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

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Abbreviations

α-HBDH	Alpha-hydroxybutyrate dehydrogenase
γH2AX	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
µg	Microgram(s)
µg/g	Microgram(s) per gram
µg/kg	Microgram(s) per kilogram
µg/L	Microgram(s) per liter
µL	Microliter(s)
µm	Micrometer(s)
$\mu m^2/cm^3$	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
$Al_2(SO_4)_3 \cdot 16H_2O$	Alum
Al_2O_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_2O_2	Hydrogen peroxide
H_2SO_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-1β	Interleukin-1β
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 triisostearate
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	Milliseviert
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
ºC	Degree(s) Celsius
OECD	Organization for Economic Co-operation and Development
OH	Hydroxyl
· OH	Hydroxyl radical(s)
· OOH	Hydroperoxl 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
p	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
p^{un}	Pink-eyed unstable
RLE-TN	Rat alveolar type II epithelial cell line
ROS	Reactive oxygen species
*r*PTM	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

Engineered nanoscale materials (nanomaterials) have been described in part as having at least one dimension on the order of approximately 1 to 100 nanometers (nm) and unique or novel properties that arise from their small size. This document is a starting point to determine what is known and what needs to be known about selected nanomaterials as part of a process to identify and prioritize research to inform future assessments of the potential ecological and health implications of these materials. Two specific applications of nanoscale titanium dioxide (nano-TiO_2) are considered: as an agent for removing arsenic from drinking water and as an active ingredient in topical sunscreen. These "case studies" do *not* represent completed or even preliminary assessments, nor are they intended to serve as a basis for risk management decisions in the near term on these specific uses of nano-TiO_2. Rather, the intent is to use this document in developing the scientific and technical information needed for future assessment efforts.

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

Each chapter and some sections of chapters have lists of questions that reflect information gaps in that portion of the document. For the most part, these information gaps can be thought of as research needs. Note that some of these needs are specific to the respective uses of nano-TiO_2 either as a water treatment agent or as an ingredient in topical sunscreen. Other research needs may apply more broadly to nano-TiO_2 irrespective of its application, and still other needs may apply even more widely to nanomaterials in general.

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

