 2, 5, 6, 8, and 10 ppm Sonicated, unfiltered nano-TiO ₂ : 50, 200, 250, 300, 400, and 500 ppm	LC ₅₀ 5.5 mg/L LOEC 2.0 mg/L NOEC 1.0 mg/L
	Primary particle <25-nm (smallest 5-nm), anatase, uncoated (photocatalytic) (Klaper, 2008); sonicated, unfiltered, secondary particle 100–500 nm in deionized water		LC ₅₀ >500 mg/L
Invertebrate (water flea, <i>Daphnia magna</i>) (Wiench et al., 2007)	20–30 nm, 80% anatase, 20% rutile, no surface coating, BET surface area 48.6 m ² /g	OECD 202, part 1 (48-hr immobility), tested concentrations: 0 (untreated control), 0.01, 0.1, 1.0, 10.0 and 100.0 mg/L	EC ₅₀ >100 mg/L
	50-nm x 10-nm, rutile, surface coating aluminum hydroxide, dimethicone/methicone copolymer, BET 100 m ² /g (T-Lite™ SF) (photostable UV filter)		EC ₅₀ >100 mg/L
	50-nm x 10-nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethicone/methicone copolymer, BET 100 m ² /g (T-Lite™ SF-S) (photostable UV filter)		EC ₅₀ >100 mg/L
	50-nm x 10-nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethoxydiphenylsilane/ triethoxycaprylsilane crosspolymer, BET 100 m ² /g (T-Lite™ MAX) (photostable UV filter)		EC ₅₀ >100 mg/L
	~300-nm, BET surface area 6 m ² /g (pigment grade)		EC ₅₀ >100 mg/L

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (water flea, <i>Daphnia magna</i>) (Lovern et al., 2007)	30-nm, anatase	1-hr exposure to 2.0 mg/L	No changes in heart rate or behaviors
Invertebrate (water flea, <i>Daphnia magna</i>) (Warheit et al., 2007a)	140-nm in water, 79% rutile:21% anatase, coated (90-wt % TiO ₂ , 7% alumina, and 1% amorphous silica) (DuPont uf-C TiO ₂) (photo-passivative/ photo-stable) (Warheit, pers. comm., 2008b)	OECD 202 (48-hr immobility) 0.1, 1.0, 10, and 100 mg/L (uf-C and fine TiO ₂)	EC ₅₀ >100 mg/L (10% immobility at 100 mg/L)
	Fine TiO ₂ : ~380-nm in water (buffered), rutile, BET surface area 5.8 m ² /g, coated with alumina (~99% TiO ₂ and ~1% alumina)		EC ₅₀ >100 mg/L (10% immobility at 10 mg/L, 0% immobility at 100 mg/L)
Invertebrates (water flea, <i>Daphnia pulex</i> and <i>Ceriodaphnia dubia</i>) (Griffitt et al., 2008)	20.5-nm primary particle, mainly 220.8- or 687.5-nm in moderately hard water, 20% rutile:80% anatase, BET surface area 45 m ² /g; sonicated (Degussa P25) (photocatalytic)	48-hr mortality, 14:10 hr light:dark cycle, for <i>D. pulex</i> adults and <i>C. dubia</i> neonates (<24 hr old) Gradient of concentrations up to 10 mg/L (The estimated median lethal concentration (LC ₅₀) from range-finder tests, and 0.6-, 0.36-, 1.67-, and 2.78-fold the estimated LC ₅₀ . However, the estimated LC ₅₀ was not specified.)	LC ₅₀ >10 mg/L for both <i>D. pulex</i> and <i>C. dubia</i>
Invertebrate (water flea, <i>Chydorus sphaericus</i>) (Velzeboer et al., 2008)	<75-nm (primary particle) nano-TiO ₂ in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer, 2008)	48-hr mortality, 17:7 hr light:dark cycle (Chydotox test) ^c	EC ₅₀ >100 mg/L ^c
Invertebrates (water flea, <i>Daphnia magna</i> ; fairy shrimp, <i>Thamnocephalus platyurus</i>) (Heinlaan et al., 2008)	25- to 70-nm powder mixture of anatase and rutile, ratio not disclosed (Sigma product 13463-67-7, Estonia) (Heinlaan, 2008)	48-hr mortality for <i>D. magna</i> 24-hr immobilization for <i>T. platyurus</i>	NOEC >20,000 mg/L for <i>T. platyurus</i> ; not tested in <i>D. magna</i>
	Conventional TiO ₂ : size and crystal form not disclosed (Sigma product 14027, Estonia; a former Riedel-de Haën product) (Heinlaan, 2008)	Up to 20000 mg/L for both nano- and conventional TiO ₂	NOEC >20,000 mg/L for <i>T. platyurus</i> ; 60% mortality at 20,000 mg/L for <i>D. magna</i>
Invertebrate (fairy shrimp, <i>Thamnocephalus platyurus</i>) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	24-hr lethality (ThamnoToxkit assay), tested concentrations not specified	LC ₅₀ >100 mg/L

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (freshwater hydra, <i>Hydra attenuata</i>) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	96-hr morphological changes, tested concentrations not specified	EC ₅₀ in 10–100 mg/L range
Fish cell (trout primary hepatocytes) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	48-hr cytotoxicity, tested concentrations not specified	TEC in 1–10 mg/L range
Fish (zebrafish, <i>Danio rerio</i>), embryo and larvae (Zhu et al., 2008)	Nano-TiO ₂ : uncoated anatase, purity >99.5%, primary particle in spindle shape, published size ≤20 nm, surface area not reported (Nanjing High Technology NANO CO., LTD, Nanjing, Jiangsu province, China); in suspension (in MilliQ water): mean measured size 230 nm, measured size range 100–550 nm, secondary particles formed by primary particles have irregular shapes Conventional TiO ₂ : anatase, purity >99.0%, published size: 10,000 nm (Third Chemical Regent Factory of Tianjin, Tianjin, China); in suspension (in MilliQ water): mean measured size 1,100 nm, measured size range 330–2,250 nm, neither primary nor secondary particles have a uniform shape	96-hr exposure to 0, 1, 10, 50, 100, or 500 mg/L nano-TiO ₂ or conventional TiO ₂ to fish eggs (started within 1.5 hr post-fertilization); light cycle 14 hr light/10 hr dark; following endpoints were measured: (1) survival of embryo and larvae (2) hatching rate at 84 hr post-fertilization (3) malformation (e.g., pericardial edema and tissue ulceration, body arcuation, etc.) in embryo and larvae	Neither nano-TiO ₂ nor conventional TiO ₂ at the tested condition caused changes in any of the three endpoints measured.
Fish (zebrafish, <i>Danio rerio</i>) (Griffitt et al., 2008)	20.5-nm primary particle, mainly 220.8- or 687.5-nm in moderately hard water, 20% rutile:80% anatase, BET surface area 45 m ² /g, sonicated (Degussa P25) (photocatalytic)	48-hr mortality on adult zebra fish and zebra fish fry (<24 hr post-hatch) at a gradient of concentrations up to 10 mg/L	LC ₅₀ >10 mg/L for both adults and fry
Fish (rainbow trout, <i>Oncorhynchus mykiss</i>) (Warheit et al., 2007a)	140-nm in water, 79% rutile:21% anatase, coated (90-wt % TiO ₂ , 7% alumina, and 1% amorphous silica) (DuPont uf-C TiO ₂) (photo-passivative or photo-stable) (Warheit, pers. comm., 2008b)	OECD 203 (96 hr) 0.1, 1.0, 10, and 100 mg/L (uf-C and fine TiO ₂)	LC ₅₀ >100 mg/L
Chronic Exposure to Aquatic Organisms			
Invertebrate (water flea, <i>Daphnia magna</i>) (Adams et al., 2006)	66-nm powder, ~35% rutile:65% anatase, average 330 nm in water, (Sigma product 634662) (photocatalytic) (Lyon, 2008)	8-day exposure to suspension at 1, 10 or 20 ppm (concentration over time was not reported)	40% mortality at 20 mg/L
Invertebrate (water flea, <i>Daphnia magna</i>) (Wiench et al., 2007)	50-nm x 10-nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethicone/methicone copolymer, BET surface area 100 m ² /g (T-Lite™ SF-S) (photostable UV filter)	OECD 211 (21-d reproduction), test concentrations: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 mg/L	NOEC 3 mg/L LOEC 10 mg/L

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Chronic Exposure to Aquatic Organisms (continued)			
Fish (rainbow trout, <i>Oncorhynchus mykiss</i>) (Federici et al., 2007)	21-nm, 75% rutile:25% anatase, sonicated (Degussa P25) (photocatalytic)	0-, 7-, or 14-day exposure to 0, 0.1, 0.5 or 1.0 mg/L (mean measured TiO ₂ concentrations were 0.089, 0.431, and 0.853 mg/L over the 12-hr period, equating to 89, 85, and 86% of the expected concentrations, respectively)	Respiratory distress, organ pathologies, and oxidative stress at as low as 0.1 mg/L; nano-TiO ₂ could be a surface acting toxicant
Acute Exposure to Terrestrial Organisms			
Photosynthetic enzyme complexes isolated from spinach leaves (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, Canada or France), characteristics in water not reported	15 min, tested concentrations not specified, measure the decrease in chlorophyll fluorescence emitted from the enzyme complexes as an indicator of inhibition of photosynthetic efficiency (Luminotox assay) (Bellemare et al., 2006)	IC ₂₀ >100 mg/L
		Mix in a 1:1 ratio with certified reference material sediment, 15 min, tested concentrations not specified, measure light output (Luminotox assay) (indirect toxicity/interaction)	Nano-TiO ₂ did not affect the toxicity of certified reference material sediment
Plant (spinach, <i>Spinacia oleracea</i>) (Linglan et al., 2008)	Nano-TiO ₂ : 5-nm, anatase, not coated Conventional TiO ₂	Soak the seeds in 0.25% nano-TiO ₂ or conventional TiO ₂ for 48 hr, and spray 0.25% nano-TiO ₂ or conventional TiO ₂ onto the leaves from 2-leaf stage to 8-leaf stage at 0.25%	Nano-TiO ₂ : Enhanced growth (size, single plant fresh weight, single plant dry weight) Increased chlorophyll content Increased net photosynthetic rate Increased mRNA, protein concentration, and activity of Rubisco activase Conventional TiO ₂ : No significant changes
Plant (spinach, <i>Spinacia oleracea</i>) (Zheng et al., 2005)	Size not specified, rutile (Shanghai Chemical Co. of China product)	Soak aged seeds for 48 hr at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0, 6.0, or 8.0 mg/L	Increased germination rate, intensity of photosynthesis, chlorophyll synthesis, and Rubisco activase activity in a dose response manner (at up to ~4.0 mg/L; peak effect at ~2 mg/L; higher concentrations have opposite effects)
Invertebrate (isopod, <i>Porcellio scaber</i>) (Jemec et al., 2008)	15-nm in diameter, 15–75 nm in length, elongated spheroid shape, anatase, surface area 190–290 m ² /g, 99.7% pure (Sigma product). 350- to 500-nm aggregates in sonicated dispersion, 780- to 970-nm aggregates in non-sonicated dispersion, sizes on dry leaves not reported	3-day dietary exposure to non-sonicated nano-TiO ₂ at 0.1, 0.5, 1, 10, 100, 1,000, 2,000, or 3,000 µg/g food or to sonicated nano-TiO ₂ at 1,000, 2,000, or 3,000 µg/g food (leaves soaked in non-sonicated or sonicated nano-TiO ₂ dispersion and then dried)	Decreased activities of catalase and glutathione-S-transferase (GST) in digestive glands at 0.5, 2,000, and 3,000 µg/g non-sonicated nano-TiO ₂ , but not in middle doses of non-sonicated nano-TiO ₂ or any doses of sonicated nano-TiO ₂

Table 5-3. Summary of nano-TiO₂ ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Terrestrial Organisms (continued)			
Invertebrate (nematode, <i>Caenorhabditis elegans</i>) (Wang et al., 2009a)	Nano-TiO ₂ , anatase, primary particle diameter 50 nm, measured BET surface area 325 m ² /g for primary particle, purity >99%, hydrodynamic diameter (of aggregates in pure water) range 338–917 nm (medium 550 nm), zeta potential at pH 7.0 = -18.9 mv (Hongchen Material Sci & Tech, Co., China) Conventional TiO ₂ , anatase, measured primary particle diameter 285 nm (by TEM), measured BET surface area 7.3 m ² /g, purity >99%, hydrodynamic diameter range 158–687 nm (medium 494 nm), zeta potential at pH 7.0 = - 33.8 mv (ACROS)	Expose synchronized worms in the L1 stage to nano-TiO ₂ or conventional TiO ₂ in ultrapure water with pH adjusted to 7.0 with HNO ₃ and NaOH Exposure for 24 hr (for lethality to the vermiform nematode) or 5 days (for growth – length of the worm, and reproduction tests – number of eggs inside the worm body, and number of offspring per worm) at 24.0, 47.9, 95.9, 167.8, and 239.6 mg/L	Lethality to the vermiform nematode: 24-hr LC ₅₀ was significantly lower for nano-TiO ₂ (79.9 mg/L) than for conventional TiO ₂ (135.8 mg/L) Length of the worm, number of eggs inside the worm body, and number of offspring per worm were all significantly decreased at 47.9 mg/L or higher concentrations of nano-TiO ₂ and at 95.9 mg/L or higher concentrations of conventional TiO ₂

^a N/A – Not applicable

ACROS – Acros Organics

BET – Surface area measured by Brunauer, Emmett, and Teller analysis

DIN – Deutsches Institut für Normung (German Institute for Standardization)

EC₅₀ – Effective concentration 50; the concentration at which 50% of subjects showed response

EU – European Union

IC₂₀, IC₂₅ – inhibitory concentration at which organisms showed 20%, 25% inhibition in measured endpoints

ISO – International Organization for Standardization

GST – Glutathione-S-transferase

LC₅₀ – Lethal concentration 50; the concentration at which 50% of subjects died

LOEC – Lowest observed effect concentration

MARA – Microbial array for risk assessment (assay)

MTC – Microbial Toxic Concentration, calculated by comparing the area under and above the growth curve (Gabrielson et al., 2003a, 2003b)

NOEC – No observed effect concentration

OECD – Organization for Economic Co-operation and Development

P25 – AEROXIDE® P25

PAM – Pulse amplitude modulation

TEC – Threshold effect concentration. The TEC for cytotoxicity is calculated using the NOEC and LOEC of cell viability reduction. $TEC = (NOEC \times LOEC)^{1/2}$

TEM – Transmission electron microscopy

UV – Ultraviolet (light/radiation), wavelengths in the range of 10-400 nm

^b Authors reported cloudy appearance or difficulty to dissolve nano-TiO₂ in preparing stock suspension. The testing concentrations (final concentrations in medium) were calculated by the volume of 10 mg/L stock suspension added into the medium. The actual concentrations of nano-TiO₂ in medium were not reported.

^c Authors reported cloudy appearance in 100 mg/L TiO₂ suspension. After centrifugation, nano-TiO₂ concentrations were no more than 10% of initial concentrations. For example, 200 µg/L nano-TiO₂ was added into pond water, and nano-TiO₂ was only 1 µg/L after centrifugation.

5.2.1.1. Effects on Bacteria and Fungi (Terrestrial and Aquatic)

1 Data for the effects of photostable nano-TiO₂ on bacteria and fungi are lacking. On the other hand,
2 photocatalytic nano-TiO₂ is known for its antibacterial and antifungal properties and has been tested for
3 various applications, including drinking water treatment (Coleman et al., 2005); surface coatings and
4 paints (Kühn et al., 2003; Tsuang et al., 2008); and food packaging (Chawengkijwanich and Hayata,
5 2007). Examples of recent studies of photocatalytic nano-TiO₂ in bacteria and fungi are provided in
6 Table 5-3.

7 Because most bacteria and fungi are non-pathogenic and are major decomposers in most terrestrial
8 and some aquatic ecosystems, chemicals with antibacterial and antifungal properties are not necessarily
9 beneficial when released into the environment. The health of decomposers is important for nutrient
10 cycling in the environment, such as carbon and nitrogen cycling in soil (Neal, 2008). Additionally, some
11 bacteria and fungi form a symbiotic relationship with plants. A well-known example is the nitrogen-
12 fixing bacteria (genus *Rhizobium*) that live in the roots of legumes. Legumes provide nutrients and a
13 relatively anaerobic environment for the rhizobia, and obtain ammonia formed from atmospheric nitrogen
14 by the rhizobia (Kimball, 2007). Thus, indiscriminant exposure to chemicals with antibacterial properties
15 could harm plants by interfering with symbiotic bacteria.

16 Sensitivity to photocatalytic nano-TiO₂ toxicity varies among species of bacteria. Adams et al.
17 (2006) reported that in the presence of sunlight, gram-negative *Escherichia coli* were more sensitive to
18 nano-TiO₂-induced growth inhibition than gram-positive *Bacillus subtilis*. With 2,000 parts per million
19 (ppm) of nano-TiO₂ in the growth medium, *E. coli* growth was decreased by 46% while *B. subtilis*
20 growth was inhibited by 99%. At 500 ppm, *E. coli* growth was decreased by only 15% and *B. subtilis*
21 growth was not inhibited (Adams et al., 2006). The different dose-response relationships of gram-positive
22 and gram-negative bacteria to nano-TiO₂ suggests the potential for nano-TiO₂ to alter microbial
23 population balance (diversity), both in wastewater treatment plants and during various phases of use and
24 disposal of nano-TiO₂. One generally accepted explanation for nano-TiO₂-induced toxicity in bacteria
25 and fungi is the generation of ROS, which can cause cell wall or cell membrane damage (Kühn et al.,
26 2003; Neal, 2008), such as lipid peroxidation (Maness et al., 1999). Although, as discussed above, UV
27 illumination increases photocatalytic nano-TiO₂ toxicity, photocatalytic nano-TiO₂ is also toxic in the dark
28 (Adams et al., 2006; Coleman et al., 2005). Because TiO₂ generates ROS (mainly highly reactive
29 hydroxyl radicals, ·OH) in the presence of UV and oxygen (Reeves et al., 2008), mechanisms other than
30 oxidative stress might also contribute to nano-TiO₂ toxicity in the dark and possibly also under UV. For
31 example, several types of nano-TiO₂ (anatase and a mixture of anatase/rutile) have been shown to adsorb

1 protein and calcium (Ca²⁺) in the medium, and cause in vitro cytotoxicity in mammalian cell lines (Horie
2 et al., 2009).

5.2.1.2. Effects on Aquatic Organisms

3 Data on the effects of nano-TiO₂ in aquatic organisms are available for freshwater algae, freshwater
4 invertebrates (water fleas and fairy shrimp), and freshwater fish (rainbow trout) (Table 5-3). Only two
5 aquatic organism studies in the literature involve photostable nano-TiO₂ (Warheit et al., 2007b; Wiench et
6 al., 2007). For other aspects of U.S. Environmental Protection Agency (EPA) tier 1 aquatic toxicity
7 testing (e.g., estuarine and marine organism acute toxicity, whole sediment acute toxicity, and bio-
8 availability/bio-magnification toxicity) (U.S. EPA, 2008d), studies have not yet been reported.

9 5.2.1.2.1. Algae

10 Algae are primary producers in ecosystems. In addition to being the food base in aquatic systems,
11 algae provide much of the earth's oxygen. Effects on algae are measured at the population level, for
12 example, in terms of population growth. In algal tests, 72-hour exposures are considered acute exposure
13 in European Union (EU) regulations, and 96-hour exposures are considered chronic by U.S. EPA (2008d).
14 A limited number of studies on the effects of either photocatalytic or photostable TiO₂ in algae have been
15 completed.

16 For photostable nano-TiO₂, EC₅₀ values determined for 72-hour growth inhibition in green alga
17 (*Pseudokirchneriella subcapitata*) were 21 mg/L (based on decreases in healthy cell numbers) and 87
18 mg/L (based on inhibition of growth rate) (Warheit et al., 2007a). In contrast, exposure to concentrations
19 of 0.001 to 1 mg/L of photostable nano-TiO₂ increased growth rate by 1–3% (green alga cell numbers
20 increased 6–19%) (Warheit et al., 2007a). U-shaped dose-response relationships are not unique to
21 nanomaterials, and it cannot be ruled out that increased growth at the low dose was a compensatory
22 response to low levels of toxicity (Calabrese and Baldwin, 1998; Davis and Svendsgaard, 1990). Fine
23 (approximately 380-nm) TiO₂ showed almost no inhibition in growth rate (or cell number) at up to
24 1 mg/L, and inhibition of growth rate was 3% at 10 mg/L and 66% at 100 mg/L (Warheit et al., 2007a).

25 For photocatalytic nano-TiO₂, the EC₅₀ values determined for 72-hr growth inhibition in green
26 algae (*Desmodesmus subspicatus*) ranged from approximately 30 mg/L to more than 50 mg/L (Blaise et
27 al., 2008; Hund-Rinke and Simon, 2006). Hund-Rinke and Simon (2006) also tested the potential for
28 TiO₂ to reduce growth by physically shading algae, and reported that as much as 50 mg/L of
29 photocatalytic nano-TiO₂ physically above the algae did not decrease algal growth, that is, it did not cause
30 a shading effect. When nano-TiO₂ and algae are in the same liquid medium, photocatalytic P25 nano-

1 TiO₂ was reported to adsorb onto the surfaces of green algae (*Pseudokirchneriella subcapitata*) and to
2 increase cellular weight by more than 130% (Huang et al., 2005). The concentration of P25 was not
3 reported. If the attached nano-TiO₂ directly blocks sunlight that otherwise could reach the algal cell
4 surface or if this extra weight causes algae to stay in deeper water, the consequent reduction in sunlight
5 could inhibit the algal growth. Because photostable nano-TiO₂ would also block UV penetration, similar
6 effects could occur with photostable nano-TiO₂. Without experimental evidence, predicting the impact of
7 nano-TiO₂ on photosynthesis is difficult because nano-TiO₂ exposure reportedly increases photosynthesis
8 in terrestrial plants, namely spinach, as discussed later in this section. Nano-TiO₂ could affect aquatic and
9 terrestrial plants differently due to exposure routes, doses, and other factors.

10 Although no marine organisms have been tested for nano-TiO₂ toxicity, the physical attachment of
11 nano-TiO₂ particles on cells could pose a risk to aquatic organisms that reproduce by external fertilization.
12 A wide variety of marine organisms fall into this category. Attached nano-TiO₂ could decrease sperm cell
13 mobility and consequently reproductive success. For comparison, carbon black nanoparticles have been
14 reported to decrease sperm frequency of seaweed (marine macroalgae) and to affect seaweed embryo
15 development (Nielsen et al., 2007). As discussed earlier (Section 5.1.1), the salinity in seawater could
16 influence the behavior and effects of nano-TiO₂, such as more aggregation as compared to pure water.

17 Nano-TiO₂ was reported to increase algal cell weight 2.3-fold by adsorbing to the algal cell surface,
18 but the tested nano-TiO₂ concentrations in water were not reported (Huang et al., 2005). If an increase in
19 weight forces surface algae into deeper water, photosynthesis could be decreased⁷ due to less sunlight
20 available in deeper water than at the surface. Because phytoplankton form the base of the food web and
21 generate half of the oxygen produced by all plants (Ramanujan, 2005), harmful effects on phytoplankton
22 from nano-TiO₂ could have wide-ranging implications.

23 5.2.1.2.2. Invertebrates

24 The endpoints used most often in ecological studies with invertebrates are mortality and
25 immobility; other endpoints include morphological changes, heart rate changes, and reproductive effects.
26 Fairy shrimp, *Thamnocephalus platyurus*, are small freshwater crustaceans and filter feeders that live in
27 temporary water bodies that dry out or periodically experience decreased water levels (Brausch et al.,
28 2006; Löhr et al., 2007). In the dry season, *T. platyurus* survives by laying resting-stage eggs (known as
29 cysts), which hatch into nauplii (first stage of crustacean larvae) within hours after being hydrated
30 (Brausch and Smith, 2009). The lethality and immobilization in *T. platyurus* larvae and adults as well as
31 the hatch rate of *T. platyurus* cysts are often used as endpoints for freshwater contaminant tests. Hydras

⁷ On the other hand, nano-TiO₂ taken up by spinach increased growth and photosynthesis by increasing the activities of enzymes important for photosynthesis (Linglan et al., 2008; Zheng et al., 2005).

1 (*Hydra attenuata*) are small simple animals with a tube-shape body (usually 1-20 mm long) and tentacles
2 on one end of the body. Intoxication of hydras can be seen in tentacle morphology, which can be normal,
3 clubbed (a sign of minor intoxication), shortened (severe intoxication), or completely retracted (lethal
4 intoxication, because this inevitably leads to death) (Environment Canada, 2007).

5 Acute and chronic toxicity of nano-TiO₂ intended for sunscreen use was studied in *Daphnia magna*
6 and reported in a poster at a scientific meeting by Wiench et al. (2007). In the acute exposure study, EC₅₀
7 values (from 48-hour mortality tests) were above 100 mg/L for all tested forms of TiO₂, which consisted
8 of three photostable forms (uncoated T-Lite™ SF, coated T-Lite™ SF-S, and coated T-Lite™ MAX), a
9 photocatalytic nano-TiO₂, and a pigment-grade TiO₂ (Wiench et al., 2007). In the chronic exposure study,
10 photostable coated T-Lite SF-S was given to *Daphnia magna* at up to 100 mg/L for 21 days, and the LC₀
11 was 30 mg/L. In this study, death was determined by the lack of swimming ability.

12 For reproductive effects after 21 days, the no observed effect concentration (NOEC) value for T-
13 Lite SF-S was 3 mg/L, and the lowest observed effect concentration (LOEC) value was 10 mg/L (Wiench
14 et al., 2007). In a different study that used photostable nano-TiO₂ intended to protect plastics against UV-
15 induced degradation, 48-hr exposure to 100 mg/L of the nano-TiO₂ induced 10% immobility in *Daphnia*
16 *magna* (Warheit et al., 2007a).

17 The effects of photocatalytic nano-TiO₂ toxicity have been studied by several research teams in
18 four types of water fleas (*Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, and *Chydorus*
19 *sphaericus*), one type of fairy shrimp (*T. platyurus*), and one type of freshwater hydra (*Hydra attenuata*).
20 For water fleas, the 48-hour mortality or immobility EC₅₀ was generally greater than 100 mg/L (Lovern
21 and Klaper, 2006; Velzeboer et al., 2008; Wiench et al., 2007), with two exceptions. One study reported
22 an LC₅₀ greater than 10 mg/L, which in this case was the highest concentration tested (Griffitt et al.,
23 2008). Another study reported a 48-hour LC₅₀ of 5.5 mg/L, using filtered nano-TiO₂ samples, which have
24 an average particle size of 30 nm after going through a 0.22-mm Nylaflo filter (Lovern and Klaper, 2006).
25 In contrast, unfiltered nano-TiO₂ samples had all sizes of nano-TiO₂ clumps, ranging from 100 to 500 nm
26 in diameter, and the mortalities never exceeded 11% at up to 500 mg/L (Lovern and Klaper, 2006).
27 Chronic exposure for 8 days caused 40% mortality at 20 mg/L in daphnids (Adams et al., 2006). For fairy
28 shrimp, the 24-hr mortality or immobility LC₅₀ was higher than 100 mg/L (Blaise et al., 2008; Heinlaan et
29 al., 2008). In the only study of hydra, the EC₅₀ of 96-hour morphological changes was less than 100 mg/L
30 (Blaise et al., 2008). The relative sensitivity among these aquatic invertebrates to nano-TiO₂ cannot be
31 determined, due to the variability of tested nano-TiO₂ formulations and experimental designs.

32 When *Daphnia magna* were exposed to photocatalytic P25 nano-TiO₂ in water, nano-TiO₂ was
33 observed on the exoskeleton and antennae and in the digestive tract (Baun et al., 2008). Baun et al.
34 (2008) noted that the aggregation of nanoparticles on the exoskeleton, at sufficient dose, might impede a

1 daphnid's mobility. Although not investigated in this study, the aggregation of nanoparticles on the
2 antennae, a chemosensory organ important for feeding and reproductive behaviors, could adversely affect
3 a daphnid's growth and reproduction (Oberdörster et al., 2006). Because nano-TiO₂ primary particles are
4 smaller than the size range of particles daphnids feed on (400–40,000 nm), the presence of nano-TiO₂ in
5 the digestive tract suggests that daphnids feed on nano-TiO₂ aggregates (Baun et al., 2008). Whether
6 nano-TiO₂ is taken up by other tissues, excreted, or transformed in daphnids is unclear (Baun et al., 2008).
7 Even if nano-TiO₂ is not absorbed into tissues, nano-TiO₂ in the digestive tract of daphnids could still
8 contribute to bioaccumulation in the food web (see Section 4.4.3).

9 The behavior and heart rate of *Daphnia magna* were evaluated in daphnids exposed to
10 photocatalytic nano-TiO₂ at 2.0 mg/L for 1 hour (Lovern et al., 2007). In this study, nano-TiO₂ had an
11 average particle diameter of 30 nm, and tetrahydrofuran, an organic solvent used to prevent aggregation,
12 was not detected in the final nano-TiO₂ suspension. The concentration of 2.0 mg/L was selected because
13 it was the lowest observed effect level (LOEL) of *Daphnia magna* mortality after 48-hour exposure
14 (Lovern and Klaper, 2006). Behavior (e.g., hopping frequency, appendage movement as an indicator of
15 feeding frequency, and postabdominal claw curling) and heart rates were not affected by the 1-hour nano-
16 TiO₂ exposure (Lovern et al., 2007).

17 5.2.1.2.3. Fish

18 Fish are used in ecological tests to represent secondary consumers in aquatic systems. Commonly
19 used fishes in ecological tests include freshwater species rainbow trout (*Oncorhynchus mykiss*), bluegill
20 sunfish (*Lepomis macrochirus*), fathead minnows, (*Pimephales promelas*) and estuarine species
21 sheepshead minnows (*Cyprinodon variegatus*). Data from zebra fish (*Danio rerio*), a model organism
22 widely used in biological and toxicological studies, can also be useful. Fish study endpoints can include
23 concentrations of chemicals, such as in fish bioaccumulation tests (see Section 4.4.1.1, Exposure);
24 mortality; behavioral markers (e.g., coughing and swimming); and pathology.

25 The toxicological studies of photostable nano-TiO₂ in fish are very limited. The 96-hr acute
26 toxicity of photostable nano-TiO₂ (DuPont uf-C) in rainbow trout produced an LC₅₀ value of greater than
27 100 mg/L (Warheit et al., 2007a). However, DuPont uf-C is designed to protect plastics from UV-induced
28 degradation, and is not known to be used in sunscreen; no fish studies of nano-TiO₂ intended for
29 sunscreen use were found.

30 In contrast, photocatalytic nano-TiO₂, which may be used in drinking water treatment, has been
31 tested in fish for acute effects (Griffitt et al., 2008; Zhu et al., 2008) and chronic effects (Federici et al.,
32 2007) (see following discussion), as well as bioaccumulation (Zhang et al., 2006) and interaction with
33 other heavy metals (see Section 4.2, Exposure). In the acute exposure study, the LC₅₀ for a 48-hr

1 exposure to an anatase/rutile mixture of uncoated nano-TiO₂ was greater than 10 mg/L for zebrafish (in
2 both female adults and <24-hr post-hatch fry) (Griffitt et al., 2008). For zebrafish eggs (blastula stage),
3 acute exposures for 96 hours at up to 500 mg/L of either nano-TiO₂ or conventional TiO₂ (both uncoated
4 anatase) did not cause developmental toxicity, as measured by survival rate of the zebrafish embryos and
5 larvae, hatching rate of embryos, and malformation in embryos and larvae (Zhu et al., 2008). In the Zhu
6 et al. (2008) study, nano-Al₂O₃ and conventional Al₂O₃ at up to 1000 mg/L also did not cause
7 developmental toxicity to zebrafish eggs, but both nano-ZnO and conventional ZnO caused decreases in
8 survival rates and hatching rate as well as increases in tissue ulceration at 1 mg/L or higher
9 concentrations.

10 Sub-lethal toxicity was observed in juvenile rainbow trout after 14 days of exposure to
11 photocatalytic P25 nano-TiO₂ (Federici et al., 2007). Respiratory toxicity and pathological changes in the
12 gill and intestine were seen after a 14-day exposure at concentrations as low as 0.1 mg/L. Furthermore,
13 there were signs of oxidative stress (increased concentrations of thiobarbituric acid substances, an
14 indicator of lipid peroxidation and oxidative stress, in multiple tissues), and activation of anti-oxidant
15 defenses (increased total glutathione levels in the gill). Na⁺K⁺-ATPase activity was also increased in the
16 gill and intestine. Disturbances were observed in the metabolism of copper and zinc, but not of Na⁺, K⁺,
17 Ca²⁺ or Mn. No major hematological disturbances were observed. Worth noting is that these effects
18 occurred without appreciable titanium accumulation in the internal organs, suggesting no nano-TiO₂
19 accumulation, as discussed earlier in Section 4.4.1. The authors suggested that surface-bound TiO₂
20 (through surface adsorption) might play a role in toxicity, similar to the case of aluminum, a surface-
21 acting toxicant that can cause systemic toxicity without significant internal accumulation. Federici et al.
22 (2007) concluded that although nano-TiO₂ was not a major hemolytic toxicant or disruptor of ion
23 regulation in this study, respiratory distress, organ pathologies, and oxidative stress were adverse effects.

24 5.2.1.2.4. Summary of Effects on Aquatic Organisms

25 Sub-lethal effects of nano-TiO₂ include decreases in daphnid reproduction by photostable nano-
26 TiO₂ (Wiench et al., 2007), as well as respiratory distress, pathological changes in gills and intestine, and
27 behavioral changes in fish (rainbow trout) by photocatalytic nano-TiO₂ (Federici et al., 2007). Several
28 studies reported visible turbidity in nano-TiO₂ stock suspensions, and the actual nano-TiO₂ concentration
29 in the liquid phase might be different from the concentration calculated from added nano-TiO₂ (Velzeboer
30 et al., 2008; Zhang et al., 2006; Zhang et al., 2008). Given that natural organic matter in the environment
31 can induce aggregation and deposition of nanoparticles or modify nanoparticle surface charges (Navarro
32 et al., 2008), the bioavailability and behavior of nano-TiO₂ in the environment are likely to be different

1 from bioavailability and behavior in pure water or simple media, although the direction of the difference
2 is difficult to predict.

5.2.1.3. Effects on Terrestrial Organisms

3 5.2.1.3.1. Plants

4 Information on nano-TiO₂ interactions with plants is available only for photocatalytic uncoated
5 nano-TiO₂ in spinach (Table 5-1). Photocatalytic uncoated nano-TiO₂ has been shown to enhance the
6 growth of spinach in several studies (Lei et al., 2008; Linglan et al., 2008; Mingyu et al., 2007a; Mingyu
7 et al., 2007b; Yang et al., 2006; Zheng et al., 2005). When a nano-TiO₂ suspension was used to soak the
8 seeds and was sprayed on the leaves, the germination rate and growth of the plant were enhanced (Zheng
9 et al., 2005). These effects were at least partially due to nano-TiO₂-induced increases in the activity of
10 several enzymes important for photosynthesis (Linglan et al., 2008), adsorption of nitrate, transformation
11 of inorganic into organic nitrogen (Yang et al., 2006), and anti-oxidative stress response (Lei et al., 2008).
12 Conventional TiO₂ suspensions showed either insignificant effects (in comparison with untreated
13 controls) or much smaller effects than nano-TiO₂ did (Linglan et al., 2008; Zheng et al., 2005).

14 5.2.1.3.2. Invertebrates

15 The only known studies on the effects of nano-TiO₂ on terrestrial invertebrates include a study on
16 an isopod, *Porcellio scaber* (Jemec et al., 2008), and a study on nematodes, *Caenorhabditis elegans*
17 (Wang et al., 2009a). Living in soil, isopods and nematodes contribute to nutrient cycling and
18 decomposition, and have been used as indicators of soil pollutants.

19 Jemec et al. (2008) investigated the effects of photocatalytic anatase nano-TiO₂ on the terrestrial
20 isopod *Porcellio scaber*, known as woodlice. Woodlice, about 16 mm long, live in the upper layer of
21 soil and surface leaf litter. They break down organic matter and contribute to soil health, and are
22 commonly used in ecological studies. In the Jemec et al. (2008) study, woodlice ate dry leaves that had
23 been soaked in nano-TiO₂ dispersions (sonicated or non-sonicated). The sonication process decreased the
24 mean agglomerate size from 780–970 nm in a non-sonicated dispersion to 350–500 nm. The activities of
25 catalase and glutathione-S-transferase (GST), two anti-oxidative stress enzymes in the digestive gland
26 (hepatopancrea) were measured. The activities of both enzymes were decreased at 0.5, 2000, and
27 3000 µg/g of non-sonicated nano-TiO₂, but not at middle concentrations (1, 10, 100, and 1000 µg/g) of
28 non-sonicated nano-TiO₂ or at any concentration (1000, 2000, and 3000 µg/g) of sonicated nano-TiO₂
29 (Jemec et al., 2008). No changes in feeding rate, defecation rate, food assimilation efficiency, weight, or
30 mortality were noted at concentrations up to 3000 µg/g of either sonicated or non-sonicated nano-TiO₂ in

1 the food. This study illustrates the importance of nano-TiO₂ dispersion preparation method on nano-TiO₂
2 toxicity.

3 Wang et al. (2009a) investigated the lethality, growth inhibition, and effects on reproduction of
4 nano-TiO₂ and conventional TiO₂ in the nematode, *C. elegans*, a small free-living (i.e., not parasitic)
5 roundworm that inhabits soil in temperate climates around the world and feeds on bacteria and fungi. In
6 the laboratory, *C. elegans* is often cultured on agar plates or in liquid medium in a Petri dish and is often
7 fed *E. coli*. In the Wang et al. (2009a) study, *C. elegans* strain Bristol N2 (wild-type) in L1 stage (larvae
8 before the first molting) was exposed to anatase nano-TiO₂ and anatase conventional TiO₂ in water. In
9 addition to lethality and growth inhibition, decreased reproduction was observed at lower mass
10 concentrations of nano-TiO₂ than conventional TiO₂. The tested reproduction parameters were eggs
11 inside body and the number of offspring per worm, which includes offspring at all stages beyond the egg
12 over the entire brood period. The mechanism of reproductive effects was not investigated. Due to the
13 lack of toxicity of supernatant of nano-TiO₂ (obtained by centrifuging the nano-TiO₂ suspension),
14 dissolution of the particle does not contribute to observed nano-TiO₂ effects on *C. elegans* (Wang et al.,
15 2009a).

5.2.1.4. Indirect and Interactive Ecological Effects

16 In addition to the direct toxicity of nano-TiO₂, indirect effects of nano-TiO₂ could also be
17 important. Nano-TiO₂ could adsorb pollutants (Nagaveni et al., 2004; Pena et al., 2006), carry the
18 pollutants into areas in an organism that the pollutants alone would not naturally appear (Moore, 2006),
19 and increase the uptake of other pollutants (a “Trojan horse” effect). Consequently, nano-TiO₂ could
20 enhance pollutant toxicity, and even cause toxicities different from those caused by exposure to the
21 pollutant alone due to differences in distribution. Also, as discussed in Section 4.2, co-exposure to nano-
22 TiO₂ in water increased the uptake of arsenic (Sun et al., 2007) and cadmium (Zhang et al., 2007) in carp,
23 but toxicity was not measured in these two studies.

24 Nano-TiO₂ was found to have no effect on the toxicity of sediment and its elutriate in a study using
25 certified reference material sediment (Blaise et al., 2008). The effects of 11 nanomaterials on sediment
26 toxicity (as measured in two direct contact assays, the Microtox solid phase assay⁸ and the Luminotox
27 solid phase assay⁹) and sediment elutriate toxicity (as measured with the MARA assay¹⁰) were studied

⁸ Microtox assay measures the reduction in light output from bioluminescent bacteria, *Vibrio fischeri*. For solid-phase assays, the concentration that causes 25% inhibition (IC₂₅) is calculated after 20 minutes of exposure.

⁹ Luminotox assay measures the inhibition of photosynthetic efficiency of photosynthetic enzyme complexes isolated from spinach leaves. For the Luminotox solid-phase assay, IC₂₀ is calculated after 15 minutes of exposure.

1 using a mixture of each nanomaterial and the certified reference material sediment at a 1:1 ratio.
2 Photocatalytic nano-TiO₂ was one of only three tested nanomaterials that did not increase the sediment or
3 elutriate toxicity in any of the three assays (Blaise et al., 2008).

5.2.1.5. Summary

4 Limited ecological toxicity information on nano-TiO₂ is currently available. Most ecotoxicological
5 studies have tested photocatalytic nano-TiO₂ that would be suitable for water treatment, but only a few
6 studies have used photostable nano-TiO₂ intended for sunscreen. Coated photostable nano-TiO₂ in
7 sunscreen could lose its coating through processes such as aging, weathering, chemical alterations (e.g.,
8 change in pH), and metabolism or biotransformation in living organisms (e.g., digestion by daphnids). If
9 so, the photocatalytic nano-TiO₂ core could be exposed and thus even photostable nano-TiO₂ could have
10 photocatalytic properties.

11 Effects of chronic exposure to nano-TiO₂ have been investigated only in water fleas and fish.
12 Although acute exposure effects have been studied in microorganisms and various aquatic
13 macroorganisms, these studies focused on lethality or immobility and provided little insight on modes of
14 action. For terrestrial organisms, only acute exposure to anatase nano-TiO₂ was investigated and only in
15 invertebrates (*P. scaber* and *C. elegans*) and spinach. Photocatalytic nano-TiO₂ decreased reproduction in
16 *C. elegans* without affecting body length. Although increased growth in spinach following acute
17 exposure to anatase nano-TiO₂ could be useful for agricultural purposes, the effects of such growth
18 promotion in an ecological system remain unclear. Photocatalytic nano-TiO₂ enhanced the uptake of
19 arsenic and cadmium in fish, indicating the possibility of interactive effects between nano-TiO₂ and co-
20 occurring toxic substances.

¹⁰ MARA assay (microbial array for risk assessment assay) measures growth inhibition in baking yeast and ten species of bacteria. A microbial toxic concentration is calculated after 18 hours of exposure.

Questions about Ecological Effects

- 5.2-1. Are current EPA standard testing protocols adequate to determine nano-TiO₂ ecotoxicity? If not, what modifications or special considerations, if any, should be made in current ecological tests? For example, what are the differences in characterization of testing material (as raw material, in media, and in organisms), dispersion methods, and realistic exposure routes between testing conventional materials and nanomaterials?
- 5.2-2. What are the ecological effects of waste and other by-products of nano-TiO₂ manufacturing?
- 5.2-3. Could ecological effects of pure nano-TiO₂ be predictive of effects from products containing nano-TiO₂ (e.g., containing stabilizers or surfactants)?
- 5.2-4. How can contributions of various nano-TiO₂ physicochemical properties to nano-TiO₂ ecological effects be identified or compared? For example, could a retrospective analysis of many studies and computer modeling identify patterns that would not be evident in individual studies? Is a structure activity relationship (SAR) approach applicable for predicting nano-TiO₂ ecological effects?
- 5.2-5. What might be the primary mechanism(s) of action of toxic effects in different species?
- 5.2-6. Are the mechanisms of cellular responses different at low and high concentrations of nano-TiO₂?
- 5.2-7. How do abiotic factors in the environment, such as UV, pH, oxygen level, and other chemicals, affect nano-TiO₂ and its ecological effects?
- 5.2-8. How do in vivo biochemical processes alter nano-TiO₂ physicochemical characteristics and toxicity?
- 5.2-9. What are the ecological effects of long-term exposure to nano-TiO₂?
- 5.2-10. What are the indirect ecological effects (e.g., on soil or water chemistry) of nano-TiO₂?
- 5.2-11. Nano-TiO₂ has anti-bacterial and anti-fungal properties. What are the effects of both photocatalytic and photostable nano-TiO₂ on the biodiversity of microorganisms?
- 5.2-12. In addition to arsenic and cadmium, do other compounds show different uptake in the presence of nano-TiO₂? Are the toxicities of arsenic, cadmium, or other chemicals affected by nano-TiO₂? Conversely, do other compounds affect the uptake and toxicity of nano-TiO₂?
- 5.2-13. Is the available ecotoxicity evidence adequate to support ecological risk assessment for nano-TiO₂? If not, what is needed?

5.3. Health Effects

1 This section summarizes and evaluates the evidence of nano-TiO₂-induced health effects from
2 epidemiological studies, laboratory animal studies, and a few selected ex vivo and in vitro studies. For a
3 review of nano-TiO₂ in vitro effects, see Fond and Meyer (2006). Organized by human and laboratory
4 animal studies and route of exposure, non-carcinogenic effects are discussed in Section 5.3.1;
5 carcinogenic effects in Section 5.3.2.

5.3.1. Non-Carcinogenic Effects

6 This section summarizes in vivo studies of nano-TiO₂ non-carcinogenic effects through dermal,
7 oral, respiratory, and other routes of exposure. The presentation is organized by exposure routes, because
8 exposure routes play a profound role in toxicokinetics, toxicodynamics, and health effects. More studies
9 have been completed on respiratory exposure (inhalation and instillation) than on other exposure routes.
10 Studies investigating solely skin penetration (not health effects) are discussed in Section 4.6.2. Most
11 studies tested photocatalytic nano-TiO₂, which could be suitable as an agent in drinking water treatment.
12 Commercial sunscreens were tested in dermal exposure studies only. Known photostable nano-TiO₂ and
13 rutile nano-TiO₂, which is expected to be photostable, were used in some studies (Chen et al., 2006; Mohr
14 et al., 2006; Nemmar et al., 2008; Oberdörster et al., 1992; Pott and Roller, 2005; Wang et al., 2007a,
15 2007b; Warheit et al., 2007a, 2007b).

5.3.1.1. Studies in Humans

16 No epidemiological studies or case reports are available for nano-TiO₂ non-carcinogenic effects. A
17 few case reports described non-carcinogenic effects in the respiratory system of workers exposed to TiO₂
18 particles of unspecified size. For example, exposure to conventional TiO₂ has been associated with
19 pneumoconiosis (Yamadori et al., 1986), pulmonary fibrosis and bronchopneumonia (Moran et al., 1991),
20 and pulmonary alveolar proteinosis (Keller et al., 1995). TiO₂ or titanium accumulation in the lung,
21 sometimes years after workplace exposures, and titanium-loaded macrophages have also been reported in
22 workers (Keller et al., 1995; Määttä and Arstila, 1975; Yamadori et al., 1986), as have titanium particles in
23 the lymph nodes (Määttä and Arstila, 1975; Moran et al., 1991) and in the liver and spleen (Moran et al.,
24 1991). None of these case reports, however, provided quantitative TiO₂ exposure data or measured
25 potentially confounding variables such as exposures to crystalline silica and tobacco smoke.

1 One epidemiological study (Chen and Fayerweather, 1988) found no consistent relationship
2 between TiO₂ (size not specified) exposure and chronic respiratory disease or fibrosis, but no conclusions
3 can be drawn because of serious limitations, including restricting subjects to workers eligible for
4 pensions; lack of information on the duration of TiO₂ exposure, asbestos or other chemical exposures; and
5 the lack of detailed information on sampling.

5.3.1.2. Animal Studies

6 For the most part (except as noted below), laboratory animal toxicity studies have investigated the
7 effects of acute or subchronic exposure to nano-TiO₂. This section presents in vivo studies of nano-TiO₂
8 (Tables 5-4 to 5-7) by route of exposure: dermal, oral, respiratory, and others. Most animal studies of
9 nano-TiO₂ focus on photocatalytic nano-TiO₂, including P25. Although sunscreen nano-TiO₂
10 formulations are intended to be photostable, the coatings that impart photostability to anatase or part-
11 anatase nano-TiO₂ in some sunscreen formulations are known to degrade over time (Barker and Branch,
12 2008; Dunford et al., 1997).

5.3.1.2.1. Toxicity from Dermal Exposure

14 Toxicity findings from studies of dermal exposure to nano-TiO₂ or sunscreen that contains TiO₂ are
15 presented in Table 5-4. For healthy unflexed skin, adverse health effects are not expected from dermal
16 exposure to photostable nano-TiO₂ in sunscreen (NANODERM, 2007; SCCP, 2007). Photocatalytic
17 nano-TiO₂, however, sometimes is used in sunscreens (Barker and Branch, 2008; Dunford et al., 1997).
18 Photocatalytic nano-TiO₂ can generate ROS when exposed to UV and can cause oxidative stress and
19 cytotoxicity in cells (cultured human fibroblasts) and in cell-free in vitro experiments (Dunford et al.,
20 1997; Lu et al., 2008). To date, the effects of long-term or repeated use of sunscreen containing nano-
21 TiO₂ have not been investigated in vivo, and no case reports of skin damage from such use are currently
22 available. As discussed earlier, most available studies indicate penetration of the outer skin layer and the
23 stratum corneum, but not penetration of living skin cells.

24 After a single topical application of photocatalytic nano-TiO₂, laboratory animals showed no skin
25 irritation 4 hours after application or sensitization 3 days after application (Warheit et al., 2007a).
26 Furthermore, although some sunscreens containing TiO₂ (size not specified) increased skin absorption of
27 herbicides and pesticides (2,4-D, paraquat, parathion or malathion), TiO₂ alone actually decreased the
28 skin absorption of the tested herbicide, 2,4-D (Brand et al., 2003). The investigators reported that a
29 solvent in the sunscreen caused increased skin absorption of herbicides, and this secondary effect can be
30 avoided by substituting phenyl trimethicone as the solvent (Brand et al., 2003).

1 Some researchers, such as Nohynek et al. (2007), have noted a discontinuity between in vitro and
2 in vivo testing results, particularly for skin toxicity. Some in vitro cultures or preparations (other than
3 those using intact skin samples) lack the stratum corneum layer, which according to currently available
4 data can block penetration, such that in vitro tests might overstate toxicity of chemicals like TiO₂. Of the
5 investigations reviewed, only three report in vivo studies of health effects after dermal exposure to TiO₂
6 [(Warheit et al., 2007a); pages 16, 17, 41–43 of (NANODERM, 2007)], and only two of those used nano-
7 TiO₂ intended for sunscreen [pages 16, 17, 41–43 of (NANODERM, 2007)]. [Warheit et al. (2007a) used
8 ultrafine particles, roughly 100 nm in size.] All three studies used a single application, and the longest
9 exposure was only 3 days. The NANODERM (2007) report concluded that “TiO₂ exposure did not
10 modify the viability, proliferation, apoptosis, and differentiation [or] adhesive properties of skin cells.” As
11 discussed previously, skin penetration studies have shown that some nano-TiO₂ can stay in hair follicles
12 for 10 days.

13 With relatively few in vivo dermal exposure studies investigating nano-TiO₂ skin absorption and
14 penetration (Table 4-5) and health effects (Table 5-4), several data gaps on the health effects of dermal
15 exposure to nano-TiO₂ are evident. First, information on the dermal penetration and effects of nano-TiO₂
16 in flexed skin and structurally compromised skin is lacking. Flexed healthy skin (Rouse et al., 2007;
17 Zhang and Monteiro-Riviere, 2008) and compromised skin (Zhang and Monteiro-Riviere, 2008),
18 including UV-exposed skin (Mortensen et al., 2008), have been shown to allow nanoparticles (other than
19 nano-TiO₂, which was not tested) to penetrate deeper than healthy non-flexed skin. Sunscreen containing
20 nano-TiO₂ is expected to be used on flexed healthy skin and misused on sunburned skin or skin with
21 micro-lesions, such as microscopic cuts due to shaving. Cytotoxicity was seen in cultured skin cells
22 treated with nano-TiO₂ (Kiss et al., 2008), and the authors postulated that, in skin with compromised
23 epidermis structure (e.g., sunburned skin or “soaked” skin), contact could occur between nano-TiO₂ from
24 sunscreen and living cells in the skin and lead to adverse effects. Second, effects from long-term,
25 repeated dermal exposures to nano-TiO₂ in sunscreen, similar to real-life exposure, have not been studied.
26 Finally, the toxicity of the various intermediate forms of nano-TiO₂ in the production process (possible
27 sources of occupational exposure, by the dermal and other routes) has not been studied.

Table 5-4. Summary of health effects of nano-TiO₂ particles in mammalian animal models: dermal route.^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse skin [female hairless (CRL:SKH1)]	Commercially available sunscreens, some of which contained TiO ₂ (size not specified)	For testing indirect dermal effect a) Commercially available sunscreens, applied at 2 mg/cm ² to skin excised from mice and placed in a diffusion chamber. 30 minutes after the sunscreen application, herbicide 2,4-D was applied on skin. b) Combination of TiO ₂ with phenyl trimethicone, ZnO, and octyl methoxycinnamate (OM) c) TiSilc Untinted sunscreen, which contains TiO ₂ , and herbicide 2,4-D. Both were applied on skin, and then again 4.5 hours after the first application d) TiSilc Untinted sunscreen and pesticides: Paraquat, Malathion, and Parathione	Some (not all) tested sunscreens increased transdermal penetration of herbicide/pesticide. Solvent, not TiO ₂ or ZnO, is responsible for sunscreen-increased skin absorption of herbicide/pesticide. a) Sunscreen effect on transdermal penetration of herbicide 2,4-D: 4 out of 7 tested sunscreens that contain TiO ₂ (and 1 out of 2 sunscreens that contain no TiO ₂) increased transdermal penetration of herbicide 2,4-D. b) Formulation effects: TiO ₂ alone, TiO ₂ plus ZnO, and TiO ₂ in trimethicone (simulation of commercial formula) decreased 2,4-D transdermal penetration. c) Repeated application of both sunscreen and herbicide: The peak penetration of 2,4-D herbicide was higher at the second application of TiSilc sunscreen and 2,4-D, compared to the first application of TiSilc and 2,4-D. However, the 2,4-D penetrations of first and second applications of TiSilc and 2,4-D were the same when skin was washed after both (but not just one) applications of TiSilc and 2,4-D. d) Sunscreen effect on transdermal penetration of other pesticides: Absorption of other pesticides (Paraquat, Malathion, and Parathione) was also increased in skin pretreated with sunscreen Ti-Silc.	Brand et al. (2003)
Human foreskin grafts on SCID mice	A commercially available sunscreen, hydrophobic emulsion containing nano-TiO ₂ (Anthelios XL SPF 60, La Roche Posay, France)	For testing dermal effects Sunscreen containing nano-TiO ₂ applied to skin at 2 mg/cm ² in occlusion for 1, 24, or 48 hours Sacrificed after exposure time; punch biopsy from the human skin graft area	No effects on cell proliferation (as measured by bromo-deoxy-uridine, BrdU, labeling); apoptosis (as measured by a double-staining method of Ki67 and TUNEL, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling); adhesive properties (as measured by the expression of P-cadherin, an adhesion molecule specific for basal epidermal keratinocytes); or differentiation (as measured by the expressions of keratin-1, keratin-10, and filaggrin) of epidermal keratinocytes. Tested sunscreen containing nano-TiO ₂ did not affect viability, proliferation, apoptosis, differentiation, or adhesive properties of skin cells.	Pages 16, 17, and 41-43 of NANODERM (2007)
Rabbit [New Zealand White]	Nano-TiO ₂ (P25—identified as uf-C in study), photocatalytic, 80% anatase/20% rutile, not coated, average particle size 129.4 nm in water, average BET surface area 53.0 m ² /g (Warheit, pers. comm., 2008b)	For testing acute dermal irritation Doses – 0 or 0.5 g Single exposure for 4 hours (nano-TiO ₂ in 0.25 mL deionized water on 6 cm ² area of skin), covered by gauze Observation at 1, 24, 48, and 72 hours after exposure	No dermal irritation effects, no clinical signs of toxicity, and no body weight loss. Not considered a skin irritant.	Warheit et al. (2007a)

Table 5-4. Summary of health effects of nano-TiO₂ particles in mammalian animal models: dermal route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [female CBA/JHsd]	Nano-TiO ₂ (P25), photocatalytic, 80% anatase/20% rutile, not coated, average particle size 129.4 nm in water, average BET surface area 53.0 m ² /g (Warheit pers. comm., 2008b) Diluting vehicle: <i>N,N</i> -Dimethyl formamide	For testing dermal sensitization (local lymph node assay) 0, 5, 25, 50, or 100% nano-TiO ₂ on both ears for 3 days Positive control group: 25% hexylcinnamaldehyde in 4:1 acetone:olive oil for 3 days (Vehicle of positive control) group: 4:1 acetone:olive oil for 3 days Sacrifice on test day 5	Increases in cell proliferation in the draining auricular lymph node of the ears treated with 50% and 100% nano-TiO ₂ compared to the vehicle control group. No dermal sensitization by nano-TiO ₂ : Stimulation index [mean disintegrations per minute of each experimental group / mean disintegrations per minute of the vehicle control group] did not exceed 3.0 in any nano-TiO ₂ treated groups. Consequently the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., where stimulation index equals 3) for nano-TiO ₂ was not calculated. Positive control group had a dermal sensitization response.	Warheit et al. (2007a)

^a BET – Brunauer, Emmett, Teller method of calculating surface area
BrdU – Bromo-deoxy-uridine
EC3 – Estimated concentration required to induce a threshold positive response, where stimulation index equals 3

OM – Octyl methoxycinnamate
TUNEL – Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling

1 5.3.1.2.2. Toxicity from Oral Exposure

2 Currently only three toxicological studies of nano-TiO₂ through oral exposure are available (Table
3 5-5). Two of them observed the toxicity for up to 2 weeks after a single oral gavage of nano-TiO₂ (Wang
4 et al., 2007a; Warheit et al., 2007a), and the other investigated genomic instability after nano-TiO₂
5 exposure through drinking water for 5 or 10 days (Trouiller et al., 2008).

6 The Warheit et al. study (2007a) was intended to provide basic hazard screening information on
7 well-characterized types of nano-TiO₂ through a “base set” of tests spanning mammalian toxicity,
8 genotoxicity, and aquatic (ecological) toxicity endpoints. The acute oral toxicity aspect of this project
9 involved female rats receiving a single oral gavage of up to 5000-mg/kg photocatalytic nano-TiO₂ (P25)
10 (3 rats per dose). The authors reported “no biologically important body weight loss” and no gross lesions
11 at necropsy 14 days after the gavage. Given that this was a basic screening study, no information on
12 organ weights, histological examinations, or blood tests (hematological or biochemical) was obtained, and
13 thus it was not meant to rule out systemic toxicity or functional changes. However, the study does
14 provide evidence that up to 5000-mg/kg nano-TiO₂ was not lethal as tested.

15 In the Wang et al. study (2007a), male and female mice received a single oral gavage of 5000-
16 mg/kg TiO₂ as 25-nm rutile spindles, 80-nm rutile spindles, or 155-nm anatase octahedrons (see Table 5-5
17 for more details). The large dose was selected because of the expected low toxicity and was
18 administered according to OECD testing procedures. No obvious acute toxicity was evident over a
19 2-week period. However, liver and kidney toxicity were indicated by biochemical parameters in the
20 serum and by pathological examination. Although no abnormal pathology was observed in the heart,
21 lung, testicle/ovary, and spleen tissues, myocardial damage was suggested by increases in serum lactate
22 dehydrogenase (LDH) and alpha-hydroxybutyrate dehydrogenase (α -HBDH), although such increases
23 might also reflect damage to other organs. Morphological changes in the brain were seen in the
24 hippocampus in both the 80-nm and 155-nm groups. The main organs with elevated TiO₂ concentrations
25 (measured only in female mice) were the liver, spleen, kidneys, lungs, and brain. Although the liver is
26 expected to receive most of the TiO₂ absorbed from the gastrointestinal tract through the portal vein,
27 elevated TiO₂ levels in the liver were observed only in the 80-nm group. The reason for this size-specific
28 elevation in hepatic TiO₂ concentration remains unknown.

29 The preliminary results of the Trouiller et al. (2008) study showed increased DNA and
30 chromosomal damage in various tissues of mice given 60–600 μ g/mL photocatalytic nano-TiO₂ (P25) in
31 drinking water for 5 days. Furthermore, the offspring of mice that were given nano-TiO₂ in drinking
32 water in the second half of the pregnancy showed increases in DNA deletions in the eye-spot assay
33 (Trouiller et al., 2008), which detects reversion of the mouse *pink-eyed unstable* (p^{un}) mutation through

1 DNA deletions of duplicated *pink-eyed dilution* (*p*) gene in the offspring of C57Bl/6J p^{un}/p^{un} mice (Reliene
2 and Schiestl, 2003; Schiestl et al., 1997). This study showed not only genotoxicity and clastogenicity, but
3 also multi-generation effects of photocatalytic nano-TiO₂ through oral exposure. Although the
4 concentrations investigated in this study are very high, the suggested modes of action and effects of
5 exposure during pregnancy are noteworthy, particularly for photocatalytic nano-TiO₂. This work is also
6 relevant to discussions of the carcinogenicity of nano-TiO₂ (see Section 5.3.2). The application of
7 genotoxicity data to the question of potential carcinogenicity is based on the premise that genetic
8 alterations are found in all cancers. Mutagenicity/genotoxicity is the ability of chemicals to alter the
9 genetic material in a manner that permits changes to be transmitted during cell division. Although most
10 tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the
11 epigenome including proteins associated with DNA or RNA, can also cause transmissible changes.
12 Genetic alterations can occur via a variety of mechanisms including gene mutations, deletions,
13 translocations, or amplification; evidence of mutagenesis provides mechanistic support for the inference
14 of potential for carcinogenicity in humans.

Table 5-5. Summary of health effects of nano-TiO₂ particles in mammalian animal models: oral route.^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [wild-type and C57Bl/6J ^{pun/pun}]	Nano-TiO ₂ (P25), photocatalytic, 80% anatase/20% rutile, not coated	For testing genotoxicity in two generations Wild-type mice: 60, 120, 300 and 600 µg/mL in drinking water for 5 days (Based the assumption of 5 mL water intake per day per mouse with a BW of 30 g, the total doses would be 50, 100, 250 and 500 mg/kg body weight) C57Bl/6J ^{pun/pun} mice for eye-spot assay: 10-day exposure, pregnant mice were given nano-TiO ₂ in drinking water from 8.5 to 18.5 days post conception. Offspring were sacrificed at 20 days old.	Increased genomic instability in exposed mice: DNA damage was increased in cells in peripheral blood at 600 µg/mL. DNA damage was measured by alkaline Comet assay, which detects DNA single strand breaks, double strand breaks, alkaline liable sites, and other lesions. DNA double strand breaks (measured by γH2AX immuno-staining) were increased in bone marrow at all tested doses. Chromosomal damage (measured by micronucleus assay) was increased in peripheral blood at 600 µg/mL. Oxidative DNA damage (measured by HPLC) was increased in liver at 600 µg/mL. Increased genomic instability in the offspring of dams exposed to nano-TiO ₂ during pregnancy: Increases in DNA deletions at the pink-eyed unstable (pun) locus [from homologous recombination or double strand breaks between the DNA fragments that contain duplicated pink-eyed dilution (p) gene (Reliene et al., 2003)] as measured by the eye-spot assay at 500 mg/kg. Increased inflammation: Increases in (mRNA levels of) pro-inflammation markers, TNF-α, IFN-γ, and IL-8 (KC) (but not anti-inflammatory markers, TGF-β, IL-10 or IL-4) in peripheral blood at 500 mg/kg as measured by real time RT-PCR.	Trouiller et al. (2008)
Rat [female, strain/stock not specified]	Nano-TiO ₂ (P25) (identified as uf-c), photocatalytic, 80% anatase/20% rutile, not coated, average particle size 129.4 nm in water, average BET surface area 53.0 m ² /g (Warheit, pers. comm., 2008b)	For testing acute effects Doses – 175, 550, 1750, or 5000 mg/kg (three rats per dose) Single oral gavage Observation for 14 days post exposure	No mortality, no biologically important body weight losses, and no gross lesions present in the rats at necropsy. Grey colored feces was observed in rats dosed at 1750 mg/kg (one rat) and 5000 mg/kg (three rats). Oral LD ₅₀ >5000 mg/kg for female rats.	Warheit et al. (2007a)

Table 5-5. Summary of health effects of nano-TiO₂ particles in mammalian animal models: oral route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [male and female CD-1 (ICR)]	<p>Nano-TiO₂ (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, uncoated, 25 nm (measured average size 21.1±5.1 nm), surface area 43.0 m²/g, column/spindle shape, purity >99% (Chen, 2008)</p> <p>Nano-TiO₂ (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, uncoated, 80 nm (measured average size 71.4±23.5 nm), surface area 22.7 m²/g, column/spindle shape, purity >99% (Chen, 2008)</p> <p>Fine TiO₂ (Zhonglian Chemical Medicine Co.), 155 nm (measured average size 155.0±33.0 nm), surface area 10.4 m²/g, anatase, uncoated, octahedrons, purity >99% (Chen, 2008)</p>	<p>Single oral gavage (acute effects)</p> <p>Dose – 5000 mg/kg</p> <p>10 female and 10 male mice per TiO₂ size group</p> <p>Necropsy at 2 weeks after the gavage</p>	<p>Hepatic toxicity:</p> <p>Increases in coefficients (wet organ weight/body weight) of liver (females in 25 nm and 80 nm groups), serum ALT (females in 25 nm group), serum ALT/AST (females in 25 nm group and males in 155 nm groups), and serum LDH (females in 25 nm and 80 nm groups).^b Decreases in AST in males in the 155 nm group (Chen, 2008).</p> <p>Pathological changes: hydropic degeneration around the central vein, spotty necrosis of hepatocytes (males and females in 80 nm and 155 nm groups).</p> <p>Nephrotoxicity:</p> <p>Increases in serum BUN (females in 25 nm group; no tin males) and serum LDH (females in 25 nm and 80 nm groups; male data not available) (Chen, 2008).^b</p> <p>Pathological changes: swelling in renal glomerules and proteinic liquid in renal tubule (males and females in 80 nm group).</p> <p>Possible brain toxicity:</p> <p>Pathological changes: increases in vacuoles in the neuron of the hippocampus (males and females in 80 nm and 155 nm groups). The vacuoles could be from reversible fatty degradation (Chen, pers. comm., 2008).</p> <p>Possible myocardial damage:</p> <p>Increase in serum LDH^b (females in 25 nm and 80 nm groups; male data not available), α-HBDH (females in 25 nm and 80 nm groups; male data not available) (Chen, pers. comm., 2008). Based on the data in this study alone, it cannot be ruled out that LDH and α-HBDH were from kidney or liver.</p> <p>No pathological changes in heart.</p> <p>No pathological changes in heart, lung, testicle/ovary or spleen in male and female mice exposed to either 80 nm or 155 nm TiO₂. No pathological changes in any organs of mice exposed to 25 nm TiO₂.</p> <p>TiO₂ distribution in female mice: increased Ti concentrations in liver (80 nm group), spleen (25, 80, 155 nm groups), kidney (25, 80 nm groups), lung (80 nm group) and brain (25, 80, 155 nm groups). For the 80 nm group, highest Ti concentration was in liver (3970 ng/g), followed by spleen, kidney, and lung (~375-625 ng/g). For 25 nm group, highest Ti concentration was in spleen (~500 ng/g).</p>	<p>Wang et al. (2007a)</p> <p>Chen (2008)</p>

^a α-HBDH – Alpha-hydroxybutyrate dehydrogenase
γH2AX – Phosphorylated form of histone H2AX (phosphorylation of H2AX at serine 139)
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
BET – Brunauer, Emmett, Teller method of calculating surface area
BUN – Blood urea nitrogen
HPLC – High performance liquid chromatography
IFN-γ – Interferon-gamma

IL-4 – Interleukin-4
IL-8 (KC) – IL-8 stands for interleukin-8 and KC for chemokine (CXC motif) ligand 1 (CXCL1)
IL-10 – Interleukin-10
LDH – Lactate dehydrogenase, a general marker of cell injury (Ma-Hock et al., 2009)
LD₅₀ – Lethal dose 50; the dosage that is lethal to 50% of the tested population
RT-PCR – Reverse transcription polymerase chain reaction
TGF-β – Transforming growth factor-beta
TNF-α – Tumor necrosis factor-alpha

^b LDH may be from heart, liver, kidney, skeletal muscle, brain, blood cells, and lungs. A test for LDH isotypes can help to narrow down the source. The primary sources for various LDH isotypes in humans are: LDH-1 from heart muscle and red blood cells; LDH-2 from white blood cells; LDH-3 from lung; LDH-4 from kidney, placenta, and pancreas; and LDH-5 from liver and skeletal muscle (Abraham et al., 2009).

1 5.3.1.2.3. Toxicity from Respiratory Exposure

2 This section discusses the health effects of nano-TiO₂ exposure through the respiratory tract (Table
3 5-6). Two methods of exposure commonly employed for studies of respiratory toxicity are inhalation and
4 instillation. Instillation can be performed in various ways, but essentially involves the direct
5 administration of a substance to the lungs rather than allowing the subject to inhale the material.
6 Intratracheal instillation “can be a useful and cost-effective procedure for addressing specific questions
7 regarding the respiratory toxicity of chemicals, as long as certain caveats are clearly understood and
8 certain guidelines are carefully followed” (Driscoll et al., 2000). Among the advantages of instillation are
9 that it permits researchers to control the doses administered into the lung and allows fast administration of
10 test material to the lower respiratory tract. Instillation studies can be useful for identifying most types of
11 effects (other than upper respiratory tract effects, such as nasal effects) and for comparing the relative
12 potency of compounds, and for this reason are of interest for screening different materials for toxicity.
13 Additionally, instillation studies require smaller amounts of test material, and chances of incidental
14 ingestion exposure (as in whole-body chamber inhalation) are lower than in inhalation studies (Driscoll et
15 al., 2000; Osier et al., 1997). On the other hand, instillation exposure involves invasive delivery,
16 bypassing of the upper respiratory tract, confounding effects from the instilled vehicle, and the use of
17 higher doses or dose rates than those tested in inhalation experiments. Confounding effects are also a
18 concern from anesthesia (needed for instillation, but not inhalation), which could affect the retention and
19 clearance of the test material (Driscoll et al., 2000). Furthermore, studies have shown that exposure to the
20 same particle through intra-tracheal instillation and inhalation can yield different responses. For example,
21 compared to inhalation, instillation caused more particles to be deposited in the basal regions of the lung
22 and caused particles to be distributed less homogenously (Osier et al., 1997). Also, results from
23 instillation cannot be extrapolated quantitatively for estimating inhalation results (Driscoll et al., 2000).

24 Interpreting and comparing results from studies with different respiratory exposure methods (such
25 as inhalation, instillation, and aspiration) requires caution. Differences among exposure methods could
26 influence uptake doses and particle distributions in the body. Also, the test material preparation required
27 for different exposure methods (such as aerosol and suspension medium preparation) could affect
28 nanomaterial aggregation. Conclusions drawn from studies using different methods should disclose
29 confounding factors to avoid misleading readers. As an illustration, consider a study that exposed mice to
30 single-walled carbon nanotubes (SWCNT) through inhalation and pharyngeal aspiration (Shvedova et al.,
31 2008). Even though the doses were designed to generate the same deposited dose in the lung, the aerosol
32 generation and agglomerate sizes of the test material differed. The authors carefully stated their
33 conclusion at the end of discussion as: “Because of exposure to smaller SWCNT structures by inhalation

1 of a dry aerosol vs. aspiration of a particle suspension containing micrometer-size agglomerates,
2 inhalation exposure was more potent than aspiration of an equivalent mass of SWCNT.”

3 The tendency of nano-TiO₂ to agglomerate raises an important issue for interpreting experimental
4 toxicology studies when the respiratory tract is the portal of entry. Upon inhalation, insoluble particles
5 will deposit in the lung according to the aerodynamic diameter of the particulate unit (i.e., the
6 agglomerate) and the physiological/morphometric characteristics of the subject. Once deposited as a
7 result of inhalation or intratracheal instillation, additional factors (e.g., physicochemistry of the particles,
8 biochemistry of the fluid lining of the lung, and other pharmacokinetic factors of the subject) may impact
9 particle size and composition and determine the ultimate dose to the target cell/molecule. The influence
10 of the lung milieu on agglomeration is discussed in more detail below.

11 It should be noted that the concentrations in available respiratory toxicity studies of nano-TiO₂ are
12 presumably much higher than likely ambient or occupational exposure levels. High concentrations of
13 fine-mode particles are known to cause the phenomenon of “particle overload.” In its simplest terms, at
14 sufficiently high concentrations, the body’s ability to clear inhaled particles is severely compromised to
15 the point that effects occur that would not occur at high-end “real-world” exposures [see ILSI Risk
16 Science Institute Workshop Participants (2000) for summary]. Thus, under particle overload conditions,
17 exposure-response relationships and even the type of responses produced can be unreliable. However, the
18 nanoparticle exposures evoking particle overload have not been fully described.

Effects in Respiratory Tract

19 As discussed below and summarized in Table 5-6, pulmonary effects studied through inhalation or
20 instillation of nano-TiO₂ include pulmonary inflammation, recruitment of neutrophils and macrophages,
21 nano-TiO₂ aggregate-loaded macrophages, disruption of alveolar spaces, alveoli enlargement,
22 proliferation of alveolar type II pneumocytes, and increases in alveolar epithelial thickness. Selected
23 instillation studies are highlighted here primarily for effects not investigated in inhalation studies (i.e.,
24 effects outside the respiratory tract and interactions with other factors).

25 Some of the factors that affect nano-TiO₂ respiratory tract toxicity were investigated by
26 Oberdörster et al. (2000). Toxicity of nano-TiO₂ could be decreased by cross-tolerance to oxidative
27 stress, because nano-TiO₂ given through an intra-tracheal instillation caused less inflammation in rats
28 previously exposed (and adapted) to Teflon fumes than in rats that were not adapted. Furthermore, nano-
29 TiO₂ induced more severe pulmonary inflammation in compromised rats, which had been given an
30 endotoxin to mimic gram-negative bacterial infections, than in healthy rats.

Inhalation and Instillation in the Same Study

1 Grassian et al. (2007a) exposed mice to nano-TiO₂ through either inhalation or intranasal
2 instillation. After instillation exposures to similar surface area doses (based on primary particle surface
3 areas) of 5-nm anatase nano-TiO₂ and 21-nm anatase/rutile nano-TiO₂, mice showed a more severe
4 inflammation response to 21-nm nano-TiO₂ than to 5-nm TiO₂. This example shows that surface area
5 alone is not a sufficient dose metric in all studies (Grassian et al., 2007a; Warheit et al., 2007a), especially
6 when the crystal form and other factors are not the same. In the Grassian et al. (2007a) study, the
7 aggregates of 21-nm and 5-nm nano-TiO₂ differed in both size and density, either of which could affect
8 the surface area that would interact with the tissues. Although the same nano-TiO₂ was used in both
9 inhalation and intranasal instillation, direct comparisons of exposure routes effects were not feasible for
10 two reasons. First, the exposure doses were not the same, whether the doses were expressed as particle
11 concentrations in air or solution, estimated particle mass per mouse, or estimated particle surface area per
12 mouse. Second, different vehicles (water for inhalation and saline for instillation) were used and the sizes
13 of agglomerates were larger in inhalation aerosols than in instillation.

14 In a study by Osier et al. (1997), acute intra-tracheal inhalation of high levels (125 mg/m³) of fine
15 and nano-TiO₂ caused less severe pulmonary response than intra-tracheal instillation. Intra-tracheal
16 inhalation involved delivering aerosols to the trachea of anesthetized rats.

Inhalation Studies

17 The effects in the respiratory tract after inhalation of nano-TiO₂ were consistent among studies.
18 With increases in exposure duration, pulmonary lesions in rodents evolve from reversible pulmonary
19 inflammation (in rats, mice, and hamsters) to impaired particle clearance or overload (in rats and mice,
20 but not hamsters) and cellular proliferation (in rats and mice, but not hamsters). In rats, but not in mice or
21 hamsters, chronic exposure leads to pulmonary alveolar fibrosis, metaplasia, and eventually lung tumors.

22 In acute and subacute studies in mice and rats, the severity of pulmonary inflammation increased
23 with increases in exposure time, and symptoms (pulmonary inflammation and increases in cell
24 proliferation in bronchi and bronchioles) were reversible when exposure ended (Grassian et al., 2007b;
25 Ma-Hock et al., 2009).

26 In subchronic studies of nano-TiO₂ exposure for 12 or 13 weeks, pulmonary inflammation,
27 pathological changes in the lung (including fibrosis), and impairment of alveolar macrophage-mediated
28 test particle clearance were reported (Baggs et al., 1997; Bermudez et al., 2002; Bermudez et al., 2004;
29 Hext et al., 2002; Hext et al., 2005; Oberdörster et al., 1994). Similar to pulmonary lesions after acute
30 and subacute exposure, pulmonary lesions after subchronic inhalation exposure were also decreased with
31 recovery time, but some lesions, such as fibrotic reactions in the lung, were not completely reversed even
32 after 1 year of recovery.

1 Species differences to nano-TiO₂ effects were observed among rats, mice, and hamsters (Baggs et
2 al., 1997; Bermudez et al., 2002; Bermudez et al., 2004; Hext et al., 2002; Hext et al., 2005; Oberdörster
3 et al., 1994). Pulmonary responses after 13 weeks of exposure were generally most severe in rats,
4 followed by mice, and least severe in hamsters. Rats and mice, but not hamsters, experienced overload at
5 10 mg/m² nano-TiO₂. Furthermore, only rats had fibroproliferative lesions and alveolar epithelial
6 bronchiolization (a type of metaplasia).

7 In chronic studies of nano-TiO₂ inhalation in rats (Creutzenberg et al., 1990; Gallagher et al., 1994;
8 Heinrich et al., 1995) and mice (Heinrich et al., 1995), lung tumors occurred in rats, but not in mice (for
9 more on carcinogenicity effects in these studies, see Section 5.3.2). In the study of Creutzenberg et al.
10 (1990), decreased pulmonary clearance (overload) was clearly demonstrated by using two sizes of tracer
11 particles after nano-TiO₂ exposure. During the 24-month exposure to nano-TiO₂ (see Table 5-6 for
12 concentrations), rats inhaled (nose-only) two types of radioactive tracers at 3, 12, and 18 months after the
13 beginning of the experiment. The half-times for pulmonary clearance of the smaller tracer particles
14 (0.35- μ m ⁵⁹Fe₂O₃) were more than 3-times longer in rats exposed to nano-TiO₂ at all three tested time
15 points, indicating overload. For the larger tracer particles (3.5- μ m ⁸⁵Sr polystyrene), overload was seen at
16 3 and 12 months, and the clearance was back to control level at 18 months, which may be due to
17 increased lung weight, altered lung structure, and altered breathing pattern, all of which could
18 consequently change the deposition of ⁸⁵Sr polystyrene particles (Creutzenberg et al., 1990).

Systemic Effects and Effects in Heart, Liver, Kidney, and Microvasculature

19 The effects of respiratory exposure to nano-TiO₂ are not limited to the respiratory system. In rats
20 exposed to 5-mg nano-TiO₂/kg BW of rutile nano-TiO₂ rods through a single intra-tracheal instillation,
21 observed effects included increases in the numbers of monocytes and granulocytes in the blood (signs of
22 systemic inflammation); decreases in the number of platelets in the blood (platelet aggregation); and
23 cardiac edema (Nemmar et al., 2008). In mice exposed to rutile and anatase nano-TiO₂ through intranasal
24 instillation, pathological changes were observed in the kidney, and temporary liver injury was suggested
25 by changes in serum biomarkers (Wang et al., 2008b).

26 Endothelium-dependent arteriolar dilation was impaired (decreased) by both fine TiO₂ and nano-
27 TiO₂ inhaled by rats, more so by nano-TiO₂ than fine TiO₂ at similar lung load mass doses (Nurkiewicz et
28 al., 2008). This microvascular dysfunction was due to fine TiO₂- and nano-TiO₂-induced increases in
29 ROS in the microvascular wall, increases in nitrotyrosine expression in spinotrapezius microcirculation,
30 and decreases in microvascular NO production (Nurkiewicz et al., 2009). In both fine TiO₂- and nano-
31 TiO₂-treated groups, vascular smooth muscle sensitivity to NO was not altered, but the microvascular NO
32 bioavailability was compromised (Nurkiewicz et al., 2009).

Effects in Brain

1 Since 1970, scientists have known that inhaled ultrafine air pollutants and engineered nanoparticles
2 translocate into the brain (Oberdörster et al., 2004). Inflammatory responses, altered neurotransmitter
3 levels, and pathological changes have been observed in rodent brains after inhalation of manganese oxide
4 (Elder et al., 2006); instillation of nano carbon black (Tin Tin Win et al., 2008); and inhalation of ultrafine
5 elemental ¹³C particles (Oberdörster et al., 2004). A few recent studies showed that anatase and rutile
6 nano-TiO₂ translocate into the brain following intranasal instillations (Wang et al., 2008a; Wang et al.,
7 2008b, 2007b).

8 The only available studies of nano-TiO₂ effects on the central nervous system are from a research
9 group that has administered nano-TiO₂ to mice using intranasal instillation (Wang et al., 2008a; Wang et
10 al., 2008b; Wang et al., 2007b). These researchers have reported increased oxidative stress and
11 inflammatory response, altered concentrations and metabolism of neurotransmitters, and pathological
12 changes in the mouse brain. When mice were given 25-nm rutile, 80-nm rutile, or 155-nm anatase nano-
13 TiO₂ through intranasal instillation (50 mg nano-TiO₂/kg BW every two days for 2, 10, 20, or 30 days),
14 changes in neurotransmitter levels in the brain were observed only in mice exposed to 80-nm and 155-nm
15 nano-TiO₂, whereas brain TiO₂ concentrations were similar for all three sizes of nano-TiO₂ (Wang et al.,
16 2007b). After intranasal instillation of 80-nm rutile or 155-nm anatase nano-TiO₂ (500 µg per mouse
17 every other day for up to 30 days), the highest titanium concentrations in the brain were in the
18 hippocampus and olfactory bulb, the two regions where most pathological changes were also seen (Wang
19 et al., 2008a; Wang et al., 2008b). The hippocampus and astrocytes seem to be the targets of nano-TiO₂
20 toxicity in the brain (Wang et al., 2008a; Wang et al., 2008b). At the ultra-structural level, mitochondria
21 appear to be a target of nano-TiO₂ in nerve cells after both in vivo and in vitro exposures (Long et al.,
22 2006; Wang et al., 2008b). For the whole brain, inflammatory responses and oxidative stress, including
23 lipid peroxidation and protein oxidation, were detected as elevated levels of oxidative markers and
24 cytokines in mice exposed to 80-nm rutile and 155-nm anatase nano-TiO₂ (Wang et al., 2008a; Wang et
25 al., 2008b).

26 Levels of several neurotransmitters, including norepinephrine, 5-hydroxytryptamine, homovanillic
27 acid, 5-hydroxyindole acetic acid, dopamine, and glutamic acid, were altered after intranasal instillation
28 of nano-TiO₂ (Wang et al., 2008a; Wang et al., 2008b; Wang et al., 2007b). Nitric oxide, which serves as
29 a neurotransmitter and an important player in inflammatory responses, was also increased in the brain of
30 mice exposed to 80-nm and 155-nm nano-TiO₂ (Wang et al., 2008a). Additionally, the activity of
31 cholinesterase, which inactivates the neurotransmitter acetylcholine, increased (Wang et al., 2008a).
32 These changes showed that the concentrations and metabolism of neurotransmitters in the brain were
33 affected by nano-TiO₂ given through intranasal instillations.

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route. ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Inhalation and Instillation in the same report				
Mouse [male C57Bl/6]	<p>Nano-TiO₂ (Nanostructured and Amorphous Materials), anatase, 5 nm, measured BET surface area 219±3 m²/g, surface functionalization: O, O-H, H₂O. Aerosol size: 119±1.56 nm (inhalation high dose), 122.9±1.55 nm (inhalation low dose)</p> <p>Nano-TiO₂ (Degussa), anatase/rutile, 21 nm, BET surface area 41±1.1 m²/g, surface functionalization: O, O-H, H₂O. Aerosol size: 138.8±1.44 m²/g (inhalation high dose), 152.9±1.38 m²/g (inhalation low dose)</p>	<p>Single inhalation exposure for 4 hours</p> <p>Particle concentration in chamber:</p> <p>5 nm TiO₂:</p> <p>Low: 0.77 mg/m³ (necropsy immediately after exposure)</p> <p>High: 7.22 mg/m³ (necropsy immediately after exposure); 7.35 mg/m³ (necropsy 20 hours after the end of exposure)</p> <p>21 nm TiO₂:</p> <p>Low: 0.62 mg/m³ (necropsy immediately after exposure)</p> <p>High: 7.16 mg/m³ (necropsy immediately after exposure); 7.03 mg/m³ (necropsy 20 hours after the end of exposure)</p>	<p>Increases in the numbers of total cell (high 5 nm, low and high 21 nm) and macrophage (high 5 nm and 21 nm) in BAL fluid immediately after exposure (not 20 hours after exposure).</p> <p>No changes in histology of the lung, total protein, LDH activity, or neutrophil number in BAL fluid.</p> <p>Nano-TiO₂ distribution (only 4 high groups examined): agglomerates were seen in macrophages, alveolar epithelial cells, and alveolar interstitium. Little difference between 5 and 21 nm exposures or necropsy time.</p> <p>Calculated/estimated particle mass per mouse (µg) and particle surface area (cm²):</p> <p>5 nm TiO₂ Low: 1.3 µg/mouse and 3.2 cm² (immediately after exposure)</p> <p>5 nm TiO₂ High: 12.5 µg/mouse and 30.3 cm² (immediately after exposure) 12.7 µg/mouse and 30.7 cm² (20 hours after exposure)</p> <p>21 nm TiO₂ Low: 1.1 µg/mouse and 2.2 cm² (immediately after exposure)</p> <p>21 nm TiO₂ High: 12.4 µg/mouse and 24.8 cm² (immediately after exposure) 12.2 µg/mouse and 24.4 cm² (20 hours after exposure)</p>	Grassian et al. (2007a)
		<p>Single intra-nasal instillation</p> <p>Particle concentration in instillation solutions:</p> <p>5 nm TiO₂:</p> <p>Low: 0.1 mg/mL</p> <p>Medium: 0.4 mg/mL</p> <p>High: 0.6 mg/mL</p> <p>21 nm TiO₂:</p> <p>Low: 0.5 mg/mL</p> <p>Medium: 2.0 mg/mL</p> <p>High: 3.0 mg/mL</p> <p>Necropsy 24 hours after instillation</p>	<p>21 nm TiO₂ induced more inflammation than 5 nm TiO₂: Increases in neutrophil number (21 nm low, medium and high; 5 nm medium and high); total cell number and IL-6 (21 nm medium and high); LDH activity and IL-1β (21 nm high) in BAL fluid.</p> <p>No pathological changes in lung; no changes in TNF-α in BAL fluid.</p> <p>21 nm anatase/rutile TiO₂ and 5 nm anatase TiO₂ do not share the same dose-response curve for neutrophil concentration in BAL fluid as a function to either particle mass or surface area.</p> <p>Calculated/estimated particle mass per mouse (µg) and particle surface area (cm²):</p> <p>5 nm TiO₂ Low: 5 µg/mouse and 12.1 cm²</p> <p>5 nm TiO₂ Medium: 20 µg/mouse and 48.4 cm²</p> <p>5 nm TiO₂ High: 30 µg/mouse and 72.6 cm²</p> <p>21 nm TiO₂ Low: 25 µg/mouse and 12.5 cm²</p> <p>21 nm TiO₂ Medium: 100 µg/mouse and 50 cm²</p> <p>21 nm TiO₂ High: 150 µg/mouse and 75 cm²</p>	

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rats [female F344]	Fine TiO ₂ (Fisher Scientific), mean primary particle size 250 nm, anatase Nano-TiO ₂ (Degussa), mean primary particle size 21 nm, anatase	Acute intra-tracheal instillation and intra-tracheal inhalation Intra-tracheal inhalation exposure for 2 hr at 125 mg/m ³ Intra-tracheal instillation exposure to the equivalent amount of TiO ₂ as in the lung at day 0 of intra-tracheal inhalation (500 µg fine TiO ₂ or 750 µg nano-TiO ₂ in 0.2 mL saline) Necropsy 0, 1, 3 or 7 days post exposure (three rats per group)	Compared to fine TiO ₂ , nano-TiO ₂ caused more pulmonary responses and slightly higher (not significant) lung TiO ₂ burden. Compared to intra-tracheal instillation, intra-tracheal inhalation to TiO ₂ generally caused less severe and less persistent pulmonary responses and slightly (not significant) higher TiO ₂ lung burden. Increases in polymorphonuclear leukocytes in BAL cell pellet on day 1 after intra-tracheal inhalation of fine TiO ₂ ; on days 1, 3, and 7 after intra-tracheal instillation of nano-TiO ₂ ; and days 0 and 1 after intra-tracheal inhalation of nano-TiO ₂ . Decreases in macrophage inflammatory protein-2 levels in BAL supernatant on days 0, 1, and 3 after intra-tracheal inhalation of nano-TiO ₂ ; and day 1 after intra-tracheal instillation of nano-TiO ₂ . Increases in macrophage inflammatory protein-2 levels in BAL cell pellets on days 1, 3, and 7 after intra-tracheal instillation of nano-TiO ₂ ; and on days 0 and 1 after intra-tracheal inhalation of nano-TiO ₂ . Increases in TNF-α protein was detected by immunocytochemistry (but not by ELISA) on days 0 and 1 after intra-tracheal inhalation of water (control); days 1 and/or 3 after intra-tracheal instillation of fine or nano-TiO ₂ and intra-tracheal inhalation of fine TiO ₂ ; and at all time points after intra-tracheal inhalation of nano-TiO ₂ . Inflammatory cell influx (polymorphonuclear leukocytes in BAL) was correlated with macrophage inflammatory protein-2 levels in BAL cell pellet (but not in BAL supernatant), but not correlated with TNF-α protein levels in BAL cell pellet or supernatant or in lung sections stained immunocytochemically.	Osier et al. (1997)
Inhalation				
Rats [male F344]	Nano-TiO ₂ , ~20 nm, anatase (Degussa) Fine TiO ₂ , ~250 nm, anatase (Fisher Scientific) Crystalline SiO ₂ , ~800 nm	Subchronic inhalation Nano-TiO ₂ : 23.5 mg/m ³ ; fine TiO ₂ : 22.3 mg/m ³ ; SiO ₂ 1.3 mg/m ³ 6 hr/day, 5 days/wk for 3 months 6- or 12-month recovery before sacrifice	Lung burden: SiO ₂ : 0.32 mg immediately after exposure. Nano TiO ₂ /fine TiO ₂ : 5.33/6.62 mg, 4.15/1.2 mg, 3.14/1.66 mg immediately, 6 months, 12 months after exposure, respectively. 6 months after exposure, in the lung: SiO ₂ caused moderate focal interstitial fibrosis and moderately severe focal alveolitis; nano TiO ₂ caused slightly less fibrosis and fine TiO ₂ caused least fibrosis. Increases in stainable collagen in all three treated groups, compared to untreated groups. 12 months after exposure, in the lung: SiO ₂ -treated rats showed decreased fibrosis; nano TiO ₂ and fine TiO ₂ treated rats showed largely normal amount of interstitial fibrosis but increases in alveolar macrophage number. Increases in stainable collagen only in SiO ₂ .	Baggs et al. (1997)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued). ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
<p>Rat [female CDF (F344)/CrIBR]</p> <p>Mouse [female B6C3F1/CrIBR]</p> <p>Hamster [female Syrian golden (Lak:LVG [SYR] BR)]</p>	<p>Fine TiO₂ (DuPont), rutile; aerosol 1.36 – 1.44 µm MMAD</p> <p>Nano-TiO₂ (P25), photocatalytic, average primary particle size 21 nm, 1.37 µm MMAD; aerosols: 1.29-1.44 µm MMAD</p>	<p>Subchronic inhalation</p> <p>Fine TiO₂: 0, 10, 50 or 250 mg/m³</p> <p>Nano-TiO₂: 0, 0.5, 2, or 10 mg/m³</p> <p>6 hr/day, 5 days/wk for 13 weeks</p> <p>0 (immediately after exposure), 4, 13, 26, or 52 (up to 46 and 49 for hamsters exposed to fine TiO₂ and nano-TiO₂, respectively) weeks of recovery before sacrifice</p>	<p>Lung burden of fine TiO₂:</p> <p>Immediately after exposure: lung burden of fine TiO₂: mice > rats > hamsters at 50 and 250 mg/m³; rats > mice > hamsters at 10 mg/m³. The lung burden decreased with time after exposure.</p> <p>The retention in lung-associated lymph nodes: rats > mice > hamsters at all concentrations. The burden in the lymph nodes increased with time after exposure (rats of all dose groups, mice of low and mid-dose groups, and hamsters of high-dose group).</p> <p>Pulmonary clearance kinetics of fine TiO₂: mice and rats in high-dose groups retained 75% initial burden after 52 weeks of recovery, while hamsters retained only 10% initial burden after 26 weeks of recovery. Overload in rats and mice at 50 or 250 mg/m³.</p> <p>Lung burden of nano-TiO₂:</p> <p>Lung burden of nano-TiO₂: rats ≥ mice > hamster. Immediately after exposure, at 10 mg/m³, rats and mice had same lung burdens for nano-TiO₂. At 2 or 0.5 mg/m³, rats had more lung burden. Mice and rats, but not hamsters, have pulmonary particle overload at 10 mg/m³.</p> <p>Pulmonary clearance kinetics of nano-TiO₂: At 10 mg/m³, rats and mice had linear fashion decreases of lung burden to ~50% after 52-week recovery, while hamsters had a biphasic fashion decrease to 3% after 48-week recovery. At 2 and 0.5 mg/m³, rats, mice and hamsters had biphasic decreases in lung burn, and rats only had detectable nano-TiO₂ after the whole recovery period.</p> <p>Burden in the lymph nodes associated with lung: During the whole recovery time, burden increased with time in rats of 10 and 5 mg/m³ groups, and in mice of 10 mg/m³ group. No nano-TiO₂ was detected in hamster lymph nodes at any time point or treatment group.</p> <p>General health of rats, mice and hamsters:</p> <p>Rats and mice at all treated groups had decreases in weight gain after exposure, and recovery occurred 3-4 week post exposure. Mice exposed to 250 mg/m³ fine TiO₂ had a consistent lower weight during the recovery period, but rats exposed to 250 mg/m³ fine TiO₂ had a consistent heavier weight. Hamster exposed to fine TiO₂ had decreases in weight gain after exposure, and recovery 6 weeks post exposure. Hamsters exposed to nano-TiO₂ had weight loss after exposure and a slow recovery over the remainder of the study. Hamsters had higher morbidity and mortality rates across treatment groups than rats and mice; this was probably due to age-related renal diseases.</p>	<p>Fine TiO₂: Bermudez et al. (2002)</p> <p>Nano-TiO₂: Bermudez et al. (2004)</p> <p>Comparison of fine and nano-TiO₂ data reported in Bermudez et al. (2002) and Bermudez et al. (2004); Hext et al. (2002, 2005)</p>

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued). ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
(continuation from previous page)			<p>Pulmonary inflammation after fine TiO₂ exposure: Rats, mice and hamsters had pulmonary inflammation, and only hamsters had full recovery.</p> <p>Rats generally had more severe inflammation, and hamsters had the least.</p> <p>Fine TiO₂ exposure: Increases in neutrophil %, lymphocyte %, and macrophage number in BAL fluid in rats and mice (in mid- and high-dose groups); increase in neutrophil % in rats at the lowest exposure. Hamsters had increased macrophage number, neutrophil %, and lymphocyte % at the highest concentration; they had an increased neutrophil % at the medium concentration. Within 26 weeks of recovery, hamsters showed normal neutrophil % and macrophage number; within 46 weeks of recovery, hamsters had normal lymphocyte %. Mice and rats showed partial recovery in neutrophil and macrophage response and no recovery in lymphocyte response after 52 weeks of recovery.</p> <p>Fine TiO₂ exposure: LDH levels in BAL fluid transiently increased in mice and rats</p> <p>Pulmonary inflammation after nano-TiO₂ exposure: Rats and mice have pulmonary inflammation.</p> <p>Nano-TiO₂ exposure: Rats and mice, but not hamsters, in the 10 mg/m³ groups have increased numbers of macrophage and neutrophil and concentrations of LDH and protein in BAL fluid.</p> <p>Pulmonary lesions are most severe in rats, and least in hamsters.</p> <p>Fine TiO₂ exposure: Alveolar cell proliferation was seen in rats (0 week post exposure at mid- and high-dose groups, 4 and 13 weeks post exposure at high-dose group) and mice (13 and 26 weeks post exposure at high-dose group), but not in hamsters.</p> <p>Only rats had a progressive fibroproliferative lesion and alveolar epithelial metaplasia (bronchiolization).</p> <p>Fine TiO₂ exposure: At 52 weeks post exposure, mouse lungs had particle-laden macrophages in alveolar and relatively normal alveolar septal structures. Rat lungs had particle-laden macrophages inside alveolar cells, fibrosis and thickening in interstitial tissue, and little alveolar epithelial metaplasia (bronchiolization) of lining epithelium. Hamster lungs did not show retained particle burden or macrophage accumulation.</p> <p>Nano-TiO₂ exposure: Alveolar epithelial proliferation, alveolar bronchiolization (alveolar epithelial proliferation of metaplastic epithelial cells around macrophages loaded with particles), alveolar septal fibrosis and interstitial particle accumulation in rats, but not mice nor hamsters, of the 10 mg/m³ group. With increasing time post exposure, the lesions became more severe.</p> <p>Species and particle differences:</p> <p>Overload was seen in rats and mice (but not hamsters) exposed to 50 and 250 mg/m³ fine TiO₂ or 10 mg/m³ nano-TiO₂.</p> <p>Lung TiO₂ burdens and tissue responses in mice, rat and hamsters exposed for 13 weeks to 10 mg/m³ nano-TiO₂ or to 50 mg/m³ fine TiO₂ were similar for all three species.</p>	

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [female Wistar]	Nano-TiO ₂ (P25), photocatalytic, 80% anatase/20% rutile, primarily particle size 15-40 nm, 0.8 µm MMAD	Chronic inhalation Rats: 24 month exposure: 7.2 mg/m ³ for the first 4 months, followed by 14.8 mg/m ³ for 4 months, 9.4 mg/m ³ for 16 months, and clean air for 6 months (concentration sometimes are reported as 7.5, 15, 10 mg/m ³) 18 or 19 hr/day, 5 days/week in whole body chamber Mice: 13.5 month exposure: Same treatment as in rats for the first 8 months, followed by 9.4 mg/m ³ for 5.5 months, and clean air for 9.5 months	Rats: Increases in lung weight, and retention of inhaled nano-TiO ₂ in lungs and lung-associated lymph nodes (mean lung retention was 39.3 mg/lung at the end of exposure). The retention slowly decreased post exposure (from 40 mg/lung after 18 months of nano-TiO ₂ exposure to 3.3 mg/lung at 4 months post exposure). Increased half-time of pulmonary clearance of tracer particles For inhaled 0.35 µm labeled tracer particles, After 3-, 12-, 18-month nano-TiO ₂ exposure and 18-month exposure plus 3-month recovery, clearance half times were 208, 403, 357, and 368 days, respectively. The controls had 61-96 days for all time points. For inhaled 3.5 µm labeled tracer particles, After 3-, 12-, 18-month nano-TiO ₂ exposure and 18-month exposure plus 3-month recovery, clearance half times were 1222, 229, 58 and 48 days, respectively. The controls had 58-70 days for all time points. The decreases in clearance half time after 12- and 18-month exposure, compared to controls, was possibly due to increases in lung weight, altered lung structure and breathing pattern, which lead to more in the tracheo-bronchial region of the lung and apparently higher clearance rates.	Creutzenberg et al. (1990)
Mouse [female NMRI]			Rats did not have increases in DNA adducts in the lung: No increases in DNA adduct 2 (nuclease P1-sensitive adduct) in the lung. Decreases in DNA adduct 1 (age-related, putative I-compound) in peripheral lung DNA compared to filtered air-exposed rats, probably due to adduct dilution through cell proliferation induced by particle exposure.	Gallagher et al. (1994)
			Rats: Increased mortality (60% vs. 42% in control) and lung wet weight, decreased mean lifetime and body weight. Increased incidence of lung tumors [18-month exposure: 5 out of 20 rats exposed to TiO ₂ (0 out of 18 in control) had lung tumors. 24-month exposure: 4/9 rats in TiO ₂ (0/10 in control)]. Mice: No increase lung tumors. Increased mortality (33% vs. 10% in control) and lung wet weight, decreased body weight. Carcinogenic in rats, but not in mice.	Heinrich et al. (1995)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [male C57Bl/6]	Nano-TiO ₂ (Nanostructured and Amorphous Materials), anatase, measured average primary particle size 3.5 ± 1.0 nm, BET surface area 219 ± 3 m ² /g, surface functionalization: O, O-H, H ₂ O (manufacturer reported primary particle size 5 nm, surface area 210 m ² /g) Aerosol size geometric mean 120-128 ± 1.6-1.7 nm for acute (two concentrations) and subacute (one concentration) exposures	Acute inhalation Doses – 0, 0.77, or 7.22 mg/m ³ Single exposure of 4 hours in whole-body chamber No recovery time	No adverse effect/Minimal pulmonary inflammation. No treatment effects on most parameters measured to gauge inflammatory response (neutrophil number in BAL fluid, total protein, and LDH activity were not changed), and no effects on lung histopathology. Increased total cell count and macrophage count in BAL fluid at highest dose.	Grassian et al. (2007b)
		Subacute inhalation Doses – 0 or 8.88 mg/m ³ 4 hr/day for 10 days in whole-body chamber 0, 1, 2, or 3 wk of recovery before sacrifice	Moderate but significant pulmonary inflammatory response that lasted for at least 2 wk but resolved by wk 3 after exposure. No changes in most parameters measured to gauge inflammatory response [total protein, LDH activity, and cytokine (IFN-γ, IL-6, or IL-1β) concentrations in BAL fluid were not changed], and no effects on lung histopathology. Increased macrophage count in BAL fluid in treated group at wk 0, 1, and 2 post exposure, but not at wk 3 post exposure. Macrophages in BAL fluid were loaded with TiO ₂ particles, and less so at wk 3 post exposure.	
Rat [male Wistar]	Nano-TiO ₂ (Baker & Collinson, Inc.), uncoated, 14% rutile/86% anatase, hydrophobic surface, average primary particle 25.1±8.2 nm (range 13- 71 nm) measured under TEM. BET surface area 51.1±0.2 m ² /g. Zeta potential was 16.5±2.2 mV in 1 mM KCl. Aerosols: 0.7-1.1 μm MMAD (geometrical standard deviations 2.3-3.4). Small and large agglomerates in the atmospheres, ranging from below 100 nm to several hundred nm. Estimated number concentrations of particles <100 nm represents only 0.1-0.4% of the total particle mass for all three atmospheres.	Short-term inhalation 0, 2, 10, and 50 mg/m ³ (actual concentrations 0, 2.4, 12.1, and 50.0 mg/m ³), 6 hr/day for 5 days, head-nose exposures to dust aerosols No recovery (immediately after the last exposure), 3- or 16-day recovery after the last exposure. In other words, necropsy on study days 5, 8, and 21, respectively.	Absolute lung weight was increased at 50 mg/m ³ immediately after exposure, but not after 16-day recovery. Lung burden: 118.4, 544.9 and 1635 μg/lung immediately after inhalation of 2, 10 and 50 mg/m ³ nano-TiO ₂ , respectively. 16 days of recovery later, the lung burdens were 93.4, 400.4 and 1340 μg/lung, respectively. Calculated clearance half-times were 47, 36 and 56 days for 2, 10 and 50 mg/m ³ groups, respectively. In the mediastinal lymph nodes, TiO ₂ was only detected in the 50 mg/m ³ group, and the nano-TiO ₂ concentrations were higher at 16 days after the last exposure (mean 11.01 μg in collected lymph nodes) than immediately after exposure (mean 2.34 μg). No TiO ₂ was detected in the liver, kidney, spleen or basal brain with olfactory bulb (detection limit 0.5 μg per organ). BAL fluid: increases in total cell count at 50mg/m ³ and polymorphonuclear neutrophils at 10 mg/m ³ and 50 mg/m ³ , but no changes in eosinophil, lymphocyte, or macrophage counts, total protein content, enzyme activities, and levels of 9 (out of tested 60) cell mediators. Among the 9 mediators, effects were only observed at 10 mg/m ³ or higher immediately after exposure. After 3 days of recovery, effects were still observed, but for clusterin and haptoglobin, they were observed at 2 mg/m ³ . Cell mediator levels were the same as controls after 16 days of recovery in 2 and 10 mg/m ³ groups, but not in 50 mg/m ³ group. Clinical pathology in blood: minor effects on serum cell mediator. No increase in serum troponin I, a biomarker for myocardial damage in rodents. Increased cell replication in large/medium bronchi and terminal bronchioles at all three groups immediately after exposure and after 3 days of recovery (not after 16 days). Macrophage diffusion also decreases over time. No change in lung cell apoptosis. Changes were most prominent immediately after the last exposure or 3 days afterward, and some endpoints returned to control levels by 16 days of recovery.	Ma-Hock et al. (2009)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued). ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [male F344]	Nano-TiO ₂ , 20 nm, anatase (Degussa); in aerosols: agglomerates 0.71 ± 1.9 µm MMAD Fine TiO ₂ , 250 nm, anatase (Fisher Scientific); in aerosols: agglomerates 0.78±1.7 µm MMAD	Subchronic inhalation Nano-TiO ₂ : 23.5 ± 2.9 mg/m ³ ; fine TiO ₂ : 22.3 ± 4.2 mg/m ³ 6 hr/day, 5 days/week, for 12 weeks Recovery for 4, 8, 12, 29 or 64 weeks before sacrifice	<p>Nano-TiO₂ caused more severe and prolonged (~1 year) pulmonary inflammatory response (i.e., increase in alveolar macrophages, polymorphonuclear neutrophils, and lavagable protein) than fine TiO₂.</p> <p>When inflammatory response was expressed as number of polymorphonuclear neutrophils and dose was expressed as surface area for retained particles (i.e., lavagable particles), nano-TiO₂ and fine TiO₂ shared the same dose response curve.</p> <p>More severe and prolonged impairment of alveolar macrophage-mediated particle clearance in rats exposed to nano-TiO₂ than rats exposed to fine TiO₂. Seven months after TiO₂ exposure, fine TiO₂ exposed (but not nano-TiO₂ exposed) rats showed normal clearance rates.</p> <p>Pathological changes in the lung: Nano-TiO₂ caused greater epithelial effects (Type II cell proliferation, occlusion of pores of Kohn) and more interstitial fibrotic foci than fine TiO₂.</p> <p>Dosimetry:</p> <p>Nano-TiO₂ and fine TiO₂ had a similar mass deposition in the lower respiratory tract and same retention in the alveolar space up to 1 year after exposure.</p> <p>Nano-TiO₂ showed longer total pulmonary retention (retention half-time: ~500 days for nano-TiO₂, ~170 days for fine TiO₂), more translocation to the pulmonary interstitium and regional lymph nodes, a greater fraction being retained, and a larger fraction of alveolar burden in the interstitium (suggesting nano-TiO₂ depends mainly on mucociliary clearance, while fine-TiO₂ depends on clearance to the gastrointestinal tract) than fine TiO₂.</p>	Oberdörster et al. (1994)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [male Wistar (strain Cri:WI(Han))]	Nano-TiO ₂ , 20-30 nm (measured by TEM), 70% anatase, 30% rutile, BET surface area 48.6 m ² /g, uncoated, isoelectric point (IEP) was pH 7 in 10 mM KCl, MMAD 1.0 µm in aerosol Fine TiO ₂ , median size 200 nm in ethanol (measured by DLS), rutile, BET surface area 6 m ² /g, IEP <pH 3 in 10 mM KCl (Kronos International), MMAD 1.1 µm in aerosol Quartz dust DQ12, median size 315 nm in ethanol, BET surface area 5.9 m ² /g, IEP <pH 3 in 10 mM KCl (Doerentrup Quarz GmbH, Germany), MMAD 1.2 µm in aerosol	Short-term inhalation: 6 hr/day for 5 consecutive days, head-nose exposure Aerosol concentration (mg/m ³): Nano-TiO ₂ : target 100 (measured concentration 88.0 ± 6.4) Fine TiO ₂ : 250 (measured 274.0 ± 30.5) Quartz dust DQ12: 100 (measured 96.0 ± 5.4). Count concentration of particles < 100 nm (particles/cm ³): Nano-TiO ₂ : 205,920 Fine TiO ₂ : 54,600 Quartz dust DQ12: 21,292 Calculated mass fraction measured <100 nm: Nano-TiO ₂ : 0.5% Fine TiO ₂ : 0.05% Quartz dust DQ12: 0.03% For distribution of the tested substance in the body, the following tissues were tested immediately after the last exposure and after 14-day recovery: lung, mediastinal lymph nodes, liver, kidney, spleen and basal brain with olfactory bulb (3 rats/group/time point) BAL at 3 or 14 days after the last exposure (5 rats/group/time point) Histological examination (6 rats/group/time point) and TEM of lung and mediastinal lymph nodes (3 rats/group/time point): immediately after the exposure and after 14 day recovery	Ti and S distribution in tissues: Immediately after 5-day inhalation/after 14 day recovery Nano-TiO ₂ : 2025/1547 µg TiO ₂ in lung, 2.2/8.5 µg TiO ₂ in mediastinal lymph nodes. Fine TiO ₂ : 9182/7257 µg TiO ₂ in lung, 8.2/108 µg TiO ₂ in mediastinal lymph nodes. Quartz DQ 12: 2190/1975 µg quartz in lung, 19/56 µg quartz in mediastinal lymph nodes. No TiO ₂ or quartz were detected in any groups in liver, kidney, spleen, or basal brain with olfactory bulb (detection limits: 0.3 µg Ti = 0.5 µg TiO ₂ per tissue, 5 µg Si = 11 µg SiO ₂ per tissue). Deposition of inhaled fine and nano-TiO ₂ in lung: Fine and nano-TiO ₂ were mainly in the lumen of the alveoli and bronchi (extracellular) and some were in the cytoplasm of alveolar macrophages. Nano-TiO ₂ was mostly agglomerates in lung, and agglomerates were roughly the same size as those in the atmosphere. No sign of desagglomeration of the inhaled agglomerates. Biological effects of fine TiO ₂ , nano-TiO ₂ and quartz: All treated groups: BAL had increased total cell count (most increases in polymorphonuclear neutrophils, slight increases in lymphocytes and monocytes); increased total protein; increased activity lactate dehydrogenase, alkaline phosphatase, γ-glutamyltransferase and N-acetyl-β-glucosaminidase. The changes in BAL parameters in the quartz group were not reversible, but changes in fine and nano-TiO ₂ groups were partly reversible by 14 days of recovery. Lung: diffuse histiocytosis Nano-TiO ₂ group: Reversible increases in absolute lung weight; mild neutrophilic inflammation in lung; inflammation declined by 14 days of recovery; lymphoreticulocellular hyperplasia in the mediastinal lymph nodes. Fine TiO ₂ group: Reversible increases in absolute lung weight; particle-loaded macrophages in the mediastinal lymph nodes. Quartz: Increase absolute lung weight, which maintained throughout recovery; multifocal infiltration of granulocytes in lung; after recovery time, pulmonary histological changes increased severity, and mediastinal lymph nodes had increased macrophage number and granulomatous inflammation.	van Ravenzwaay et al. (2009)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued). ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rats [female Sprague- Dawley [H1a:(SD)CVF]]	<p>Fine TiO₂, primary particle <5 μm, 99% rutile (reported vendor), BET surface area 2.34 m²/g [reported in Sager et al. (2008)] (Sigma-Aldrich, product # 224227); MMAD of the aerosols 402 nm with a GSD of 2.4, CMD of the aerosols 710 nm</p> <p>Nano-TiO₂ (P25), primary particle 21 nm, 80% anatase, 20% rutile (reported by vendor), BET surface area 48.08 m²/g [reported in (Sager et al., 2008)]; MMAD of the aerosols 138 nm with a GSD of 2.2, CMD of the aerosols 100 nm</p>	<p>Short-term inhalation</p> <p>Whole body chamber exposure</p> <p>Exposures selected for not alter BAL markers of pulmonary inflammation or lung damage</p> <p>Exposure to fine TiO₂: aerosol concentration x exposure time (actual deposition in lung)</p> <p>15 mg/m³ x 480 min (90 μg)</p> <p>16mg/m³ x 300 min (67 μg)</p> <p>12 mg/m³ x 240 min (36 μg)</p> <p>6 mg/m³ x 240 min (20 μg)</p> <p>3 mg/m³ x 240 min (8 μg)</p> <p>Exposure of nano-TiO₂: aerosol concentration x exposure time (calculated/actual deposition in lung)</p> <p>10 mg/m³ x 720 min that took place over 3 days (38 μg)</p> <p>12 mg/m³ x 240 min (19 μg)</p> <p>6 mg/m³ x 240 min (10 μg)</p> <p>3 mg/m³ x 480 min (10 μg)</p> <p>12 mg/m³ x 120 min (10 μg)</p> <p>3 mg/m³ x 240 min (6 μg)</p> <p>1.5 mg/m³ x 240 min (4 μg)</p> <p>Shame exposure (control): 0 mg/m³ x 240 min</p> <p>24 h post exposure, sample collection, including exteriorizing spintrapezius muscle with rats under anesthesia while leaving its nerves supply and all feed vessels intact for the test of arteriolar dilation</p>	<p>Histology of the lung:</p> <p>No significant inflammation.</p> <p>Particle accumulation in alveolar macrophage. Anuclear alveolar macrophages were seen in both nano-TiO₂ and fine TiO₂ exposed rats, but not in shame exposed rats. Anuclear alveolar macrophages are presumed to be an apoptotic change.</p> <p>Endothelium-dependent arteriolar dilation as measured after intraluminal infusion of the Ca²⁺ ionophore A23187 in exteriorized spintrapezius muscle:</p> <p>Both fine TiO₂ and nano-TiO₂ exposures impaired arteriolar dilation in a dose-dependent manner, and nano-TiO₂ exposure produced greater impairment than fine TiO₂ at similar pulmonary load doses. No-effect dose of fine TiO₂ was 8 μg (as in lung deposition), and for nano-TiO₂ was 4 μg.</p> <p>On a mass base, nano-TiO₂ was about one order of magnitude more potent than fine TiO₂; on total particle surface area base calculated by BET surface area, fine TiO₂ would be more potent than nano-TiO₂ (the authors suspected overestimation of the total nano-TiO₂ surface area delivered, since no agglomeration was considered).</p> <p>Additional nano-TiO₂ exposure conditions (12 mg/m³ x 2 h; 4 mg/m³ x 6 h; 8 mg/m³ x 3 h) yielded the same level of impairment of systemic arteriolar dilation, suggesting the response is dependent on the exposure concentration (of product) x time.</p>	Nurkiewicz et al. (2008)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
		<p>Same exposure conditions as above (Nurkiewicz et al., 2008) for endogenous microvascular NO production tests, but only three groups in all other tests: aerosol concentration x exposure time (actual deposition in lung)</p> <p>Shame exposure (control): 0 mg/m³ x 240 min</p> <p>Fine TiO₂: 16mg/m³ x 300 min (67 µg)</p> <p>Nano-TiO₂: 6 mg/m³ x 240 min (10 µg)</p> <p>24 hr post exposure, sample collection, including and exteriorizing spintrapezius muscle as described in (Nurkiewicz et al., 2008) and excising spintrapezius muscles from separate groups of rats for measurement of NO, microvascular oxidative stress, and nitrotyrosin staining</p>	<p>Same impairment of arteriolar dilation at 67 µg fine TiO₂ and 10 µg nano-TiO₂: more than 50% decrease compared to shame treated controls after Ca²⁺ ionophore A23187 injection at 20 and 40 psi ejection pressures.</p> <p>No change in arteriolar dilation in response to sodium nitroprusside (NO donor) in either 67 µg fine TiO₂ or 10 µg nano-TiO₂ exposed rats, indicating no change in vascular smooth muscle sensitivity to NO.</p> <p>Increased ROS amount in the microvascular wall in both 67 µg fine TiO₂ and 10 µg nano-TiO₂ groups at the same level as measured by ethidium bromide fluorescence.</p> <p>Increased nitrotyrosine expression in 10 µg nano-TiO₂ treated rats (not measured in fine TiO₂ group) in lung (3 folds) and spinotrapezius microcirculation (4 folds), as compared to shame exposure, suggesting nitrosative injury in lung and systemic microcirculation.</p> <p>Decreased Ca²⁺ ionophore A23187-stimulated endogenous microvascular NO production in fine TiO₂ and nano-TiO₂ treated groups in a dose-dependent manner: Similar to shame control, the NO production was sensitive to nitric oxide synthase inhibition caused by N^ε-monomethyl-L-arginine.</p> <p>Radical scavenging (by superoxide dismutase mimetic 2,2,6,6-tetramethylpiperidine-N-oxyl and catalase); inhibition of NADPH oxidase (by apocynin); and inhibition of myeloperoxidase (by 4-aminobenzoic hydrazide) all restored stimulated NO production and partially restored arteriolar dilation (stimulated by Ca²⁺ ionophore A23187) in 67 µg fine TiO₂ and 10 µg nano-TiO₂ groups.</p>	Nurkiewicz et al. (2009)
Instillations				
Mouse [male ICR]	<p>Nano-TiO₂ (Degussa), rutile, highly dispersed and hydrophilic fumed nano-TiO₂, diameter 19–21 nm (average primary particle size 21 nm), surface area of 50±15 m²/g, purity ≥99.5%</p> <p>To avoid aggregation, the nano-TiO₂ suspension was ultrasonicated before it was used to treat animals or cells; each sample was vortexed just before an aliquot was drawn for instillation. However, authors did not report the sizes of aggregates before or after sonication.</p>	<p>Single intra-tracheal instillation</p> <p>0, 0.1, or 0.5 mg/mouse</p> <p>3 days (for hyper-acute response), 1 wk (acute) or 2 wk (chronic) of recovery before sacrifice</p>	<p>Gross morphology and histology of the lung: Emphysema-like lung injuries were seen at 0.1 and 0.5 mg/mouse (more severe at 0.5 mg) at 3 days, 1 wk, and 2 wks after the instillation.</p> <p>Pulmonary changes included disruption of alveolar space, alveolar enlargement, proliferation of alveolar type II pneumocyte, increases in alveolar epithelial thickness, and accumulation of particle-laden macrophages.</p> <p>1 wk after instillation, 0.1 mg/mouse increased alveolar macrophage infiltration, type II pneumocyte proliferation, and apoptosis in macrophage and type II pneumocyte.</p> <p>Gene expression in lung 1 wk after instillation of 0, 0.1, and 0.5 mg/mouse:</p> <p>cDNA microarray showed up-regulation in pathways involved in cell cycle regulation, apoptosis, chemokines, and complementary cascades.</p> <p>RT-PCR showed up-regulation in <i>plgf</i>, chemokines (<i>cxc11</i>, <i>cxc15</i>, and <i>ccl3</i>), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and prostaglandin E receptor 4.</p> <p>Western blot and ELISA showed increases in placenta growth factor (PIGF) protein (a prechemokine that regulates the expression of several chemokines, leading to inflammatory cascade) in cells and in serum.</p>	Chen et al. (2006)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [female Wistar (HsdCpb:WU)]	Nano-TiO ₂ (P25), photocatalytic, hydrophilic, 80% anatase/20% rutile, primarily particle size 25 nm, BET specific surface area 52 m ² /g	Repeated weekly intra-tracheal instillation Instilled doses: 5 instillations x 3 mg 5 instillations x 6 mg 10 instillations x 6 mg	Increased primary benign tumors and malignant cancers in lung in all tested doses.	Mohr et al. (2006) Pott and Roller (2005) ^b
	Nano-TiO ₂ (Degussa T805 / P805), ^b crystal form not specified, coated with an organic silicon compound; 21 nm; 32.5 m ² /g ^b	Repeated weekly intra-tracheal instillation Instilled doses: 15 instillations x 0.5 mg 30 instillations x 0.5 mg	High initial acute mortality, lowered dose to 0.5 mg. No conclusion on carcinogenicity.	
	Fine TiO ₂ , hydrophilic, anatase, primary particle 200 nm, BET specific surface area 9.9 m ² /g	Repeated weekly intra-tracheal instillation Instilled doses: 10 instillations x 6 mg 20 instillations x 6 mg	Increased primary benign tumors and malignant cancers in lung in all tested doses.	
Rat [male Wistar]	Nano-TiO ₂ , rutile, primary particle diameter 4-6 nm, rod shape (synthesized in the lab by a soft chemistry technique); BET surface for instilled nano-TiO ₂ rods was 14.64 cm ² for dose of 1 mg/kg, 82.30 cm ² for 5 mg/kg. Aggregates appeared to be in a radial arrangement and usually less than 1 μm.	Single intra-tracheal instillation (acute effects) 1 or 5 mg/kg nano-TiO ₂ or vehicle only (150 μL) Single intra-tracheal instillation Nano-TiO ₂ was suspended in saline containing 0.01% Tween 80 (a surfactant and emulsifier) Blood collection and necropsy at 24 hours after instillation	Pulmonary inflammation: increases in macrophage and neutrophil numbers in BAL fluid at 5 mg/kg. Most nano-TiO ₂ aggregates in BAL fluid were inside macrophages. Pulmonary and cardiac edema: increases in the wet weight-to-dry weight ratios of lung and of heart at 1 and 5 mg/kg. Systemic inflammation: increases in monocyte and granulocyte (but not lymphocyte) numbers in blood at 5 mg/kg. Platelet aggregation: decreases platelet number in blood of rats exposed to 5 mg/kg nano-TiO ₂ , suggesting platelet aggregation [in vitro supporting evidence: adding 2 or 10 μg/mL (but not 0.4 μg/mL) nano-TiO ₂ directly into untreated rat whole blood caused platelet aggregation].	Nemmar et al. (2008)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rats [male F344]	Nano-TiO ₂ , ~20 nm, anatase Fine TiO ₂ , ~250 nm, anatase	Single intra-tracheal instillation (acute effects) 500 µg of either anatase nano-TiO ₂ or anatase fine TiO ₂ A single intra-tracheal instillation, followed by 24-hr recovery	Anatase nano-TiO ₂ induced more inflammatory response and higher interstitial access in the lung than anatase fine TiO ₂ of the same mass dose.	Oberdörster et al. (1992)
	Nano-TiO ₂ , ~20 nm, anatase (free anatase nano-TiO ₂) Alveolar macrophage collected 24 hrs after donor rat received 200 µg anatase nano-TiO ₂ via intra-tracheal instillation (containing phagocytized anatase nano-TiO ₂) Alveolar macrophage collected from untreated rat lung PMNs from peripheral blood of untreated rats Serum-exposed anatase nano-TiO ₂ (incubated in rat serum for 1 hr and then washed twice)	Single intra-tracheal instillation (acute effects) Free anatase nano-TiO ₂ , 104 µg Phagocytized anatase nano-TiO ₂ 104 µg + 9.5 x 10 ⁶ alveolar macrophages + 3.9 x 10 ⁶ polymorphonuclear neutrophils Alveolar macrophages 6.8 x 10 ⁶ Polymorphonuclear neutrophils 2.2 x 10 ⁶ Serum-exposed anatase nano-TiO ₂ 100 µg A single intra-tracheal instillation, followed by 24-hr recovery	Free anatase nano-TiO ₂ and serum-exposed anatase nano-TiO ₂ caused pulmonary inflammatory reaction (same level) and interstitial distribution. Phagocytized anatase nano-TiO ₂ alone did not contribute significantly to inflammatory reaction, because the reaction can be explained by the alveolar macrophages and polymorphonuclear neutrophils. Phagocytized anatase nano-TiO ₂ showed less interstitial distribution than free anatase nano-TiO ₂ .	
	Fine TiO ₂ , ~250 nm, anatase Nano-TiO ₂ , ~20 nm, anatase Fine TiO ₂ , ~220 nm, rutile (from Dr. Siegal at Argonne National Laboratory, Argonne, IL) Nano-TiO ₂ , ~12 nm, rutile Carbon black, ~30 nm (Cabot, 660R)	A single intra-tracheal instillation of 500 µg each; anatase fine TiO ₂ was also tested at 1000 µg; anatase nano-TiO ₂ was also tested at 65, 107, 200, and 1000 µg 24-hr recovery	When inflammatory response was expressed as number of PMN and dose was expressed as surface area for retained particles (i.e., lavagable particles), all particles shared the same dose-response curve, except anatase and rutile nano-TiO ₂ at high doses. When inflammatory response was expressed as lavage protein and dose was expressed as retained particle surface area, all particles shared the same dose response curve. Higher fractions of nano-TiO ₂ (anatase and rutile nano-TiO ₂) were interstitialized (translocated into interstitium or epithelium cells) than other particles.	

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [strain / stock not specified]	Nano-TiO ₂ , ~20 nm, surface area is estimated to be 10 times of surface area of ~250 nm TiO ₂ Fine TiO ₂ , ~250 nm	Single Intra-tracheal instillation (acute effects) Nano-TiO ₂ : 30, ~150, 500 µg Fine TiO ₂ : ~150, 500, 2000 µg	Pulmonary inflammation (neutrophil % in lung lavage) was seen at 24 hr post exposure. At the same mass dose, nano-TiO ₂ induced more inflammation than fine TiO ₂ . When doses are expressed as surface area, fine TiO ₂ and nano-TiO ₂ shared the same dose-response curve.	Oberdörster (2000)
	Nano-TiO ₂ Polytetrafluoroethylene (PTFE) (Teflon) fume, count median diameter ~18 nm	Repeated inhalation of PTFE fume (5 x 10 ⁵ particles/cm ³ = ~50 µg/cm ³ , 5 min/day for 3 days) followed by a single intra-tracheal instillation of 100 µg nano-TiO ₂	Cross tolerance: Nano-TiO ₂ induced less pulmonary inflammation (neutrophil % in BAL fluid) in rats that had adapted to PTFE fumes for previous three days than in rats that were not adapted (not exposed to PTFE fume). The author suggested this cross tolerance is from adaptation to oxidative stress.	
	Nano-TiO ₂ , ~20 nm Fine TiO ₂ , ~250 nm Lipopolysaccharide (LPS), an endotoxin found in gram negative bacteria	Inhalation of LPS followed by a single intra-tracheal instillation of nano-TiO ₂ and fine TiO ₂ (acute effects) LPS: ~12 min exposure, ~70 endotoxin units (estimated alveolar dose) Nano-TiO ₂ and fine TiO ₂ : 50 µg Within 30 minutes of inhalation of LPS or saline, intra-tracheal instillation of nano-or fine TiO ₂ 24 hours of recovery	LPS alone: mild pulmonary inflammation (~10% neutrophil in lung lavage at 24 hr post exposure). The treatment of LPS was to mimic an early stage of infection with gram negative bacteria (compromised host). 50 µg nano-TiO ₂ , but not fine TiO ₂ , further increased inflammatory response in compromised hosts with mild pulmonary inflammation. Neutrophil % in rats exposed to (LPS and then nano-TiO ₂) > (LPS and then fine TiO ₂), LPS alone, nano-TiO ₂ alone > fine TiO ₂ alone, negative control. It is unclear whether fine TiO ₂ at a dose that increases inflammatory response would further increase inflammatory response in compromised hosts.	
Rat [Wistar]	Nano-TiO ₂ (P25), photocatalytic, 80% anatase/20% rutile, untreated, hydrophilic surface, primarily particle size ~20 nm Nano-TiO ₂ (Aeroxide® T805), photostable, 80% anatase/20% rutile, silanized, trimethoxyoctylsilane-treated hydrophobic surface, primarily particle size ~20 nm Crystalline silica and quartz particles (DQ-12) as positive reference	Single intra-tracheal instillation (subchronic effects) Doses: 0, 0.15, 0.3, 0.6, or 1.2 mg nano-TiO ₂ (positive control: 0.6 mg quartz DQ12) in 0.2 mL saline supplemented with 0.25% lecithin 3, 21, or 90 days of recovery	Transient pulmonary inflammatory responses to both types of nano-TiO ₂ (mostly only at 1.2 mg dose, some at 0.6 mg groups) (most responses returned to normal by day 90). P25 induced more pulmonary inflammatory responses than T805 in some tests, but T805 induced more proliferation changes in the lung (as percentage of Ki67-positive cells) than P25 on days 3 and 21. Neither P25 nor T805 increased oxidative DNA adduct (as 8-oxoguanine) in the lung on day 90. Quartz induced persistent inflammatory response and increased 8-oxoguanine on day 90.	Rehn et al. (2003)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [male Wistar]	Nano-TiO ₂ (Degussa), mean diameter 29 nm, BET surface area 49.78 m ² /g Fine TiO ₂ (Tioxide Ltd), mean diameter 250 nm BET surface area 6.6 m ² /g Carbon black, mean diameter 260.2 nm, BET surface area 7.9 m ² /g Ultrafine carbon black, mean diameter 14.3 nm, BET surface area 253.9 m ² /g	Single intra-tracheal instillation (acute effects) 0, 125, and 500 µg particles in saline 24 hours of recovery before sacrifice	Nano-TiO ₂ at 500 µg (but not nano-TiO ₂ at 125 µg or fine TiO ₂ at either 125 or 500 µg) increased neutrophil number (inflammation), LDH activity (cytotoxicity), GGT activity (epithelial damage), total protein in bronchoalveolar lavage fluid (membrane permeability), and macrophage activity to migrate toward chemotaxin C5a (chemotaxis). Both nano- and fine TiO ₂ (at 500 µg, but not at 125 µg) decreased phagocytic function of macrophage. Carbon black caused same changes as fine TiO ₂ , with the exception of increases in LDH activity at 500 µg. Ultrafine carbon black caused same changes as nano-TiO ₂ , but increases in inflammation and LDH and GGT activities were significant at 125 µg (nano-TiO ₂ caused significant changes at 500 µg only).	Renwick et al. (2004)
Rat [male Cri:CD(SD)IGS BR]	Fine TiO ₂ (DuPont): primary particle ~300 nm, anatase, ~99 wt % TiO ₂ /~1 wt % alumina, BET surface area ~6 m ² /g (R-100) Nano-TiO ₂ rods (synthesized hydrothermally): primary particle length 92 - 233 nm x width 20 - 35 nm, anatase, BET surface area 26.5 m ² /g Nano-TiO ₂ dots (synthesized hydrothermally): primary particle diameter 5.8 - 6.1 nm, sphere, anatase, BET surface area 169.4 m ² /g Quartz (Min-U-Sil quartz): median primary particle ~1.5 µm (range 1 - 3 µm), crystalline silica, BET surface area 4 m ² /g	Single intra-tracheal instillation (subchronic effects) 0, 1 or 5 mg/kg of each testing material in PBS with polytron dispersant BAL fluid analysis at 24 hr, 1 week, 1 month, and 3 months post exposure (5 rats per group per dose per time point) Morphological studies at the same time points (4 rats per group per high dose per time point; 4 rats per group per low dose for the first two time points)	Like fine TiO ₂ , nano-TiO ₂ rods and nano-TiO ₂ dots caused only transient pulmonary inflammation, and not significant lung toxicity. All 5 mg/kg TiO ₂ (fine, nano rods, and nano dots), but not 1 mg/kg TiO ₂ , caused transient, short-lived inflammation (increases in neutrophil % in BAL fluid at 24 hr post exposure only; increases in LDH by 5 mg/kg nano-TiO ₂ rods at 24 hr post exposure only). No changes in lung weight, tracheobronchial cell proliferation (measured in high dose groups only) or lung morphology (pathological changes). TiO ₂ in macrophages was seen in all three types of TiO ₂ . Transient lung parenchymal cell proliferation in low and high fine TiO ₂ at 1 week post exposure (different from previous studies in similar conditions). Quartz caused sustained pulmonary inflammation and early sign of pulmonary fibrosis. Sustained pulmonary inflammation (increases in neutrophil % in BAL fluid at 1 mg/kg at 24 hr after exposure, 5 mg/kg at all time points) (increases in LDH at 5 mg/kg at all time points) (increase in neutrophils and foamy alveolar macrophages). Prelude of fibrosis (thickening of lung tissue) (persistent lung parenchymal cell proliferation at 5 mg/kg at 1 month and 3 month post exposure). Absolute lung weight was increased at 5 mg/kg at 1 wk, 1 month, and 3 months post exposure. Increased tracheobronchial cell proliferation at 5 mg/kg (not measured in low dose) at 24 hr post exposure only.	Warheit et al. (2006)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [CrI:CD@/SD)IG S BR]	<p>Nano-TiO₂ (DuPont), photostable, rutile, coated with alumina, (~98% titanium dioxide, ~2% alumina), average particle size of 136 nm in water and average BET surface area of 18.2 m²/g (uf-1)</p> <p>Nano-TiO₂ (P25) (Evonik), photocatalytic, 80% anatase/20% rutile, not coated, average particle size 129.4 nm in water, average BET surface area 53.0 m²/g</p> <p>Nano-TiO₂ (DuPont), photostable, rutile, coated with silica and alumina surface coating (~88 wt % titanium dioxide, ~7 wt % amorphous silica and ~5 wt % alumina), average particle size of ~149.4 nm in water, average BET surface area 35.7 m²/g (uf-2)</p> <p>Fine TiO₂ (DuPont), photostable, rutile, coated with alumina (~99% titanium dioxide and ~1% alumina), an average particle size 382 nm in water, average BET surface area 5.8 m²/g</p> <p>Quartz</p>	<p>Single intra-tracheal instillation (subchronic effects)</p> <p>0, 1, or 5 mg/kg</p> <p>90 days recovery period</p>	<p>No sustained adverse pulmonary effects for photostable nano-TiO₂ (both types of coated rutile).</p> <p>Pulmonary inflammation and cytotoxic effects at highest exposure of photocatalytic nano-TiO₂ increased bronchoalveolar lavage fluid LDH and BAL fluid microprotein concentrations.</p> <p>Increased tracheobronchial and lung parenchymal cell proliferation rates at highest exposure of photocatalytic nano-TiO₂.</p> <p>Lung inflammation/cytotoxicity/cell proliferation and histopathological responses: quartz > nano-TiO₂ P25 (anatase and rutile) > fine TiO₂ (rutile) = nano-TiO₂ uf-1 (rutile) = nano-TiO₂ uf-2 (rutile).</p>	<p>Warheit et al. (2007a)</p> <p>Warheit et al. (2007c)</p>

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [female CD1(ICR)]	Nano-TiO ₂ (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, 80 nm, measured average size 71.4 ± 23.5 nm, purity >99% Fine TiO ₂ (Zhonglian Chemical Medicine Co.), anatase, 155 nm, measured average size 155.0 ± 33.0 nm, purity >99%	Repeated intranasal instillation ~500 µg TiO ₂ in pure water per mouse very other day for 2, 10, 20, or 30 days (1, 5, 10 or 15 instillations, respectively) Necropsy 1 day after last instillation For translocation of TiO ₂ into brain: 6 mice per group for each time point. For effects in brain: 10 mice per group	TiO ₂ distribution (measured after 15 instillations): first into olfactory bulb, and then to hippocampus. Ti concentrations: hippocampus, olfactory bulb > cerebellum, cerebral cortex > thalamus. Serum biomarkers for liver function (ALT, AST, ALP), kidney function and cholesterol levels: No consistent change. Only changes were increased ALT (80 nm group after 1 and 5 instillations, 155 nm group after 5 instillation), increased AST (80 nm group after 5 instillations) and increase ALP (155 nm group after 1 instillation). Pathological changes in kidney: atrophy of renal glomerulus, infiltration and dwindling of interstitially inflammatory cells in the lumen of Bowman's capsules. No changes in organ weight. No pathological changes in heart, liver, spleen, cerebral cortex or cerebellum. No change in proinflammatory cytokine TNF-α in serum. Brain: Oxidative stress: GSH-Px and GST activities and GSH levels were increased in the 80 nm group after 5 instillations, but not in other groups or other time points. Malondialdehyde levels (indicator for lipid peroxidation) and soluble protein carbonyl content (indicator for protein oxidation; measured only after 15 instillations) were increased in both 80 and 155 nm group after 15 instillations. SOD activity was decreased in 155 nm after 15 instillations. Catalase activity (measured only after 15 instillations) was increased in 80 and 155 nm groups. Pathological changes in olfactory bulb and C1A regions of hippocampus: Olfactory bulbs showed increased neuron numbers, irregular arrangement of neuron cells, and ultra-structural changes in both 80 and 155 nm groups. CA1 region of the hippocampus showed enlarged and elongated pyramidal cell soma, dispersed arrangement and loss of neurons, fewer Nissl bodies, fewer mitochondria, and increased rough endoplasmic reticulum. Astrocytes may be damaged (only measured after 15 instillations): Hippocampus had increased glial fibrillary acidic protein (GFAP) levels, particularly in CA4 region. Activity of cholinesterase (which inactivates acetylcholine, a neurotransmitter) was increased. Both changes were in 80 and 155 nm groups. Neurotransmitters: Levels of glutamic acid (a neurotransmitter) and nitric oxide (NO, as neurotransmitter and from inflammatory response) were increased in both 80 and 155 nm groups (measured only after 15 instillations). Cytokines: Increased THF-α and IL-1β, but not IL-6 (155 nm after 15 instillations).	Wang et al. (2008a) Wang et al. (2008b)

Table 5-6. Summary of health effects of nano-TiO₂ particles in mammalian animal models: respiratory route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [CD-1(ICR)]	Nano-TiO ₂ (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, 25 nm, purity >99% Nano-TiO ₂ (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, 80 nm, purity >99% Fine TiO ₂ (Zhonglian Chemical Medicine Co.), anatase, 155 nm, purity >99%	Repeated intranasal instillation (subacute effects) 10 µL of 50 mg/kg TiO ₂ or water every two days Blood and brain were collected from anesthetized mice after 2, 10, 20, or 30 days	No changes in water and food consumption or body weight. Brain TiO ₂ content (measured in all brain samples): increased in treated mice and was highest in 25 nm treated group at 2 and 10 days; decreased slightly and was similar in all treated groups at 20 and 30 days. Neurotransmitters (measured in 20 and 30 day brain samples): Changed in 80 nm and 155 nm TiO ₂ -treated mice compared to control, but not in 25 nm TiO ₂ -treated mice. All changes were after 20 days, with the exception of decreased dopamine in 80 nm group after 30 days. After 20 days: Norepinephrine was significantly increased in 80 and 155 nm TiO ₂ -treated mice; 5-hydroxytryptamine was significantly increased in 155 nm TiO ₂ -treated mice; homovanillic and 5-hydroxyindole acetic acid were decreased in 80 and 155 nm TiO ₂ -treated mice; dopamine was decreased in 80 nm TiO ₂ -treated mice.	Wang et al. (2007b)

^a ALP – Alkaline phosphatase, a marker of type II epithelial cell toxicity (Ma-Hock et al., 2009) or liver toxicity
 ALT – Alanine transaminase
 AST – Aspartate aminotransferase
 BAL – Bronchoalveolar lavage
 BET – Brunauer, Emmett, Teller method of calculating surface area
 CMD – Count median diameter
 DLS – Dynamic light scattering
 ELISA – Enzyme-linked immunosorbent assay
 F344 – Fischer 344
 GFAP – Glial fibrillary acidic protein
 GGT – γ -glutamyltransferase, a marker for damage to Clara and type II epithelial cells (Ma-Hock et al., 2009)
 GSD – Geometric standard deviation
 GSH – Reduced glutathione
 GSH-Px – Glutathione peroxidase
 GST – Glutathione-S-transferase
 IEP – Isoelectric point
 IL-1 β – Interleukin-1 beta

IL-6 – Interleukin-6
 IFN- γ – interferon-gamma
 LDH – Lactate dehydrogenase, a general marker of cell injury (Ma-Hock et al., 2009)
 LPS – Lipopolysaccharide
 MMAD – Mass median aerodynamic diameter
 MTP – Microsomal triglyceride
 NADPH – Nicotinamide adenine dinucleotide phosphate
 P25 – AEROXIDE® P25
 PBS – Phosphate buffered saline
 PIGF – Placenta growth factor
 PMN – Polymorphonuclear neutrophils
 PTFE – Polytetrafluoroethylene
 ROS – Reactive oxygen species
 RT-PCR – Real-time polymerase chain reaction
 SOD – Superoxide dismutase
 TEM – Transmission electron microscopy
 TNF- α – Tumor necrosis factor-alpha

^b According to Pott and Roller (2005): "Titanium dioxide T 805 from Degussa was ordered from Sigma-Aldrich, but the supplier only offered an amount of at least 40 kg P 805. Neither Sigma-Aldrich nor Degussa answered at all clearly when questioned insistently as to the difference between T 805 and P 805. So, it is not proven that P 805 is identical with T 805 from Degussa." The primary particle size and surface area in the table were from Pott and Roller (2005). Currently available T805 is photostable nano-TiO₂ (80% anatase, 20% rutile) that has been treated with octylsilane to achieve a hydrophobic surface. Degussa T805 primary particle is still 21 nm, but specific surface area (BET) is 45 m²/g (Liames, 2008a).

1 5.3.1.2.4. Toxicity by Other Exposure Routes

2 Ocular exposure, intravenous injection, and subcutaneous injection have also been investigated in
3 nano-TiO₂ toxicity studies (Table 5-7). Ocular exposure to sunscreen containing nano-TiO₂ could occur
4 accidentally when sunscreen spray and sunscreen lotion are applied. At least one brand of sunscreen
5 lotion that contains nano-TiO₂ is in a tear-free formula and marketed for children (Project on Emerging
6 Nanotechnologies, 2007). A single ocular exposure to photocatalytic nano-TiO₂ caused conjunctival
7 redness for 1 or 2 days in rabbits (Warheit et al., 2007a).

8 One journal article and two professional meeting abstracts are available on the effects of injected
9 nano-TiO₂ in rats and mice. In the Fabian et al. (2008) study, an intravenous injection of 5 mg/kg nano-
10 TiO₂ with unknown photoreactivity did not induce changes in blood tests diagnostic for inflammatory
11 responses, kidney toxicity, or liver toxicity. Two meeting abstracts presented immunological effect
12 studies in mice exposed to nano-TiO₂ through subcutaneous and intravenous injections (Miller et al.,
13 2007b; Weaver et al., 2007). Preliminary results showed that photocatalytic nano-TiO₂ in suspension
14 (Degussa W740X) appeared to have very limited inflammatory ability, and very high doses (560 mg/kg
15 for intravenous injections and 5,600 mg/kg for subcutaneous injections) were needed to produce
16 immunological effects (Weaver, 2008).

Table 5-7. Summary of health effects of nano-TiO₂ particles in mammalian animal models: other (injection, ocular) route. ^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Injection				
Rat [male Wistar (strain Cri:WI(Han))]	Nano-TiO ₂ , primary particle 20-30 nm (measured by TEM), BET surface area 48.6 m ² /g, 70% anatase/ 30% rutile, uncoated, IEP was pH 7 in 10 mM KCl Fine TiO ₂ (Kronos International), median size 200 nm in ethanol (measured by DLS), rutile, BET surface area 6 m ² /g, IEP < pH 3 in 10 mM KCl	A single intravenous injection via tail vein Saline (control) or 5 mg/kg nano-TiO ₂ Nano-TiO ₂ stock 0.5% in rat serum, then diluted in saline, injection of ~1 mL of test substance preparation/kg of rat BW Aggregates in serum are mostly <1000 nm, with 10 wt % <100 nm Necropsy at 1, 14, and 28 days after the injection (12 rats total for four treatment groups) Ti concentrations were measured in lung, liver, kidney, spleen, brain, blood cells, plasma, and popliteal lymph nodes at 1, 14, and 28 days after injection	No inflammation, kidney toxicity, or liver toxicity detected: no changes in concentrations of cytokines, enzymes and other indicators in the blood (total of 67 parameters) for inflammatory responses, kidney function, and liver function. TiO ₂ distribution: TiO ₂ concentrations 1 day after injection: liver > spleen >> lung > kidney. The time for the TiO ₂ concentration to return to normal levels were in the same sequence. Liver had same TiO ₂ levels after 14 and 28 days. Spleen had slightly decreased TiO ₂ levels 14 and 28 days after injection. Lung and kidney had no elevated TiO ₂ 14 days after injection. No TiO ₂ detected in blood cells, plasma, brain or lymph nodes (mediastinal, mesenteric, popliteal) at any three time points tested (detection limit 0.3 µg Ti = 0.5 µg TiO ₂ per tissue).	Fabian et al. (2008); van Ravenzwaay et al. (2009)
Mouse [Balb/C]	Nano-TiO ₂ (Degussa W740X), dispersion of photocatalytic uncoated nano-TiO ₂ (80% anatase/ 20% rutile) at 40 wt%, primary particle 4.7 nm, mean aggregate size ≤ 100 nm; (Evonik, 2008; Llames, 2008b; Weaver, 2008)	Intravenous injections 5.6 mg/mouse/day for 2 days (total dose 11.2 mg/mouse) 1 or 3 days of recovery before sacrifice	Lung, liver, and spleen showed white discoloration and phagocytosis of nano-TiO ₂ aggregates by macrophages under light microscope.	Miller et al. (2007b)
Mouse [sex, strain/stock not specified]	Nano-TiO ₂ (Degussa W740X), dispersion of photocatalytic uncoated nano-TiO ₂ (80% anatase/ 20% rutile) at 40 wt%, primary particle 4.7 nm, mean aggregate size ≤ 100 nm; (Evonik, 2008; Llames, 2008b; Weaver, 2008)	Subcutaneous injections: total 0 or total 5600 mg/kg over two days Intravenous injections: total 0 or total 560 mg/kg over two days 1 or 5 days of recovery	Subcutaneously injected mice: Day 1: No changes in any cell population in peripheral blood, except CD8+ T cells. Day 5: Increases in granulocytes in circulation and spleen; decreases in circulating lymphocyte percentages; no changes in macrophage percentages or any cell population in draining lymph nodes. Lack of Con-A stimulated T-cell proliferation in lymph nodes. Intravenously injected mice: Macrophage in the marginal zone of the spleen white pulp contained nano-TiO ₂ aggregates, suggesting interaction between T-cells and nano-TiO ₂ . No changes in Con-A stimulated T-cell proliferation.	Weaver et al. (2007)

Table 5-7. Summary of health effects of nano-TiO₂ particles in mammalian animal models: other (injection, ocular) route (continued).^a

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Ocular exposure				
Rabbit [male New Zealand White]	Nano-TiO ₂ , (P25), photocatalytic, 80% anatase/20% rutile, not coated, average particle size was 129.4 nm in water, average BET surface area was 53.0 m ² /g (Warheit, pers. comm., 2008b)	Acute ocular irritation Doses – 0 or 57 mg to one eye of each animal Single exposure (the eye remained unwashed following treatment) Observation at 1, 24, 48, and 72 hours following administration of the nano-TiO ₂	Reversible conjunctival redness in the treated eye (normal by 24 or 48 hours after administration of nano-TiO ₂). No corneal injury evident, no clinical signs observed, and no body weight loss occurred.	Warheit et al. (2007a)

^a BET – Brunauer, Emmett, Teller method of calculating surface area
 BW – Body weight
 CD8 – Cluster of differentiation 8
 CD8 + T cell – Cytotoxic T cell with CD8 surface protein

DLS – Dynamic light scattering
 IEP – Isoelectric point
 P25 – AEROXIDE® P25
 TEM – Transmission electron microscopy

5.3.1.3. Summary of Non-carcinogenic Effects

1 Some of the non-carcinogenic effects shared by conventional and nano-TiO₂ were similar in the
2 nature or type of the effects, but differed in dose-response. For example, pulmonary inflammation in
3 laboratory animals and overload in rats were observed after respiratory tract exposures to either
4 conventional TiO₂ or nano-TiO₂, and nano-TiO₂ often caused more severe or more persistent responses
5 than conventional TiO₂ at the same mass concentrations/doses. Systemic effects were also observed:
6 increased inflammatory cell numbers and decreased platelet numbers in the blood, renal pathology,
7 potential hepatic toxicity, and changes in the brain morphology and neurotransmitters. Except for the
8 effects in the brain, the aforementioned effects outside the lung have been reported only once and have
9 not been confirmed by other laboratories. While topically applied photostable nano-TiO₂ is not expected
10 to cause adverse effects in healthy skin, data are lacking on the effects in healthy flexed human skin and
11 damaged human skin.

5.3.2. Carcinogenic Effects

12 The carcinogenicity of TiO₂ to humans has been reviewed by various international health
13 organizations and workplace regulatory agencies. Currently, TiO₂ (including nano-TiO₂, but not
14 considered separately) is classified as “possibly carcinogenic to humans” (Group 2B) by the International
15 Agency for Research on Cancer (IARC) (Baan, 2007; IARC, 2006) and as “carcinogenic” (Class D2A) by
16 the Workplace Hazardous Materials Information System (WHMIS), a program administered by the
17 Canadian Centre for Occupational Health and Safety (CCOHS) (2006).

18 In a 2005 draft evaluation, TiO₂ was not designated as a “potential occupational carcinogen,” due
19 to insufficient evidence (NIOSH, 2005). For nano-TiO₂, NIOSH expressed concern in the 2005 draft
20 about the potential carcinogenicity of ultrafine TiO₂ (nano-TiO₂) if exposure levels were at the current
21 mass-based occupational limits of 1.5 mg/m³ for respirable dust or 15 mg/m³ for total dust, and
22 recommended controlling exposure to as low as feasible below the recommended exposure limit (NIOSH,
23 2005). Based on calculated lung cancer risks, NIOSH (2005) stated a draft recommendation for an
24 exposure limit of 0.1 mg/m³ for ultrafine TiO₂, which is more than 10-fold lower than the exposure limit
25 of 1.5 mg/m³ for fine TiO₂ (less than 2.5 μm), as time-weighted average concentrations for up to 10 hr/day
26 during a 40-hour work week.

27 This section reviews studies in humans and in animals on carcinogenicity of nano-TiO₂ and briefly
28 discusses the mode of action of conventional TiO₂ and nano-TiO₂ carcinogenicity. Conventional TiO₂ has
29 been shown to induce lung cancer through inhalation in rats at 250 mg/m³ (6 hr/day, 5 days/week for 24

1 months) (Lee et al., 1985a, 1985b), but not at 50 mg/m³ or below (Lee et al., 1985a, 1985b; Muhle et al.,
2 1991). No increases in tumors were observed in mice receiving a single intra-tracheal instillation of 0.5
3 mg of TiO₂, in mice and rats fed with TiO₂ in the diet at up to 5.0% daily for 103 weeks, or in hamsters
4 given 3 mg of TiO₂ via intra-tracheal instillation weekly for 15 weeks (Baan, 2007). Similarly,
5 epidemiological studies did not show increased lung cancer in people exposed to conventional TiO₂
6 (Boffetta et al., 2001; Boffetta et al., 2004; Chen and Fayerweather, 1988; Fryzek et al., 2003;
7 Ramanakumar et al., 2008; Siemiatycki, 1991). The carcinogenicity studies of conventional TiO₂ are not
8 discussed in detail in this case study, and readers are referred to studies cited here and in the IARC
9 Monographs Working Group report (Baan, 2007).

5.3.2.1. Studies in Humans

10 Seven epidemiological studies of TiO₂ carcinogenicity have been reported: two population-based
11 case-control studies (one for lung cancer (Boffetta et al., 2001) and the other for 20 types of cancer
12 (Siemiatycki, 1991)); two retrospective cohort mortality studies (Boffetta et al., 2004; Fryzek et al.,
13 2003); one mortality, morbidity, and case-control study (lung cancer and chronic respiratory diseases)
14 (Chen and Fayerweather, 1988); and two case-control studies (lung cancer) (Ramanakumar et al., 2008).
15 Based on these studies, IARC (2006), the Canadian Centre for Occupational Health and Safety (CCOHS)
16 (2006), and NIOSH (2005) concluded that the evidence is insufficient to conclude that TiO₂ exposure
17 increases the risk of lung cancer in human beings. Furthermore, none of these studies were designed for
18 nano-TiO₂ exposure, and none of them provided information on TiO₂ particle sizes. The risks posed by
19 nano-TiO₂ (ultrafine primary particles) and the relationship between particle size and lung cancer risk in
20 humans cannot be discerned from these studies.

5.3.2.2. Animal Studies

21 Carcinogenicity of nano-TiO₂ was observed in three animal studies using photocatalytic nano-TiO₂
22 in rodents (Borm et al., 2000; Heinrich et al., 1995; Pott and Roller, 2005). Increased lung tumor
23 incidences were observed in rats (Borm et al., 2000; Heinrich et al., 1995; Pott and Roller, 2005), but not
24 in mice (Heinrich et al., 1995), exposed to P25 through inhalation or intra-tracheal instillation.
25 Photocatalytic nano-TiO₂ given through intraperitoneal injections did not increase tumors in the
26 abdominal cavity in rats (Pott et al., 1987). Intramuscular implantation of nano-TiO₂ with unknown
27 photo-reactivity also did not increase tumors at the sites of implantation in rats (Hansen et al., 2006).
28 Data specifically on photostable nano-TiO₂ carcinogenicity are inconclusive (2005).

1 5.3.2.2.1. Intratracheal Instillation

2 Female Wister CRP/WU rats received fine and nano-TiO₂ via intra-tracheal instillations, and the
3 tumor incidence and pulmonary inflammation were measured 2.5 years after administration (Borm et al.,
4 2000). Fine TiO₂ (250 nm) was given 6 times at 10 mg each, and the photocatalytic nano-TiO₂ (21 nm,
5 80% anatase, 20% rutile, uncoated, P25) was given 5 times at 6 mg each (Borm, pers. comm, 2008). At
6 these total doses (60 mg for fine TiO₂ and 30 mg for nano-TiO₂), lung clearance might be expected to be
7 severely compromised. The authors found evidence of alveolar and interstitial inflammation 2.5 years
8 after instillation. The histologically confirmed tumor incidences were 27% for fine TiO₂ and 66% for
9 nano-TiO₂, while the macroscopic tumor incidences were only 20.9% for fine TiO₂ and 50% for nano-
10 TiO₂. In vehicle-treated controls, the microscopic tumor incidences were between 5 and 6%. Although
11 particles that induce high tumor incidences generally also cause high inflammatory cell counts, nano-TiO₂
12 caused a high tumor incidence and low inflammatory cell counts. Borm et al. (2000) suggested that
13 tumor formation was directly related to high interstitialization rather than overload and subsequent tissue
14 response, similar to the premise that lung burden is correlated to surface area of the particles (Oberdörster
15 et al., 1994).

16 Pott and Roller (2005) reported increases in pulmonary tumors in rats exposed to hydrophilic fine
17 TiO₂ and hydrophilic nano-TiO₂, but were unable to draw conclusions about the carcinogenicity of
18 hydrophobic nano-TiO₂. Female Wistar (HsdCpb:WU) rats received weekly intra-tracheal instillations of
19 three types of TiO₂: hydrophilic nano-TiO₂ (P25), hydrophobic nano-TiO₂ (Aeroxide[®] P805/Degussa
20 P805, see Footnote c in Table 5-8), and hydrophilic fine TiO₂ (232033 from Sigma). If the products used
21 in the study are the same as those currently available, both the hydrophilic nano-TiO₂ and fine TiO₂ were
22 photocatalytic and the hydrophobic nano-TiO₂ was photostable. The tested TiO₂ physicochemical
23 properties, doses, and key results are listed in Table 5-8. The types of primary benign lung tumor were
24 adenoma and epithelioma, and the primary malignant tumors were adenocarcinoma and squamous cell
25 carcinoma. At the tested doses, 42–46 rats out of 48 rats/group survived in the hydrophilic nano-TiO₂ and
26 hydrophilic fine TiO₂ groups, and statistically significant increases in benign or malignant lung tumors, or
27 both, were observed in these two groups.

Table 5-8. Treatments and pulmonary tumor incidences in rats exposed to fine and nano-TiO₂ through intra-tracheal instillation in Pott and Roller (2005) study.

Treatment	Crystal form; primary particle size; specific surface area (BET)	Photo-stability	Dose (number of instillations x mg per instillation)	Rats at start/at risk ^a	Survival 50% (wks)	Lungs with primary benign tumors (%)	Lungs with primary malignant tumors (%)	Lungs with tumors, total (%)	Lungs with metastases of other tumors (%)
Nano-TiO ₂ , hydrophilic (P25)	Majority anatase; 25 nm ^b (21 nm and 30 nm were also reported); 52 m ² /g	Photo-catalytic	5 x 3.0	48/42	114	21.4	31.0	52.4	14.3
			5 x 6.0	48/46	114	17.4	50.0	67.4	15.2
			10 x 6.0	48/46	104	23.9	45.7	69.6	15.2
Nano-TiO ₂ , hydrophobic (Degussa P805) ^c (Sigma AL 900032) ^c	(Data of Degussa T805) ^c Crystal form not specified, coated with an organic silicon compound; 21 nm; 32.5 m ² /g	(Currently available Degussa T805 is a photostable UV filter)	15 x 0.5	24/11	86	0.0	0.0	0.0	9.1
			30 x 0.5	48/15	114	6.7	0.0	6.7	6.7
Fine TiO ₂ , hydrophilic (Sigma AL 232033)	Anatase; 200 nm; 9.9 m ² /g	(Untreated anatase is photo-catalytic)	10 x 6.0	48/44	108	15.9	13.6	29.5	11.4
			20 x 6.0	48/44	113	38.6	25.0	63.6	2.3
No treatment	--	--	--	48/46	113	0.0	0.0	0.0	13.0

BET – Brunauer, Emmett, Teller method of calculating surface area

P25 – AEROXIDE® P25

UV – Ultraviolet (light/radiation), wavelengths in the range of 10-400 nm

^a Rats at risk were "sufficiently examined rats which survived at least 26 weeks after first instillation" according to Pott and Roller (2005).

^b Regarding particle characteristics, Pott and Roller (2005) noted "There are no clearly measured values or more than one piece of information." The value listed in the table was assumed to be close to the correct value and was used for further calculations by Pott and Roller (2005).

^c According to Pott and Roller (2005): "Titanium dioxide T805 from Degussa was ordered from Sigma-Aldrich, but the supplier only offered an amount of at least 40 kg P 805. Neither Sigma-Aldrich nor Degussa answered at all clearly when questioned insistently as to the difference between T805 and P805. So, it is not proven that P805 is identical with T805 from Degussa." The primary particle size and surface area in the table were from the Pott and Roller (2005) study. Currently available T805 is photostable nano-TiO₂ (80% anatase, 20% rutile) that has been treated with octylsilane to achieve a hydrophobic surface. The primary particle size is still 21 nm, but the specific surface area (BET) is 45 m²/g.

1 Hydrophobic nano-TiO₂ (Degussa P805) showed high acute mortality in the Pott and Roller (2005)
2 study. Nano-TiO₂ P805 was given at a much lower amount in each instillation than nano-TiO₂ P25 and
3 fine TiO₂, because instilled P805 showed acute lethality. A single intra-tracheal instillation of P805 at 0.5,
4 1.0, and 1.5 mg caused death in 25%, 58%, and 92% female Wistar rats, respectively, within 24 hours.
5 Pott and Roller (2005) originally ordered Degussa T805 for their study, and were unable to confirm that
6 the received P805 was the same as T805. The physicochemical properties of T805, but not P805, were
7 used for calculation and reported in the study (Pott and Roller, 2005). In contrast to the high acute
8 toxicity of hydrophobic nano-TiO₂ reported in the Pott and Roller (2005) study, very low toxicity of
9 hydrophobic nano-TiO₂ was reported in an earlier study by Rehn et al. (2003). Rehn et al. (2003)
10 reported that a single intra-tracheal instillation of P805 at 0.15, 0.3, 0.6, or 1.2 mg caused no death in

1 female Wistar rats. Furthermore, P805 induced only mild, reversible inflammatory responses in the lung,
2 and was less biologically active than P25 (Rehn et al., 2003). The reasons for the great discrepancy in the
3 toxicity of hydrophobic nano-TiO₂ (P805 vs. T805 manufactured by Degussa) remain unclear.

4 5.3.2.2.2. Inhalation

5 Heinrich et al. (1995) reported increased lung cancer in rats (but not in mice) that inhaled
6 photocatalytic nano-TiO₂. Animals were exposed to P25 aerosols (18 hours/day, 5 days/week) in whole-
7 body exposure chambers. Generated by a dry dispersion technique, the nano-TiO₂ aerosol had a mass
8 median aerodynamic diameter of 0.80 μm, with a geometric standard deviation of 1.80.

9 For female NMRI (Naval Medical Research Institute) mice, the nano-TiO₂ exposure was stopped
10 after 13.5 months and followed by clean air exposure for 9.5 months. The 13.5-month nano-TiO₂ aerosol
11 exposure was 4 months at 7.2 mg/m³, 4 months at 14.8 mg/m³, and 5.5 months at 9.4 mg/m³. Although
12 nano-TiO₂ exposures decreased lifespan in mice (50% mortality at 17 months after birth, compared to 20
13 months in controls), the exposures did not increase lung tumor incidence at the end of the study (13.8% in
14 nano-TiO₂ exposed, compared to 30% in controls). Even though the reported spontaneous lung tumor
15 rate seemed to be higher than historical data (20.7% lung cancer in the natural lifespan of female NMRI
16 mice (Lohrke et al., 1984); 12% bronchiole-alveolar lung adenoma and 10% bronchiolo-alveolar lung
17 carcinoma in female Han:NMRI mice up to 104 weeks old (Rittinghausen et al., 1997), 13.8% would not
18 be considered as an increase compared to historical controls.

19 For female Wistar rats, the nano-TiO₂ exposure was stopped after 24 months, and followed by
20 clean air exposure for 6 months. The 24-month nano-TiO₂ aerosol exposure consisted of 4 months at 7.2
21 mg/m³, 4 months at 14.8 mg/m³, and 16 months at 9.4 mg/m³. At the end of the 30-month study, 32 of
22 100 nano-TiO₂-exposed rats had benign or malignant lung tumors (20 benign squamous cell tumors, 13
23 adenocarcinoma, 4 adenoma, and 2 squamous cell carcinoma), while only 1 of 217 control rats had lung
24 adenocarcinoma (Heinrich et al., 1995). The lung particle loading was 23.2 mg/lung after 6 months, and
25 39.2 mg/lung after 24 months (Gallagher et al., 1994). The exposure to nano-TiO₂ did not increase the
26 levels of DNA adducts in the lung (Gallagher et al., 1994). This study showed that inhaled photocatalytic
27 nano-TiO₂ is a lung carcinogen in female rats, but no dose-response relationship can be calculated due to
28 the dosing design. In a parallel study, decreased pulmonary clearance (overload) was clearly
29 demonstrated (Creutzenberg et al., 1990).

30 The aerosol concentrations used in this study, ranging from 7.2 mg/m³ to 14.8 mg/m³, are
31 occupationally relevant, for example, the OSHA PEL (Occupational Safety and Health Administration
32 permissible exposure limit) is 15 mg/m³ and the ACGIH TLV (American Conference of Governmental
33 Industrial Hygienists threshold limit value) is 10 mg/m³.

1 5.3.2.2.3. Intraperitoneal Injection

2 Pott et al. (1987) intraperitoneally injected Wistar and Sprague-Dawley rats with photocatalytic
 3 nano-TiO₂ (P25)¹¹ and examined abdominal cavities for tumors. The treatment doses ranged from a
 4 single intraperitoneal injection of 5 mg nano-TiO₂ to 5 injections of 20 mg nano-TiO₂ (for a total of 100-
 5 mg nano-TiO₂) over 5 weeks (Table 5-9). Tumor incidences were based on rats with sarcoma,
 6 mesothelioma, or carcinoma in the abdominal cavity. Rats with uterine tumors were excluded from the
 7 rats-with-tumor count, because 5–10% of the controls had malignant tumors of the uterus and some with
 8 metastases. Tumor incidences in the abdominal cavity in nano-TiO₂-treated rats ranged from 0% to 10%
 9 in the 5 experiments using nano-TiO₂ (Table 5-9). Although controls were not available in all
 10 experiments, Pott et al. (1987) concluded there were no increases in tumor incidence (in the abdominal
 11 cavity) in nano-TiO₂ treated rats.

Table 5-9. Incidence of tumor in the abdominal cavity of rats intraperitoneally injected with photocatalytic nano-TiO₂.

Animal, age at the beginning of the experiment	Nano-TiO ₂ treatment	Rats with sarcoma, mesothelioma, or carcinoma, other than uterine tumors, in the abdominal cavity (percentage)
Rats sacrificed when in bad health or 2.5 years after treatment		
Wistar rat, 9 weeks old	i.p. injection of 18 mg/rat, once per week for 5 weeks (total dose 90 mg/rat)	6 of 113 rats examined (5.3%)
Sprague-Dawley rats, 8 weeks old	i.p. injection of 5 mg/rat	2 of 52 rats examined (3.8%)
Wistar rats, 4 weeks old	i.p. injection of 5 mg/rat	0 of 47 rats examined (0%)
Wistar rats, 5 weeks old	i.p. injections of 2, 4, and 4 mg/rat (total dose 10 mg/rat)	0 of 32 rats examined (0%)
Preliminary results at 28 months after i.p. injection		
Wistar rats, 8 weeks old	i.p. injection of 20 mg/rat, once per week for 5 weeks (total dose 100 mg/rat)	5 of 53 rats (36 rats examined and 17 rats survived) (9.4%)

i.p. – intraperitoneal

Source: Data from Pott et al. (1987).

¹¹ Data from Pott et al. (1987) reported the P25 as anatase and did not specify particle size in the 1987 publication. Currently available P25 is 80% anatase and 20% rutile (primary particle size approximately 21 nm), and a representative of Degussa stated that the company has never changed the formula since Degussa P25 was introduced to the market (Clancy, pers. comm. 2008).

1 5.3.2.2.4. Intramuscular Implantation

2 No tumors were observed in rats receiving implantations of either conventional TiO₂ or nano-TiO₂
3 for up to 12 months (Hansen et al., 2006). Each of the 10 male Sprague-Dawley rats was surgically
4 implanted with conventional TiO₂ (a 9-mm x 2-mm disk containing 100% rutile) subcutaneously on the
5 left side, and with nano-TiO₂ (20–160 nm, mean size 70 nm, 90% anatase and 10% rutile) intramuscularly
6 on the right side of paravertebral muscle. The implanted doses were one disk of conventional TiO₂ and
7 0.1 mL nano-TiO₂. Four rats were sacrificed after 6 months, and the remaining six were sacrificed after
8 12 months. Inflammation (but not granuloma) was observed at the site of conventional TiO₂ implantation,
9 and granuloma (localized nodular inflammation; non-cancerous inflammation) was observed at the site of
10 nano-TiO₂ implantation at both 6 and 12 months. No tumors were observed at either time.

5.3.2.3. Modes of Action for Carcinogenicity

11 The mode of action of lung cancer induced by poorly soluble particles with no specific toxicity is
12 believed to be particle deposition in respiratory epithelium, decreased lung clearance (to the degree of
13 overload), persistent inflammation, cellular injury and persistent cell proliferation, fibrosis, and secondary
14 genotoxicity (mutation) in the lung cells (Baan et al., 2006; Muhle and Mangelsdorf, 2003). TiO₂ is
15 traditionally considered chemically inert and falls into the category of poorly soluble particles with no
16 specific toxicity. When dose-response is expressed as surface area (dose) to tumor proportion (response),
17 TiO₂, nano-TiO₂, and other poorly soluble particles with no specific toxicity appear to share the same
18 dose-response curve¹² (Dankovic et al., 2007).

19 With the exception of mutation, all the events described in the previous paragraph (Baan et al.,
20 2006; Muhle and Mangelsdorf, 2003) have been reported in rats exposed to both fine TiO₂ and
21 photocatalytic nano-TiO₂ through inhalation or instillation (Borm et al., 2000; Heinrich et al., 1995; Hext
22 et al., 2002; Pott and Roller, 2005). Figure 5-1 illustrates that, at low or medium exposure levels, lungs
23 with normal clearance show inflammation that diminishes over time after exposure ceases. When the
24 exposure level is high enough to decrease clearance, rats show persistent pulmonary inflammatory
25 responses (even after exposure ends), cell proliferation and fibrosis, and eventually tumors. In mice,
26 when the exposure is high enough to cause decreases in clearance, pulmonary inflammatory responses
27 gradually decrease after the exposure ceases and no persistent pathological changes or tumors are

¹² Because the nano-TiO₂ data used in this dose-response curve were from studies using the same photocatalytic nano-TiO₂ product, this dose-response curve might not be applicable to nano-TiO₂ with a different crystalline type/ratio, purity, shape, surface treatment, or some other characteristic. Although such factors are known to affect nano-TiO₂ toxicity, their role in carcinogenicity remains unknown.

1 observed in the lung. In hamsters, no overload has been observed and therefore no prediction of the
2 outcome of overload in hamsters is presented here.

3 Increased mutation frequency in hypoxanthine-guanine phosphoribosyl transferase (hprt) was seen
4 in type II alveolar cells isolated from rats exposed to 100 mg/kg fine TiO₂ through intra-tracheal
5 instillation (Driscoll et al., 1997). No studies that investigated mutations in lungs of rats exposed to nano-
6 TiO₂ are available. In vitro studies also support the mode of action stated above. Both macrophage- and
7 neutrophil-enriched BAL cell populations from rats exposed to high concentrations of fine TiO₂ showed
8 increased mutations in cultured cells (rat alveolar type II epithelial cell line; RLE-TN) in vitro (Driscoll et
9 al., 1997). Because catalase, an enzyme that catalyzes the decomposition of hydrogen peroxide to water
10 and oxygen, decreased BAL-cell-induced mutation in RLE-TN cells, ROS released from inflammatory
11 cells could contribute to secondary genotoxicity and eventually to the carcinogenicity of TiO₂ (Driscoll et
12 al., 1997). This sequence of events, however, does not appear to occur in mice. At an inhalation dose that
13 causes overload, nano-TiO₂ does not appear to increase lung tumors in mice. More specifically, overload
14 occurs in mice at an inhalation concentration of 10 mg/m³ nano-TiO₂ (P25), based on the increase of
15 clearance half-life of nano-TiO₂ from 40 days at 2 mg/m³ to 395 days at 10 mg/m³, after 13 weeks (6
16 hr/day, 5 days/week) of exposure (Hext et al., 2002). After 13.5 months of inhalation exposure to the
17 same type of nano-TiO₂ (P25) at approximately 10 mg/m³ (including 4 months of exposure at 14.8
18 mg/m³), mice showed no increased lung tumors over the 2-year study period (Heinrich et al., 1995).

19 Although the evidence available to date for nano-TiO₂ carcinogenesis is consistent with the mode
20 of action of other poorly soluble particles and suggests that particle overload is a sufficient condition for
21 nano-TiO₂ to induce lung cancer, this does not definitively establish that particle overload is a necessary
22 condition for nano-TiO₂-induced lung cancer. For example, it has been suggested that nano-TiO₂-induced
23 lung tumors are directly related to high interstitialization rather than overload (Borm et al., 2000). Given
24 the paucity of nano-TiO₂ cancer studies and the lack of consensus on exposure-dose metrics, the question
25 arises whether there may be other effects or modes of action unique to nano-TiO₂ or nanomaterials in
26 general that are yet to be found.

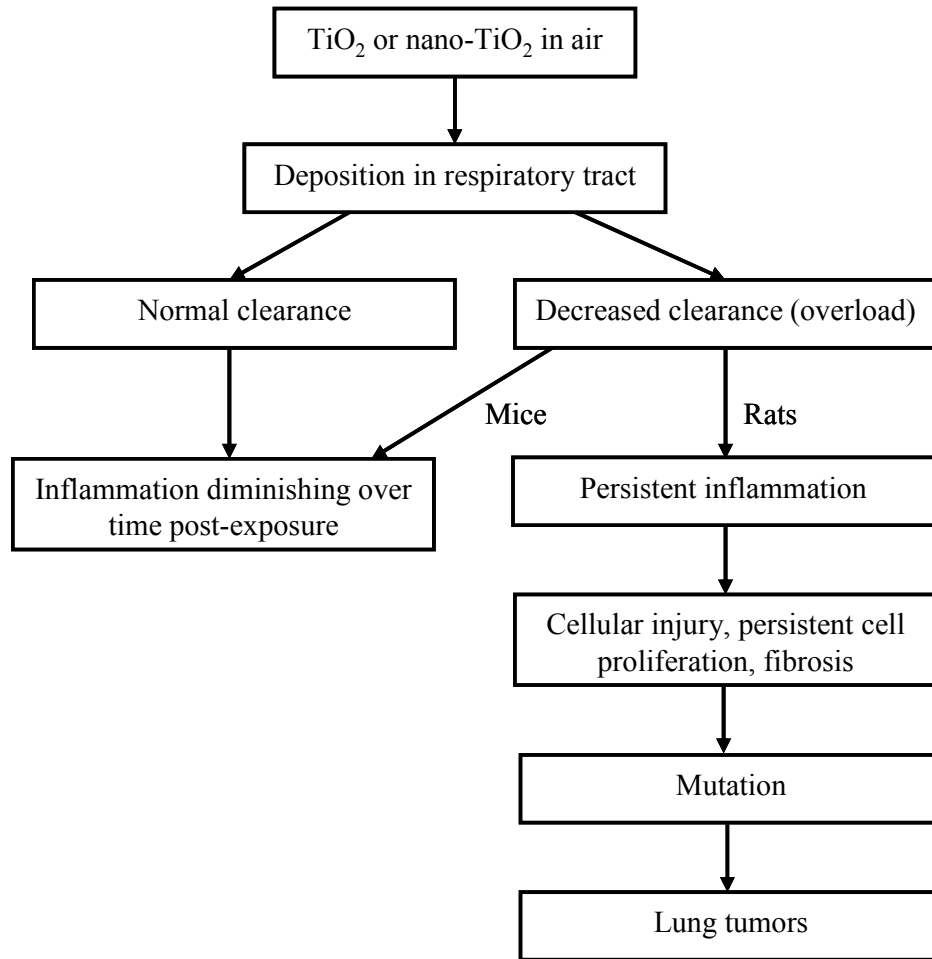


Figure 5-1. The pulmonary effects of fine TiO₂ and nano-TiO₂ exposure through inhalation or instillation.

1 Although the carcinogenicity of TiO₂ and nano-TiO₂ in rats at high doses has been shown
 2 repeatedly in inhalation and instillation studies, the relevance of this rat-specific response to human health
 3 is under debate. Rats have been suspected to be more sensitive to poorly soluble particle-induced lung
 4 cancer because they are more prone to pulmonary inflammation (Muhle and Mangelsdorf, 2003).
 5 Furthermore, lung tumors induced by poorly soluble low-toxicity particles are limited to rats with
 6 severely compromised particle clearance in lung (overload) (Hext et al., 2005). In human exposures,
 7 people working in dusty environments, such as coal miners, could encounter high concentrations of
 8 particles and have impaired lung clearance (Baan et al., 2006). Coal miners, however, are likely to be
 9 exposed to a mixture of particles (i.e., not limited to poorly soluble low-toxicity particles). Evidence of
 10 *persistent* or *chronic* inflammation in humans exposed to TiO₂ is suggested only by case studies of

1 workers exposed to TiO₂ and other minerals (Keller et al., 1995; Moran et al., 1991; Yamadori et al.,
 2 1986).

5.3.2.4. Summary of Carcinogenic Effects

3 The results of nano-TiO₂ carcinogenicity studies in animals are summarized in Table 5-10. No data
 4 are available for nano-TiO₂ carcinogenicity in humans or for photostable nano-TiO₂ in animals. TiO₂ (not
 5 specific to nano-TiO₂) was classified as “possibly carcinogenic to humans” (Group 2B) by an IARC
 6 Monographs Work Group in 2006 (Baan, 2007), and “carcinogenic” (Class D2A) by WHMIS (CCOHS,
 7 2006). NIOSH (2005) proposed not designating TiO₂ as a “potential occupational carcinogen” because of
 8 insufficient evidence, but expressed concern about the potential carcinogenicity of ultrafine TiO₂ (nano-
 9 TiO₂) at the current exposure limits. Based on calculated lung cancer risks, the draft NIOSH
 10 recommendation was an exposure limit of 0.1 mg/m³ for ultrafine TiO₂ and 1.5 mg/m³ for fine TiO₂ (less
 11 than 2.5 μm), as time-weighted average concentrations. The relevance of rat-specific nano-TiO₂
 12 carcinogenicity to human health remains to be elucidated.

Table 5-10. Results of nano-TiO₂ carcinogenicity studies in animals.

Exposure route	Species	Result	Lowest effective dose (highest ineffective dose)	References
Photocatalytic nano-TiO₂				
Intra-tracheal instillation	Wistar rats, female	Increased lung tumors (benign and malignant)	5 instillations at 6.0 mg/instillation	Borm et al. (2000)
			5 instillations at 3.0 mg/instillation	Pott and Roller (2005)
Inhalation	Wistar rats, female	Increased lung tumors	Approximately 12 mg/m ³ for 24 months ^a	Heinrich et al. (1995)
	NMRI mice, female	No increases in lung tumors	(Approximately 10 mg/m ³ for 13.5 months) ^b	Heinrich et al. (1995)
Intraperitoneal injection	Wistar and Sprague-Dawley rats	No increase in abdominal tumors	(5 intraperitoneal injections at 18 mg/rat per injection)	Pott et al. (1987)
Nano-TiO₂ with unspecified photoreactivity^c				
Intra-tracheal instillation	Wistar rats, female	No conclusion ^d	(30 instillations at 0.5 mg/instillation)	Pott and Roller (2005)
Intramuscular implantation	Sprague-Dawley rats, male	No increases in tumor at implantation sites	(not specified)	Hansen et al. (2006)

NMRI = Naval Medical Research Institute

^a 7.2 mg/m³ for 4 months, followed by 14.8 mg/m³ for 4 months and then 9.4 mg/m³ for 16 months

^b 7.2 mg/m³ for 4 months, followed by 14.8 mg/m³ for 4 months and then 9.4 mg/m³ for 5.5 months

^c Nano-TiO₂ particles not specified or have questionable identification

^d Unexpected high acute toxicity; problem with ascertaining the identity of testing material

Questions about Health Effects

General

- 5.3-1. Are the current EPA harmonized health test guidelines for assessing toxicity adequate to determine the health effects/toxicity of nano-TiO₂?

Dermal toxicity

- 5.3-2. Is the current information on nano-TiO₂ skin penetration sufficient for risk assessment?
- 5.3-3. Would nano-TiO₂ penetrate into living cells in flexed, "soaked," or damaged skin (such as sunburned, scratched, eczematous skin)?
- 5.3-4. How important is testing nano-TiO₂ skin penetration on different races and at different ages?
- 5.3-5. Do certain formulations of nano-TiO₂ sunscreens generate hydroxyl radicals when applied to skin?
- 5.3-6. Given that nano-TiO₂ is a good antimicrobial agent, how does it affect skin flora? Does application of sunscreen promote the colonization of skin by potentially harmful bacteria (e.g., staph)?
- 5.3-7. To what extent do photocatalytic properties of nano-TiO₂ contribute to dermal effects?

Respiratory toxicity

- 5.3-8. What kind of studies would provide the most suitable data to understand dose-response of nano-TiO₂ occupational exposure and health effects in humans?

Reproductive toxicity

- 5.3-9. What is the potential for reproductive and developmental effects of nano-TiO₂?

Carcinogenicity

- 5.3-10. Is ingested nano-TiO₂ carcinogenic?
- 5.3-11. Is inhaled nano-TiO₂ carcinogenic at exposure levels below those that induce particle overload?

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Appendix A. Nano-TiO₂ in Sunscreen: Background Information

1 Nanoscale titanium dioxide (nano-TiO₂) has been used in topical sunscreen products since around
2 1990 (Environmental Working Group, 2008). Between 1995 and 2002, the market for inorganic
3 sunscreen ingredients (both nanoscale and non-nanoscale) increased from a value of roughly \$30 million
4 to a value of about \$38 million, and has maintained about a 20% share of the sunscreen ingredient market
5 as a whole (Dransfield, 2005). Dransfield (2005) projected that the inorganic active sunscreen ingredient
6 market would grow to approximately \$75 million by 2010, and that inorganic active ingredients would
7 account for one-third of the total active sunscreen ingredient market. Dransfield (2005) suggested that the
8 projected increase in the popularity of inorganics can be attributed to improved transparency in the
9 products, which would imply particularly rapid growth in the market for nanoscale inorganics. In 2006,
10 the Australian Therapeutic Goods Administration (TGA) estimated that 70% of titanium sunscreens and
11 30% of zinc sunscreens in Australia were formulated with nanoparticles (TGA, 2006).

12 The U.S. topical sunscreen market in 2000 was approximately \$553 million (65% of the \$853
13 million “sun-care” market (a category that includes self-tanning products, after-sun products, etc.)
14 (Packaged Facts, 2001). The size of the U.S. sunscreen market had apparently not changed substantially
15 since 1993, when retail sales were reportedly in the range of \$550–575 million (Davis, 1994). The total
16 U.S. sun-care market reached \$1.1 billion in 2005, and is projected to reach \$1.2 billion by 2010 (Jeffries,
17 2007). If sunscreens continue to account for 65% of the U.S. sun-care market, that would translate to
18 \$715 million in sunscreen sales in 2005, and a projected \$780 million in sunscreen sales in 2010.
19 Globally, sales of sun protection products that presumably include topical sunscreens and cosmeceuticals
20 were expected to exceed \$820 million in 2006 (Newman, 2006). As a “mature” market in the United
21 States, sun protection products are expected to have a growth rate of only about 2% per year (Jeffries,
22 2007). Between 2005 and 2010, however, growth in the sun-care market was expected to be much faster
23 abroad than in the United States (Jeffries, 2007). If the growth in cosmeceuticals has dampened demand
24 for conventional sunscreen, this growth has led to even greater demand for active sunscreen ingredients,
25 including micronized TiO₂ (Davis, 1994).

A.1. Sunscreen Chemistry, and the Role and Properties of Nano-TiO₂

1 Ultraviolet (UV) radiation is classified by wavelength into three types: UV-A (320–400
2 nanometers [nm]), UV-B (290–320 nm), and UV-C (200–290 nm). The shorter the wavelength, the more
3 energy the UV radiation transmits. Consequently, the shorter wavelength rays can cause more damage to
4 skin than the longer wavelength rays. About 10% of the solar radiation that reaches Earth’s surface is UV,
5 and about 95% of that is UV-A. The long wavelengths of UV-A contribute to skin aging, skin wrinkling,
6 and skin cancer. UV-B is in the middle range of UV, and contributes to burning and tanning, skin aging,
7 and skin cancer. Although UV-C has the shortest wavelength and can be dangerous, it is blocked by
8 ozone in the atmosphere and does not reach Earth’s surface (Jeffries, 2007; Shao and Schlossman, 1999).

9 The traditional sunburn protection factor (SPF) rating system measures protection against UV-B
10 radiation only. The Food and Drug Administration (FDA) proposed an official rating system that also
11 takes UV-A radiation into account, awarding sunscreens between one and four stars based on their UV-A
12 protection. This system was expected to go into effect in November 2008 or later (72 FR 49070).

13 Various other UV-A protection ratings systems are in use or have been proposed in Australia, New
14 Zealand, Europe, Japan, China, and Korea (Moyal, 2008).

A.1.1. Size of Nano-TiO₂ Particles (Mean and Distribution)

15 The composition of nano-TiO₂-based sunscreens is determined or constrained by several factors,
16 including peculiar properties of nano-TiO₂, general principles of sunscreen chemistry, and aesthetic and
17 other concerns. The size of nano-TiO₂ particles (both the primary particle size and the effective particle
18 size of aggregates and agglomerates) affects protection against UV-A and UV-B radiation, the opacity of
19 the sunscreen, and the stability of the dispersions. In most cases, a range of nano-TiO₂ sizes is present
20 due to various primary particle sizes and aggregation.

21 The size of nano-TiO₂ particles affects how much UV-A and UV-B the particles transmit and
22 scatter, and therefore, the degree of protection the particles provide against UV-A and UV-B radiation.
23 Shao and Schlossman (1999) found that a nano-TiO₂ dispersion with a primary particle size of about
24 15 nm transmitted less UV-B and more UV-A and visible light than did dispersions with primary particle
25 sizes of 35, 100, and 200 nm. (The particles were present in aggregates of mean sizes 125.3, 154.1,
26 251.1, and 263.4 nm, respectively.) The results of this study indicate that smaller nano-TiO₂ particles are
27 better for UV-B protection, and larger nano-TiO₂ particles are better for UV-A protection. Dransfield
28 (2005) presented data indicating that TiO₂ particles (not specifying whether they were primary or
29 secondary particles) in the range of 40–100 nm provide the best UV-A protection, and particles in the

1 range of 60–220 nm provided the best UV-B protection. According to Hewitt (2002), theoretical
2 calculations suggest that the optimal mean TiO₂ primary particle size for good UV-B and UV-A protection
3 is about 50 nm. Chaudhuri and Majewski (1998) noted that nano-TiO₂ with a primary crystal size of 10–
4 20 nm and an effective particle size of about 100 nm is expected to have a “very high UV scattering
5 effect.”

6 Particle size also determines the opacity of nano-TiO₂ formulations. Larger primary particles
7 transmit less visible light (Shao and Schlossman, 1999). Aggregation will also make a formulation more
8 opaque (Chaudhuri and Majewski, 1998). TiO₂ particles larger than 200 nm in sunscreen or cosmetics
9 leave a white hue on the skin and are considered aesthetically unacceptable in many applications. Nano-
10 TiO₂ particles smaller than 100 nm are generally not visible, and the sunscreen appears transparent when
11 applied. A presentation by Schlossman et al. (2006) included pictures demonstrating the opacity of
12 formulations with different particle sizes when applied to skin. Formulations with an effective
13 agglomerated particle size of 100–120 nm (primary particle size of 10 nm) or 120–150 nm (primary
14 particle size of 15 nm) were transparent or nearly transparent. Schlossman et al. (2006) noted that, in
15 addition to particle size, two other factors affected the opacity/transparency of formulations: the
16 difference between the refractive index of the particle and that of the media, and the uniformity of particle
17 dispersion.

18 Chaudhuri and Majewski (1998) noted that particle size also affects the stability of sunscreen
19 dispersion. The reason for this was not made clear in the article, but in a discussion of pigmentary
20 particles in paints, Himics and Pineiro (2008) explained that smaller pigmentary particles produce a better
21 dispersion because the larger surface area creates a higher viscosity, which prevents settling and
22 clumping. The phenomenon that Chaudhuri and Majewski (1998) noted could have a similar explanation.

23 A range of particle sizes provides a range of UV protection, but too wide a range could pose a risk
24 of opacity or of compromising the stability of the dispersion (e.g., if too many particles are too large). In
25 the past, controlling the range of particle sizes produced by manufacturing processes was difficult, and
26 distributions with a mean particle size of 50 nm included particles in the visible range. As technology has
27 improved, creating particles of desired size and size distributions with much greater accuracy (Hewitt,
28 2002) has become possible.

A.1.2. Active Ingredient Purity

29 The U.S. Pharmacopeia (USP) sets reference standards for TiO₂ and other active ingredients in
30 over-the-counter and prescription drugs. The 2006 edition of the USP national formulary monographs,
31 USP-NF 30 (U.S. Pharmacopeia, 2006), declares that TiO₂ “contains not less than 99.0% and not more
32 than 100.5 percent of TiO₂.” For “attenuation grade” TiO₂, that determination is made on an ignited basis.

1 USP specifies tests for water-soluble impurities, acid-soluble impurities, arsenic, and organic volatile
2 impurities, and notes that FDA also has set limits on acceptable lead, antimony, and mercury
3 contamination. USP also specifies that the material must be stored in well-closed containers, and that it
4 be properly labeled as attenuation grade (with names and amounts of added coatings, stabilizers, and
5 other treatments listed) if intended for UV-attenuation.

A.1.3. Photostability and Surface Coating/Doping

6 Nano-TiO₂ is a natural semiconductor with photocatalytic properties. Its electrons can easily
7 become excited by energy absorbed from UV radiation. When the electrons return to ground state, longer
8 wavelength radiation is emitted. Alternatively, if the energized electrons escape from the particle, they
9 can catalyze chemical reactions (oxidation/reduction processes) in nearby molecules. These reactions can
10 create free radicals, which can damage skin cells or degrade other sunscreen ingredients. The choice of
11 nano-TiO₂ crystal affects photostability. In particular, rutile is much more photostable than anatase
12 (Chaudhuri and Majewski, 1998; Maynard, 2008). Although anatase is less photostable, it appears to be
13 in common use. Barker and Branch (Barker and Branch, 2008) studied five TiO₂ sunscreens purchased
14 over the counter and found that one was pure rutile and the other four were anatase/rutile mixes in which
15 anatase predominated.

16 To increase TiO₂ and nano-TiO₂ photostability (i.e., to reduce the likelihood that excited electrons
17 will escape), the crystals are commonly given a surface coating. Coating TiO₂ with silicon dioxide and
18 alumina (3.5% by weight) can reduce photocatalytic activity by 99% (SCCNFP, 2000). Other TiO₂ or
19 nano-TiO₂ surface coatings mentioned in the literature include inorganic oxides (Bird, 2002), simethicone
20 (Chaudhuri and Majewski, 1998), methicone, lecithin (Schlossman et al., 2006), stearic acid, glycerol,
21 silica, aluminum stearate, dimethicone (SCCNFP, 2000), metal soap, isopropyl titanium triisostearate
22 (ITT), triethoxy caprylylsilane, and C9-15 fluoroalcohol phosphate (Shao and Schlossman, 1999).
23 Alumina is often used in combination with other coating materials. The amount of surface coating
24 applied varies substantially from product to product. For examples of common coating concentrations
25 and combinations, see Appendix B, Table B-2.

26 Another technique for increasing photostability is “doping” the TiO₂ or nano-TiO₂ particles by
27 embedding within them minute amounts of metals such as manganese, vanadium, chromium, and iron
28 (Park et al., 2006). Doping rutile nano-TiO₂ with manganese is reported to increase UV-A absorption,
29 reduce free radical generation, and increase free radical scavenging behavior (Reisch, 2005; Wakefield et
30 al., 2004). Doped TiO₂ is colored instead of white, which can have desirable cosmetic effects in products
31 such as skin lighteners (Park et al., 2006).

1 Recent research by Barker and Branch (2008) has found that the surface coatings on nano-TiO₂ in
2 many sunscreens might not be stable or effective. The investigators studied the weathering of paint in
3 contact with sunscreen. Out of five nano-TiO₂ sunscreens tested, four released photocatalytically
4 generated hydroxyl radicals that accelerated the weathering of the paint. All four of those sunscreens
5 used an anatase/rutile mix. The one nano-TiO₂ sunscreen that showed no appreciable effect on paint
6 weathering was Oxonica's Optisol, which is 100-percent rutile, and is doped with manganese rather than
7 surface-coated. It is not know whether nano-TiO₂ sunscreens generate hydroxyl radicals when applied to
8 skin or whether such hydroxyl radicals would penetrate the skin and pose a threat to the health of the
9 sunscreen user (Brausch and Smith, 2009; Maynard, 2008).

A.1.4. Dispersion and pH Considerations

10 Nano-TiO₂ can exist as a dry powder, but most sunscreen applications require the particles to be
11 suspended in a fluid medium. This liquid is called a "dispersion" because special care must be taken to
12 ensure that nano-TiO₂ will be distributed evenly and to minimize further aggregation and agglomeration
13 (which could negatively impact UV scattering performance, transparency, etc., by increasing the effective
14 particle size). Sunscreen manufacturers can purchase nano-TiO₂ powder and formulate their own
15 dispersion, or they can purchase ready-made "predispersions."

16 In an effective dispersion, suspended particles are attracted to the dispersion medium and repel
17 each other. Surface coatings influence the interaction of nano-TiO₂ with the dispersion medium, which
18 can be water-based (aqueous), oil-based, or silicone-based. Early TiO₂ dispersions were generally oil-
19 based (Bird, 2002). Surface coatings that make TiO₂ dispersible in non-aqueous media can be lipophilic
20 (e.g., metal soap, ITT, lecithin); hydrophobic (e.g., methicone, dimethicone, triethoxy caprylylsilane); or
21 both (e.g., C9-15 fluoroalcohol phosphate) (Shao and Schlossman, 1999). For methicone and C9-15
22 fluoroalcohol phosphate, silicone might be the preferred medium (Shao and Schlossman, 1999). Bird
23 (2002) states that coatings have been developed to enable TiO₂ to be dispersed effectively in aqueous
24 media as well, but provides no examples. Chaudhuri and Majewski (1998) describe one product, an
25 "amphiphilic" powder (Eusolex[®] T-2000) containing about 80-percent USP-grade rutile coated with
26 alumina and simethicone, that is easily dispersible in both water and oil.

27 Two related concepts that are useful in discussing the dispersion of particles are the pH at the point
28 of zero charge (pH_{pzc}), which is the point at which the surface charge density of a particle is zero, and the
29 isoelectric point (IEP), which is the pH at which the net surface electric charge of a particle is zero. In
30 situations where no ions other than H⁺ and OH⁻ are adsorbed at the particle surface, pH_{pzp} is identical to
31 the IEP.

1 At most pH values, nano-TiO₂ particles suspended in a dispersion have a positive electrical charge
2 or a negative electrical charge and repel each other. At the pH_{pzc}/IEP, however, there is no electrostatic
3 repulsion, and particles tend to agglomerate (Hewitt, 1995). To maintain electrostatic repulsion and
4 prevent agglomeration, the dispersed product must be maintained at a pH other than the IEP (usually at a
5 lower pH) at every stage of production and storage.

6 Surface coating can affect a particle's pH_{pzc}/IEP and can potentially extend the pH range at which
7 the dispersion can be handled. For example, uncoated nano-TiO₂ has an IEP of pH 6, and nano-TiO₂
8 coated with alumina and simethicone has an IEP of pH 9 (Chaudhuri and Majewski, 1998). Bird (2002)
9 cites lecithin as another coating that is advantageous for electrostatic reasons.

10 Experimental tests show additional pH considerations. Nano-TiO₂ performance can be adversely
11 affected by strongly acidic formulations (effects include more agglomeration, lower SPF, and greater
12 opacity), unless special formulating techniques are used (Hewitt, 1995).

13 Additional compounds can be added to the dispersion as “dispersants.” “[The] proper dispersant
14 can help particles to disperse into [the] vehicle so as to shorten the dispersion time and increase the degree
15 of dispersion. It can reduce the viscosity and yet stabilize the dispersion by either electrostatic or steric
16 repellency” (Shao and Schlossman, 1999). Different dispersants are used in water- and oil- (or silicone-)
17 based formulations. PEG-10 dimethicone is used as a dispersant for nano-TiO₂ in a cyclopentasiloxane
18 carrier in the predispersion CM3K25VM Kobo Products, Inc. manufactures. Polyhydroxystearic acid is
19 used as a dispersant in a C12-15 alkyl benzoate carrier in Kobo's TNP40TPPS predispersion (Shao and
20 Schlossman, 2004). Mitchnick and O'Lenick (1996) mention lecithin and phosphate esters as potential
21 “dispersing aids” for TiO₂ dispersions, but they also use language suggesting that they might actually
22 mean surface coatings.

A.1.5. Distribution of Active Ingredient in Emulsion

23 Most sunscreens are emulsions – mixtures of two fluids (called “phases”) that are immiscible (do
24 not combine easily). For instance, water and oil, two immiscible fluids, may be mixed in an emulsion by
25 an energetic process such as stirring or shaking. In some cases, the two fluids tend to quickly separate
26 again. To prevent separation, an emulsifier (typically a surfactant or a polymer) can be added. In an
27 emulsion containing two types of liquids, generally, droplets of one fluid are dispersed in a larger amount
28 of the other fluid. The two fluids are referred to as the “dispersed phase” and the “continuous phase,”
29 respectively.

30 Types of emulsion used in sunscreens and other cosmetic products include oil in water (in which an
31 oil phase is dispersed in a water phase, abbreviated “o/w”); water in oil (w/o); water in water (w/w); and
32 occasionally water in oil in water (w/o/w). In “oil-free” formulations, oil is substituted by silicones

1 (w/Si, Si/w) (Hewitt, 2000). As noted above, nano-TiO₂ is most easily dispersed in oil, but emulsions can
2 be formulated with nano-TiO₂ in a water phase, an oil phase, or a silicone phase. The nano-TiO₂ can be
3 present in the dispersed phase or the continuous phase of a sunscreen emulsion (Dransfield, 2005).

4 The emulsifiers used to keep the two phases from separating are typically partially hydrophilic and
5 partially hydrophobic (or even lipophilic). By gathering on the interface between the dispersed phase and
6 the continuous phase, emulsifiers bind the two phases (this is the principle behind soaps, shampoos, and
7 detergents, which enable water to wash away oils and other normally hydrophobic particles), or at least
8 prevent the two phases from repelling each other. Emulsifiers used in sunscreen emulsions include
9 glyceryl stearate, PEG-100 stearate, and polyglyceryl-3-methyl glucose distearate (Oxonica, 2005).

A.1.6. Other Ingredients, Active and Inactive

10 Nano-TiO₂ can be combined with other physical UV blockers, such as zinc oxide (ZnO) (which can
11 also be micronized), or with chemical UV filters to improve the UV protection the sunscreen provides.
12 The sunscreen formula can also include a diverse array of inactive compounds for a variety of purposes.

13 TiO₂ and ZnO can form agglomerates. This attribute presents an obstacle to using TiO₂ and ZnO in
14 the same sunscreen. A solution is to put one active ingredient in the oil phase of the emulsion and the
15 other in the water phase (Hewitt, 1995).

16 Combining nano-TiO₂ with chemical UV filters often provides better UV-B protection than
17 expected, based on the SPF of each ingredient. The improved protection is probably due to the scattering
18 the physical UV blocker provides, which increases the optical path length of the radiation and creates
19 more opportunities for absorption by the chemical filter (Bird, 2002; Chaudhuri and Majewski, 1998).

20 Emollients are often included in sunscreens to make the products feel more pleasing on the skin or
21 to moisturize. In excessive quantities, emollients could break down the dispersion microstructure. This
22 effect can be counteracted by using suitable surfactants or polymers (Hewitt, 1996).

23 Increasingly, nano-TiO₂ is found in “cosmeceuticals,” products that combine a variety of active
24 ingredients to perform multiple health and beauty functions. These products include moisturizers and
25 color cosmetics (see below for more on cosmeceuticals). The manganese added to some nano-TiO₂
26 formulations to prevent formation of free radicals during UV exposure can also help scavenge free
27 radicals generated by other means, thus providing extra skin-protection benefits.

28 Inert ingredients can be added to achieve the right viscosity or liquidity, spray-ability, color or
29 transparency, pH, water-resistance, or spreadability. Silicones and related compounds can be added to
30 impart water-resistance, improve skin feel, serve as emulsifiers in various formulations, and enhance the
31 SPF of oil-based dispersions (Hewitt, 2000).

A.2. Some Sunscreens with Nano-TiO₂ or Micronized TiO₂ as Active Ingredient

1 Table A-1 was compiled in 2007 from information contained in the Environmental Working
2 Group's cosmetic database "Skin Deep" (Environmental Working Group, 2008) and from on-line
3 shopping sources. Products labeled as containing TiO₂ of unspecified particle size were excluded. The
4 list of products provided in Table A-1 is likely not exhaustive. Also, product formulations and labels
5 could change over time.

Table A-1. Titanium dioxide (TiO₂) content in various sunscreen products.

Brand/ Manufacturer	Product	Percentage TiO ₂
Abella	Solar Shade, SPF 45	N/A
Alba Botanica	Chemical Free Sunscreen, SPF 18	7.0%
B. Kamins	Chemist Bio-Maple Sunbar Sunscreen, SPF 30 Fragrance-Free	2.04%
BABOR	High Protection Lotion, SPF 30	N/A
BABOR	Moderate Protection Sun Cream, SPF 20	4.5%
BENEV	Pure TiO ₂	N/A
Bliss	Oil-free Sunban Lotion for the Face, SPF 30	6%
California Baby	SPF 30 & Fragrance Free Sunscreen; also available as Sunblock Stick, SPF 30	4.5%
California Baby	Sunscreen SPF 30+ - Everyday Year Round; also available as Sunblock Stick	4.5%
California Baby	Water Resistant, Hypo-Allergenic Sunscreen, SPF 30	N/A
Cellex-C	Sunscreen, SPF 15	2%
Cellex-C	Water Resistant Sunscreen, SPF 30	2%
Cellex-C	Sun Care Broad Spectrum UV-A, UV-B Sunblock & Moisturizer, SPF 15	N/A
Cellex-C	Sun Care, SPF 30	2%
Colorescience	SPF 30 All Clear Sparkles Shaker Jar; SPF 30 Perfectly Clear Sparkles Shaker Jar; SPF 30 Almost Clear Sparkles Shaker Jar; these variations also available in trial size, brushable, and rock and roller ball forms	12%
Dermalogica	Oil Free Matte Block, SPF 20	4%
Dermalogica	Ultra Sensitive FaceBlock, SPF 25	14%
EmerginC	Sun 30 (and tinted version)	N/A
Fallene/Total Block	Total Block Clear, SPF 65	4%
Fallene/Total Block	CoTZ, SPF 58	10%
Fallene/Total Block	Total Block Cover-Up/Make-Up, SPF 60	10%
Fallene/Total Block	Total Block Tinted, SPF 60	10%
Jan Marini	Bioglycolic Facial Lotion, SPF 15	5.5%
June Jacobs	Micronized Sheer, SPF 30	14.5%
Lancôme	Soleil High Protection Face Cream – Gel, SPF 30	4.5%
Lancôme	Soleil Soft-Touch Moisturizing Sun Lotion, SPF 15	4.5%
Peter Thomas Roth	Instant Mineral, SPF 30	15%
Pevonia Botanica	Pevonia Soleil Sun Block, SPF 15	N/A
ProCyte	Ti-Silc Sheer, SPF 45	N/A
ProCyte	Ti-Silc Sheer, SPF 45 (tinted)	3.5%
ProCyte	Ti-Silc Sunblock, SPF 60+	8%
ProCyte	Ti-Silc Untinted, SPF 45	3.5%
ProCyte	Z-Silc Plus Sunblock, SPF 30+	4.0%
Total Skin Care LLC	pH Advantage Basics Sun Blocker, SPF 15	N/A
Wilma Schumann	Wilma Schumann Sunscreen, SPF 20	N/A

N/A – Not available.

Source: Skin Deep Database (Environmental Working Group, 2008).

A.3. References

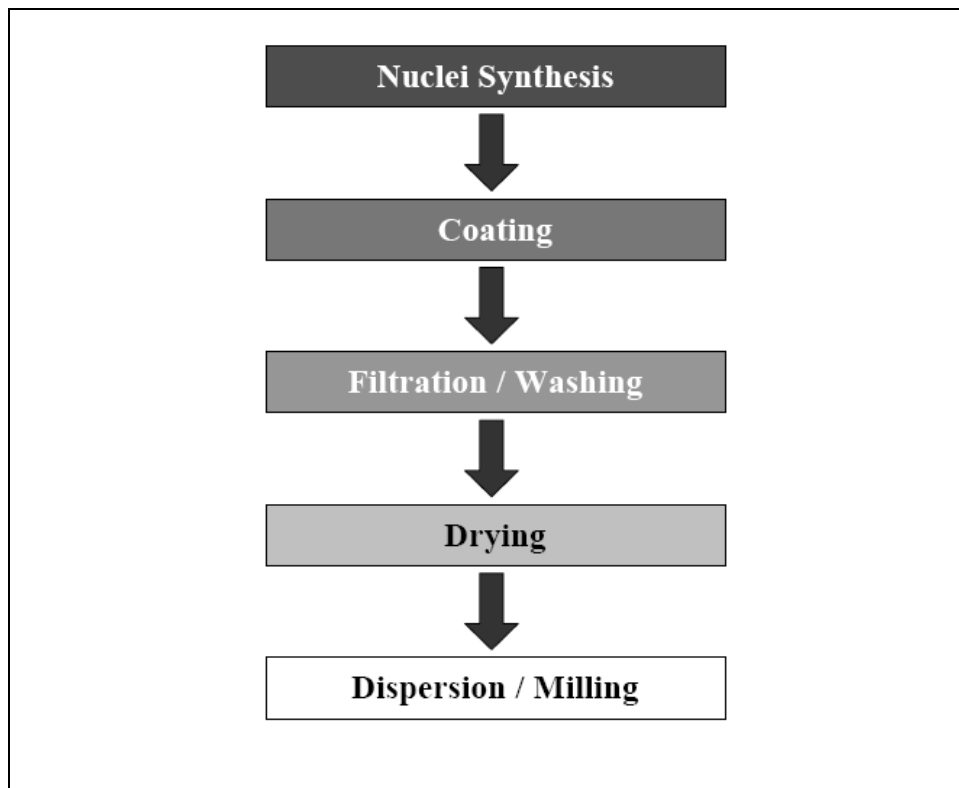
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Appendix B. Nano-TiO₂ in Sunscreen: Manufacturing Processes

B.1. Overview of Nano-TiO₂ Manufacturing Process

- 1 A generic manufacturing process for nano-TiO₂ for sunscreen applications is outlined in
- 2 Figure B-1.

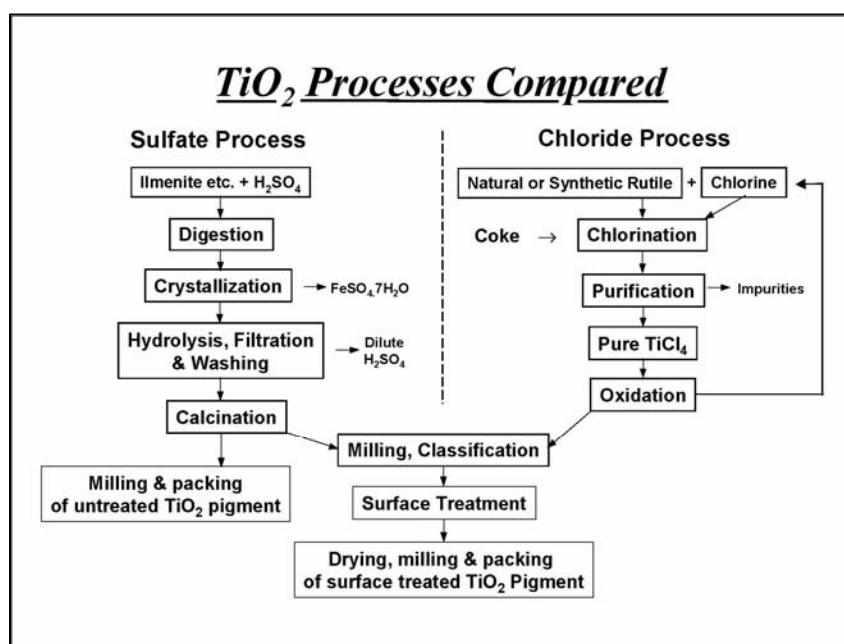


Source: Reprinted with permission from Dransfield (2005).

Figure B-1. Generic manufacturing process for nano-TiO₂ for sunscreens.

B.1.1. Titanium Dioxide Nuclei Synthesis

1 Commercial-scale TiO₂ synthesis is mostly by sulfate or chloride processes. In this section, a
2 sulfate process, chloride process, and patented Altair process are described. These three processes can be
3 used to synthesize both conventional (or pigmentary) and nanoscale TiO₂. There are many new processes
4 being developed in the laboratory, but it is outside the scope of this Appendix to cover them (see review
5 of nano-TiO₂ synthesis by (Chen and Mao, 2007). The sulfate process and the chloride process,
6 illustrated in Figure B-2, are two common methods used to produce TiO₂ in a variety of grades for many
7 different applications.



Source: Reprinted with permission from Millennium Inorganic Chemicals (2007).

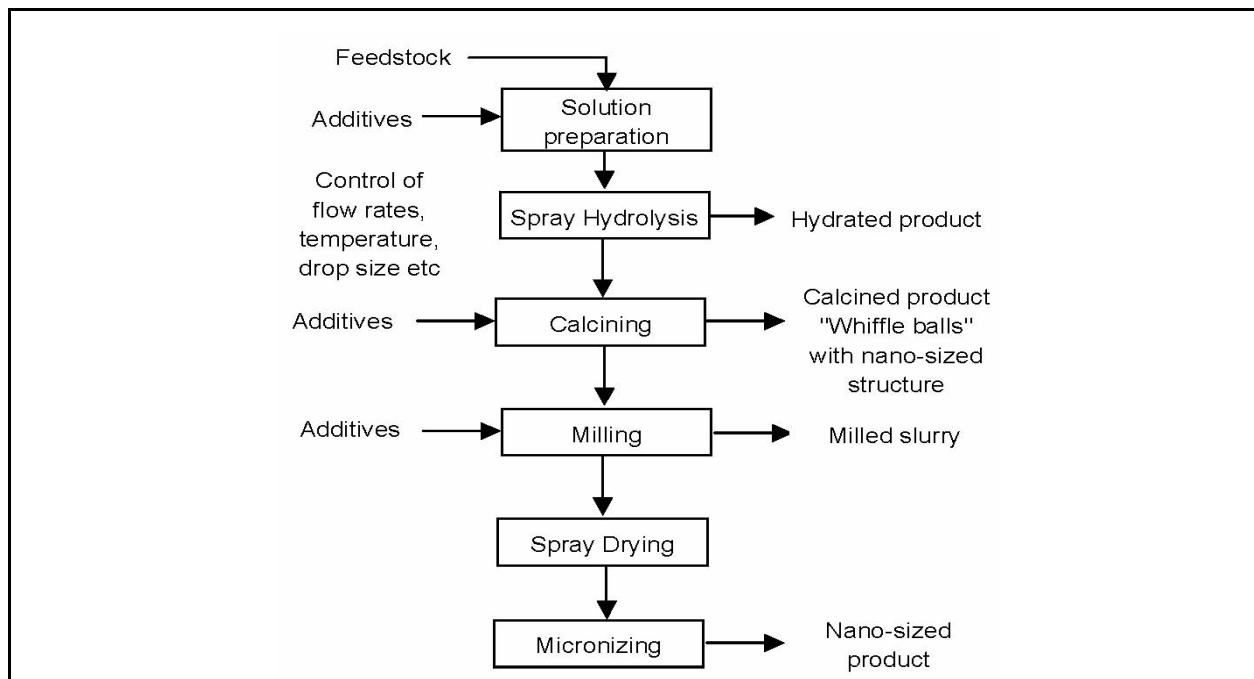
Figure B-2. Sulfate and chloride processes for TiO₂ manufacture.

8 The sulfate process, a wet process for creating pigmentary TiO₂, dates from around 1930, and it
9 was the dominant method used to produce TiO₂ until the chloride process was developed in the 1950s
10 (Hext et al., 2005). The chloride process now accounts for about 60% of worldwide TiO₂ pigment
11 production (Hext et al., 2005). The chloride process, a gas-phase process, is more energy efficient than
12 the wet-phase sulfate process; it can produce finer particles and particles with specific morphologies

1 (Osterwalder et al., 2006). The sulfate process is used primarily to create pigmentary particles. Because
2 attenuation-grade TiO_2 can be produced using “the same processes as larger pigmentary grades”¹
3 (Schlossman et al., 2006), the sulfate process and the chloride process are considered in this document as
4 possible manufacturing techniques for nano- TiO_2 in sunscreen.

5 The sulfate process and the chloride process differ in the feedstock and techniques for nuclei
6 synthesis. In both processes, particles are milled and surface-treated to prepare them for the intended
7 application. The “surface treatment” step in Figure B-2 corresponds to the “coating” step in Figure B-1.

8 The Altair process, a patented, spray-hydrolysis-based process, is illustrated in Figure B-3. This
9 process is used by Altair Nanotechnologies, Inc. to produce not only coated nano- TiO_2 for sunscreen
10 applications, but also uncoated and larger TiO_2 particles and several ceramic oxides (Verhulst et al.,
11 2003). The feedstock for this process is titanium oxychloride. This patented process is comparable in
12 many respects to the sulfate process. What makes it unique, according to Verhulst et al. (2003), is the
13 spray hydrolysis step, which eliminates the aqueous filtration step.



Source: Reprinted with permission from Verhulst et al. (2003)

Figure B-3. Nano- TiO_2 manufacturing process used by Altair Nanotechnologies, Inc.

¹ Pigment-grade refers to a classification of particles of size 200 nm or larger. However, any grade of particles will contain a range of particle sizes, and “[a]lthough pigment-grades of TiO_2 are usually considered to consist of micron sized particles, particles below 100 nm may be present in such grades” (SCCP, 2007).

1 Details of the sulfate process, chloride process, and the Altair Process (derived from spray
2 hydrolysis) are provided in the following paragraphs. The steps unique to each process are presented
3 first, followed by steps shared in these processes. Additionally, processes specific to manufacturing nano-
4 TiO_2 include an additional gas-phase process ($\text{TiCl}_4 + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{HCl}$) and three additional wet
5 processes ($\text{TiOCl}_2 + 2\text{NaOH} \rightarrow \text{TiO}_2 + 2\text{NaCl} + \text{H}_2\text{O}$; $\text{Na}_2\text{TiO}_3 + 2\text{HCl} \rightarrow \text{TiO}_2 + 2\text{NaCl} + \text{H}_2\text{O}$; and
6 $\text{Ti}(\text{OR})_4 + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{ROH}$) (Dransfield, 2005). The gas-phase process is similar to the chloride
7 method except that the titanium tetrachloride is hydrolyzed rather than oxidized. It is also similar in some
8 aspects to the Altair method. These three wet processes rely on feedstocks that are not found in nature,
9 and thus require some additional, unspecified preparatory steps. Waste products from the various
10 processes include hydrochloric acid, salt, water, and compounds formed from impurities.

11 **Specific Steps in the Sulfate Process.** The sulfate process begins with ilmenite ore (FeTiO_3),
12 which is dried, ground, and treated with concentrated sulfuric acid (H_2SO_4) in an exothermic digestion
13 reaction, producing a cake of titanyl sulfate (TiOSO_4) and other metal sulfates. This cake is then
14 dissolved in water or a weak acid. After chemical flocculation, a clear solution and an insoluble mud are
15 produced. The clear solution is cooled to crystallize ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, known
16 as “copperas”). The ferrous sulfate heptahydrate is separated and sold as a by-product (Millennium
17 Inorganic Chemicals, 2007).

18 The insoluble mud is washed, filtered, and evaporated to produce a concentrated TiOSO_4 liquor.
19 The liquor is hydrolyzed to produce a suspension or “pulp” that consists mainly of colloidal hydrous
20 titanium oxide clusters (Millennium Inorganic Chemicals, 2007).

21 The TiO_2 is precipitated from the suspension, which is typically facilitated by a seeding technique
22 to control particle size (no description of the seeding technique was provided). After further washing, heat
23 is applied to crystallize the particles in a process known as calcination, which is also used in other
24 processes. Either anatase or rutile crystals can be produced, depending on the additives applied before
25 calcination (Millennium Inorganic Chemicals, 2007).

26 The following equations represent the chemical processes involved in the sulfate process
27 (Dransfield, 2005):

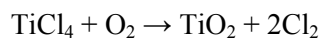
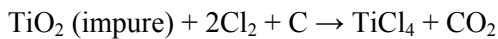


30 **Specific Steps in the Chloride Process.** Natural or synthetic rutile is the feedstock material for
31 the chloride process. During the chlorination step, rutile is added to chlorine and a source of carbon in a
32 fluidized bed at 900 degrees Celsius ($^\circ\text{C}$). The exothermic reaction produces titanium tetrachloride
33 (TiCl_4) plus a variety of impurities. As the gas cools, low-volatile impurities (e.g., iron, manganese, and

1 chromium chlorides) condense out. A stable, very pure liquid TiCl_4 is achieved following condensation
2 and fractional distillation (Millennium Inorganic Chemicals, 2007).

3 The pure TiCl_4 is then oxidized to TiO_2 in a second exothermic reaction. Temperature and other
4 reaction parameters determine the mean particle size, size distribution, and crystal type of the resulting
5 TiO_2 . The TiO_2 is cooled, and impurities are removed. Chlorine released by the oxidation reaction is
6 recycled for reuse (Millennium Inorganic Chemicals, 2007).

7 The following equations represent the chemical processes involved in the chloride process
8 (Dransfield, 2005):



11 **Specific Steps in the Altair Process–Spray Hydrolysis.** The patented Altair process (Verhulst et
12 al., 2003) was derived from a spray hydrolysis method for TiO_2 synthesis. The feed is a titanium
13 oxychloride aqueous solution. The feed solution can be produced by hydrating liquid TiCl_4 in a dilute
14 hydrogen chloride (HCl) solution. In spray hydrolysis, heat (from hot air or a hot receiving surface)
15 causes rapid and complete evaporation of the water in the feed solution as the solution is sprayed. An
16 amorphous, homogeneous, dense, thin film remains on the receiving surface. The film is composed of
17 dry, hollow, almost completely amorphous, TiO_2 particles containing some free or hydration water and
18 some HCl (Verhulst et al., 2003).

19 **Calcination for Sulfate and Altair Processes.** Calcination is the process of heating a solid
20 material to a temperature high enough to change its chemical composition (though generally not high
21 enough to liquefy it). In wet processes like the sulfate and Altair processes, calcination generally occurs
22 after the hydrolysis step. Verhulst et al. (2003) describe the calcined product as a porous crystalline
23 structure of nanoparticles. The crystalline structure retains the shape of the original droplets from the
24 hydrolysis step and will eventually be broken down by milling. The duration and temperature of
25 calcination and the additives introduced during calcination directly influence the structure, particle size,
26 and particle-size distribution of the calcined product. For example, the anatase structure can be stabilized
27 by adding phosphates during calcination (Verhulst et al., 2003).

28 **Milling and Micronizing for Sulfate, Chloride, and Altair Processes.** Milling breaks apart the
29 hollow crystalline lattice ² structure produced in the calcination step, but has to be mild enough not to

² Lattice is the geometrical arrangement of atoms in a crystal.

1 break the individual crystallites (Verhulst et al., 2003). Milling also breaks down agglomerates or
2 aggregates into smaller particles.

3 Both a wet media mill (e.g., with zirconia beads) and ultrasonic milling can be effective (Verhulst
4 et al., 2003). After spray drying, the milled particles (“loosely agglomerated balls”) can be “further
5 micronized to produce a dispersed powder.” How, if at all, micronizing differs from milling is not clear.

B.1.2. Surface Treatments and Doping

6 Some, but not all, nano-TiO₂ particles used for sunscreen undergo surface treatment to prevent the
7 creation of free radicals, which could degrade the sunscreen or damage the skin (DuPont, 2007;
8 Schlossman et al., 2006; Wakefield et al., 2004). Surface coatings for nano-TiO₂ in sunscreen can include
9 combinations of inorganic oxides, simethicone, methicone, lecithin, stearic acid, glycerol, silica,
10 aluminum stearate, dimethicone, metal soap, isopropyl titanium triisostearate (ITT), triethoxy
11 caprylsilane, and C9-15 fluoroalcohol phosphate.

12 In a patent they hold, Mitchnik and O’Lenick (1996) describe a sample protocol for applying a
13 silicone surface treatment to TiO₂ for sunscreen. The patent does not specify the size of the TiO₂
14 particles. A quantity of silicone compound (generally between 0.1% and 25% by weight of the total
15 formulation) is combined with TiO₂ powder. The mixture is heated to 40-100 °C for 2-10 hours, or long
16 enough to remove 97% of the alcohol produced in the reaction. The patent holders claim that the
17 resultant coated particles provide superior performance because the coating “preserves the structure of the
18 TiO₂ crystals, eliminates the reactivity in water, and makes them hydrophobic.”

19 Nano-TiO₂ particles can also be doped with various metals such as manganese, vanadium,
20 chromium, and iron. Park et al. (2006) listed examples of doping methods, including: (1) combining
21 particles of a host TiO₂ lattice with a second component in solution or suspension, and then baking at no
22 lower than 300 °C. The second component is typically a salt, such as a chloride, or an oxygen-containing
23 anion, such as a perchlorate or a nitrate; (2) mixing solutions of the dopant salt and of a titanium alkoxide,
24 and then heating the solution to convert the alkoxide to the oxide and precipitate out the doped material;
25 and (3) flame pyrolysis³ or plasma routes (no additional detail provided).

³ Flame pyrolysis is a synthesis method in which flame heat is applied to vaporize stock material (gas phase precursors) and to initiate chemical reaction for particle (including nanoparticles) production.

B.2. Nano-TiO₂ Particles and Products Used in Sunscreens

1 Several commercially-available nano-TiO₂ particles intended for sunscreen application and some of
 2 their characteristics are summarized in Table B-2 (SCCNFP, 2000). Although these nano-TiO₂ particles
 3 were selected for their applicability to the European market, they are likely to be fairly representative of
 4 nano-TiO₂ active ingredients used in the United States.

Table B-1. Selected list of nano-TiO₂ particles used in sunscreen.

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%
Solaveil fine particle powder	Uniquema	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%

nm = nanometer

Source: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers) (2000).

5 Three manufacturers of United States Pharmacopeia (USP)-grade nano-TiO₂ for sunscreen
 6 applications provided information on their products and processes: Kobo Products Inc., which specializes

1 in powders and dispersions; Oxonica, a European nanomaterials group; and Uniqema, a manufacturing
2 company specializing in oleochemicals ⁴ and specialty chemicals for cosmetics and personal care
3 products. Uniqema was acquired by Croda in 2006 (Cosmetics and Toiletries, 2006).

4 Kobo manufactures a line of 26 attenuation grade TiO₂ dispersions containing nano-TiO₂. The
5 primary particle sizes are mostly 10-35 nm in 25 of 26 dispersions; one dispersion contains 90 nm
6 primary TiO₂ particles. The nano-TiO₂ aggregate sizes in dispersions (measured by dynamic light
7 scattering [DLS]) are mostly 103-165 nm in 25 of 26 dispersions, including the dispersion with 90 nm
8 primary particles; one dispersion contains 230 nm aggregates (Kobo Products Inc., 2009). One of the
9 Kobo TiO₂ dispersions called TNP40VTTS contains nano-TiO₂ particles coated with alumina and an
10 isopropyl titanium tri-isostearate/triethyl caprylsilane crosspolymer (Kobo Products Inc., 2009; Shao and
11 Schlossman, 2004). Polyhydroxystearic acid is used to disperse the product in the solvent/carrier, C12-15
12 alkyl benzoate, which is an ester (Kobo Products Inc., 2009; Shao and Schlossman, 2004). The particles
13 in another dispersion, CM3K40T4, are surface-treated with alumina and methicone and are dispersed in
14 the cyclopentasiloxane carrier with the help of PEG-10 dimethicone (Kobo Products Inc., 2009; Shao and
15 Schlossman, 2004).

16 Optisol™ UV Absorber, a nano-TiO₂ product, is the first commercial product from Oxonica
17 Materials (a branch of Oxonica), and the first commercial health product from Oxonica. Optisol™ is a
18 powder composed of uncoated rutile nano-TiO₂ (size not specified) with approximately 0.67% manganese
19 in the crystal lattice (Kobo Products Inc., 2009; Shao and Schlossman, 2004). Doping with manganese
20 gives the sunscreen the advantages of increased ultraviolet-A (UV-A) absorption, reduced free radical
21 generation, and increased free radical scavenging behavior (Reisch, 2005; Umicore, 2008).

22 Uniqema/Croda⁵ manufactures several TiO₂ sunscreens, including a line of Solaveil™ Clarus using
23 nano-TiO₂ (Chandler, 2006). Solaveil CT-100 and Solaveil CT-200, two of the products in the Solaveil
24 Clarus line, are discussed here as examples. Solaveil CT-100 has more than 50% C12-C15 alkyl
25 benzoate, 25-50% nano-TiO₂, and 1-5% each of aluminum stearate, polyhydroxysteric acid, and alumina
26 (Croda, 2007). Solaveil CT-200 has 15-40% nano-TiO₂, 10-30% isohexadecane, 10-30% glycerol tri(2-
27 ethylhexanoate), 3-7% aluminum stearate, and 1-5% each of polyhydroxysteric acid and aluminum oxide
28 (Croda, 2008). The TiO₂ particle size distribution is very narrow, with the vast majority of particles
29 falling in the nano range (Croda, 2008). Uniqema (no date) recommends using CT-200 at a concentration
30 of 2-30%. The dispersion can be included in the oil phase in an oil-in-water (o/w) emulsion, or in the

⁴ Oleochemicals, e.g., fatty acids, fatty alcohols, and fatty esters, are derived from biological oils or fats.

⁵ Croda acquired Uniqema in 2006 (Cosmetics and Toiletries, 2006). In this Appendix, information sources are cited as it was presented at the time of publication.

1 water phase in water-in-oil (w/o) emulsion, or added separately to a w/o emulsion after emulsification
2 (Uniqema, no date).

B.3. Formulations for Sunscreen Containing Nano-TiO₂

3 Sunscreen formulations that major manufacturers use are proprietary. Companies that produce
4 sunscreen ingredients, however, promote their products by publicizing suggested formulations. These
5 suggested formulations indicate the types of ingredients and processes that might be typical in sunscreen
6 formulation. Two such suggested formulations are discussed here.

7 Generally, compatible ingredients are combined into a number of fluid phases. These phases are
8 then energetically mixed in a particular sequence (sometimes at specified temperatures) to form an
9 emulsion. Formulators have to take care not to allow the pH of the mixture to reach the isoelectric point
10 (IEP) of the nano-TiO₂ or any other dispersed ingredient.

11 Table B-3 shows a sample formulation using Croda Solaveil CT-100W and Solaveil CT-200
12 (Croda, 2009). Table B-4 lists a sample formulation that uses nano-TiO₂ from Kobo for SPF 35 sunscreen
13 that appears transparent when applied on skin (Kobo Products Inc., 2009).

Table B-2. Formula SC-383-1 for “Weightless Morning Dew with Sun Protection.”

Ingredients	%
Part A	
Water	QS
Hydroxypropyl starch phosphate ^a	1.00
Arlatone V-150 [steareth-100 (and) steareth-2 (and) mannan (and) xanthan gum]	0.50
Arlatone LC	2.00
Pricerine™ 9088 (glycerin)	4.00
Solaveil CT-10W [water (and) titanium dioxide (and) isodeceth-6 (and) oleth-10 (and) aluminum stearate (and) alumina (and) simethicone]	5.00
Part B	
Solaveil CT-200 [titanium dioxide (and) isohexadecane (and) triethylhexanoin (and) aluminum stearate (and) alumina (and) polyhydroxystearic acid]	2.00
Ethyl methoxycinnamate ^b	4.00
BRIJ™ 721 (steareth-21)	2.00
Arlamol PS15E (PPG-15 stearyl ester)	5.00
Part C	
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben ^c	1.00

pH: 6.75 ± 0.5; viscosity: 223.5 ± 10% (centipoise) cps

Procedure:

Disperse Arlatone V-150 in water. Then disperse the preservative. Add Pricerine 9088 and heat to 60 °C and add Arlatone LC. Continue heating to 80°C and add Solaveil CT-10W. Combine and heat Part B to 80 °C. Add Part B to Part A. Homogenize for 2 minutes. Return to stirring and cool to 40 °C. Add Part C. Stir to room temperature.

Note: QS means a sufficient quantity.

^a Structure XL, National Starch

^b Eusolex 2292, Merck KGaA

^c Phenonip XB, Clariant

Source: Croda (2009).

Table B-3. Formula KSL-17 for High SPF Transparent Sunscreen.

Ingredients	%
Part 1	
Rose Talc-MS2 – Kobo Products : <i>Talc (and) Methicone</i>	1.00
Velvesil 125 – Momentive/Kobo Products : <i>Cyclopentasiloxane (and) C30-45 Alkyl Cetearyl Dimethicone Crosspolymer</i>	3.00
Net-WO – Barnet : <i>Cyclopentasiloxane (and) PEG-10 Dimethicone (and) Distearidimonium Hectorite</i>	0.20
CM3K40T4 – Kobo Products : <i>Cyclopentasiloxane (and) Titanium Dioxide (and) PEG-10 Dimethicone (and) Alumina (and) Methicone</i>	35.00
Uvinul MC80 – BASF : <i>Ethylhexyl Methoxycinnamate</i>	7.00
Salacos 99 – Nisshin Oil : <i>Isononyl Isonanoate</i>	5.00
Lexol EHP – Inolex Chemical : <i>Ethylhexyl Palmitate</i>	4.00
Squalane – Fitoderm : <i>Squalane</i>	0.20
Tocopherol – Cognis : <i>Tocopherol</i>	0.20
SF96-350 – Momentive/Kobo Products : <i>Dimethicone</i>	1.00
SF96-100 – Momentive/Kobo Products : <i>Dimethicone</i>	1.00
SF1202 – Momentive/Kobo Products : <i>Cyclopentasiloxane</i>	27.10
Propyl Paraben NF – International Sourcing : <i>Propylparaben</i>	0.10
Part 2	
Sodium Citrate – Roche : <i>Sodium Citrate (and) Water</i>	2.00
Net-DG – Barnet : <i>Dipotassium Glycyrrhizinate</i>	0.10
Sodium Hyaluronate – Centerchem : <i>Sodium Hyaluronate (and) Water</i>	1.00
Keltrol CG-T – CP Kelco : <i>Xanthan Gum (and) Water</i>	2.00
Butylene Glycol – Ruger : <i>Butylene Glycol</i>	4.00
Methyl Paraben NF – International Sourcing : <i>Methylparaben</i>	0.10
Water	6.00

Manufacturing Procedure:

* Use explosion-proof mixers and equipment during batching process *

1. Mix each Part separately. Make sure Net-WO is dispersed in Part 1.
2. Heat both Parts to 40 °C and add Part 2 to Part 1 while stirring with homogenizer at 3,000 rotations per minute (rpm).
3. Increase the rotation to 5,000 rpm and continue to emulsify for 5 minutes.
4. Cool down to room temperature with sweeping mixer.

Source: Kobo Products Inc. (2009).

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Appendix C. Nano-TiO₂ Exposure Control in the Workplace and Laboratory

C.1. Workplace Exposure Controls

1 This section summarizes strategies that are currently in place or recommended to decrease
2 exposures to nanomaterials in the workplace (Nanosafe, 2008b; NIOSH, 2009) and to ensure the
3 effectiveness of personal protective equipment (PPE) against nano-TiO₂ (Golanski et al., 2008; Guizard
4 and Tenegal, 2008; Nanosafe, 2008b). While this section focuses on workplace practice of nanomaterial
5 manufacturers, some of the principles and use of PPE are also applicable to laboratories and other
6 settings.

7 The NanoSafe dissemination report (Nanosafe, 2008b) provided several tiers of approaches to
8 decrease nanomaterial exposure in the workplace. During production, the first and preferred approach is
9 to avoid free air flowing particles. If this avoidance is not possible, the process should be contained. If
10 process containment is not possible, extended PPE (which includes double gloves of nitrile, a mask [FFP3
11 or powered respirators incorporating helmets], a protective suit, and safety shoes) and an effective local
12 exhaust system, such as a high efficiency particulate air (HEPA) H14 filter, should be used.

13 During loading and unloading of reactors, and while packing containers, exposure can be decreased
14 by process containment (e.g., by using a glove box or emptying the reactor using an industrial vacuum
15 with a HEPA filter through a liquid trap) (Nanosafe, 2008b). Less preferred alternatives are to transfer
16 nanoparticles within a laminar air-flow booth or extraction hood, or to conduct the transfer in an isolated
17 area equipped with HEPA H14 filter. These alternative options would require the use of extended PPE
18 (Nanosafe, 2008b).

19 During cleaning, special vacuums to avoid dust explosion can be used to trap nanoparticles. The
20 vacuums should be cleaned in a room equipped with a HEPA H14 filter and a washer to clean the
21 protective suites (Nanosafe, 2008b). Alternatively, particles can be drawn into a powder-collection
22 system using a variable-speed fan. Components should be cleaned in a hood equipped with a HEPA filter
23 and an explosion vent panel.

24 The National Institute for Occupational Safety and Health (NIOSH) has a nanotechnology program
25 to increase safety and decrease potential exposures to nanomaterials in the workplace (NIOSH, 2009). In
26 a NIOSH document for safe nanotechnology (NIOSH, 2009), occupational health surveillance and
27 guidelines for working with engineered nanomaterials are discussed, among other topics. Some of these

1 programs could also encourage the general public to reduce environmental releases. Some companies
2 that manufacture nanoscale titanium dioxide (nano-TiO₂) have engineering safeguards and additional
3 programs in place to reduce or eliminate occupational and environmental exposures (e.g., BASF, 2008;
4 DuPont, 2007). Various production methods to decrease worker exposure are also being investigated [for
5 nano-TiO₂, see Guizard and Tenegal (2008)].

6 With a goal to manage nanotechnology safely and effectively within industry, the Nanoparticle
7 Occupational Safety and Health (NOSH) Consortium has investigated methods for monitoring workplace
8 exposure and testing protective technologies. The NOSH Consortium has measured the effectiveness of
9 standard respiratory filters with silicon dioxide (SiO₂) aerosol nanoparticles. With the exception of
10 prolonged exposure (400 minutes or longer), the filter efficiencies for both charged and re-neutralized
11 SiO₂ aerosol nanoparticles met the specifications of the filter type (Ostraat, 2009). The longest exposure
12 time within which the N100 filter performed at or exceeded the efficiency specified by the filter ranking
13 (>99.97-percent filtration efficiency) was 210 minutes (Ostraat, 2009). No PPE specific for
14 nanomaterials exists or is under development (Klaessig, 2008). (For filter efficiency against nano-TiO₂
15 aerosol penetration tested by NanoSafe, see below.)

16 In the following section, two types of PPE are briefly discussed in terms of their protection against
17 nano-TiO₂ aerosols: 1) filters for inhalation protection and 2) protective clothing and gloves for skin
18 protection. Eye-protective gear is available as a third type of PPE commonly used for protection against
19 nano-TiO₂ aerosols, but no information was found on this subject.

20 Each type of nanomaterial is different, and the methods for testing PPE efficiency (such as using
21 charged or neutralized particles) could greatly affect the measured barrier effectiveness. For example,
22 fibrous filters often remove more charged aerosol nanoparticles than uncharged or neutralized aerosol
23 nanoparticles (Kim et al., 2006; Ostraat, 2009). Other physicochemical properties of nanoparticles that
24 affect filtration efficiency include size, chemical composition, and shape. The size of the particle that
25 penetrates most effectively into a specific filter is called the maximum penetrating particle size (MPPS).
26 For particles smaller than the MPPS, the particle penetrations decrease with decreasing particle size; for
27 particles larger than the MPPS, the particle penetrations decrease with increasing particle size. Particles
28 smaller than the pore size of the filter may be filtered out when the Brownian movement of the particles
29 leads to collision of the particle and filter [page 400 and 401 of McKeytta (1984)].

30 Electrostatic filters are charged polypropylene fibers, classified as FFP3—minimum filtration
31 efficiency 99%—based on European Norm (EN) certification. When an electrostatic filter was tested
32 with nano-TiO₂ aerosols, for which size ranged from 16 nm to greater than 76 nm, the MPPS was
33 approximately 35 nm, which was very similar to graphite MPPS (Golanski et al., 2008). At the MPPS,
34 however, nano-TiO₂ penetration was nearly five times higher than that for graphite. Near the MPPS, the
35 differences between nano-TiO₂ and graphite particle penetration increase by an order of magnitude.

1 HEPA filters have a minimum filtration efficiency of 99.97%, are composed of glass fibers, and are
2 classified as H12 for particles <1 micrometer (μm). Like electrostatic filters, HEPA filters showed one
3 order of magnitude higher penetration of nano-TiO₂ (10–19 nm) than that of graphite (10–19 nm), with
4 the highest penetration at approximately 0.2% for 19-nm TiO₂ (Golanski et al., 2008). The penetration of
5 platinum (Pt) through HEPA filters was only slightly lower than that of nano-TiO₂. Golanski et al.
6 showed that particle size alone might not be a sufficient indicator of HEPA filter performance and
7 suggested that nano-TiO₂ might penetrate fibrous filters more than other nanomaterials, namely graphite
8 and Pt (2008). The exposure duration of the Golanski et al. (2008) study was not reported, and therefore,
9 it could be possible that the filtration efficiency of HEPA filters for nano-TiO₂ might decrease with
10 prolonged exposure, as was found for the N100 filter for more than 400 minutes of exposure to SiO₂
11 aerosol nanoparticles (Ostraat, 2009).

12 The efficiency of protective clothing in preventing nano-TiO₂ penetration by diffusion was higher
13 for non-woven fabric than woven cotton and polyester fabric (Golanski et al., 2008). Air-tight, non-
14 woven, polyethylene Tyvek (115 μm thick) was more efficient against nanoparticle penetration than
15 woven cotton (650 μm thick) and woven polyester (160 μm thick) for 10-nm nano-TiO₂ (Golanski et al.,
16 2008), 10-nm nano-Pt (Golanski et al., 2008), and 40- and 80-nm graphite (Nanosafe, 2008a).

17 Nitrile, latex, and Neoprene gloves were reported to be efficient against nano-TiO₂ aerosol
18 penetration via diffusion for a short exposure time (minutes). No penetration through gloves was detected
19 when the gloves were exposed to aerosols of approximately 10-nm nano-TiO₂ and 10-nm Pt (Golanski et
20 al., 2008) or 20- to 100-nm graphite (Nanosafe, 2008a). As these authors pointed out, aerosol penetration
21 test results that examine diffusion do not indicate penetration against dispersion. In addition, continuous
22 flex of gloves could lead to cracks and holes in the gloves (Schwerin et al., 2002), so changing gloves
23 throughout the day is recommended (Harford et al., 2007).

C.2. Manufacturer and Laboratory Practices

24 In 2006, the University of California-Santa Barbara completed a study of nanomaterial
25 manufacturers and laboratories for the International Council on Nanotechnology by surveying
26 organizations about their manufacturing and laboratory practices. Survey results indicated that only 36%
27 of the 64 responding organizations stated that they monitored exposure to the nanomaterials in their
28 workplace. Additionally, 38% of the organizations surveyed believed their nanomaterials posed no
29 special risks, 40% had safety concerns, and 22% were unaware whether the materials they work with or
30 manufacture pose safety risks (Gerritzen et al., 2006).

31 Subsequently, the same research team published additional findings based on a larger sample size.
32 Of the 82 responding firms and laboratories, 89% had a general environmental health and safety program,

1 and 70% provided some type of special training on nanomaterial safety. Nanomaterial safety training was
2 more prevalent in North American firms and laboratories (88%) than in European (64%) or Asian (61%)
3 organizations. Nearly 82% of respondents made nano-specific PPE recommendations to employees.
4 Those tended to be the same firms and laboratories that used advanced engineering controls (i.e., beyond
5 fume hoods) to prevent exposure. Controls included exhaust filtration, air filtration, wet scrubbers, and
6 automated or enclosed operations. Approximately 56% of North American respondents practiced
7 workplace monitoring for nanoparticles, compared to 32% of all respondents. Waste-containing
8 nanomaterials were disposed of as hazardous waste in 78% of North American organizations, compared
9 to 60% of all respondents (Conti et al., 2008).

10 A survey of 43 New England nanotechnology firms found that larger companies (with 500 or more
11 employees) tended to better recognize environmental health and safety (EHS) risks potentially posed by
12 nanoparticles and had EHS measures in place. Many smaller firms either did not perceive risks or did not
13 implement EHS measures (due both to staff and resource constraints and a lack of information on how to
14 quantify nanoparticle risks) (Lindberg and Quinn, 2007).

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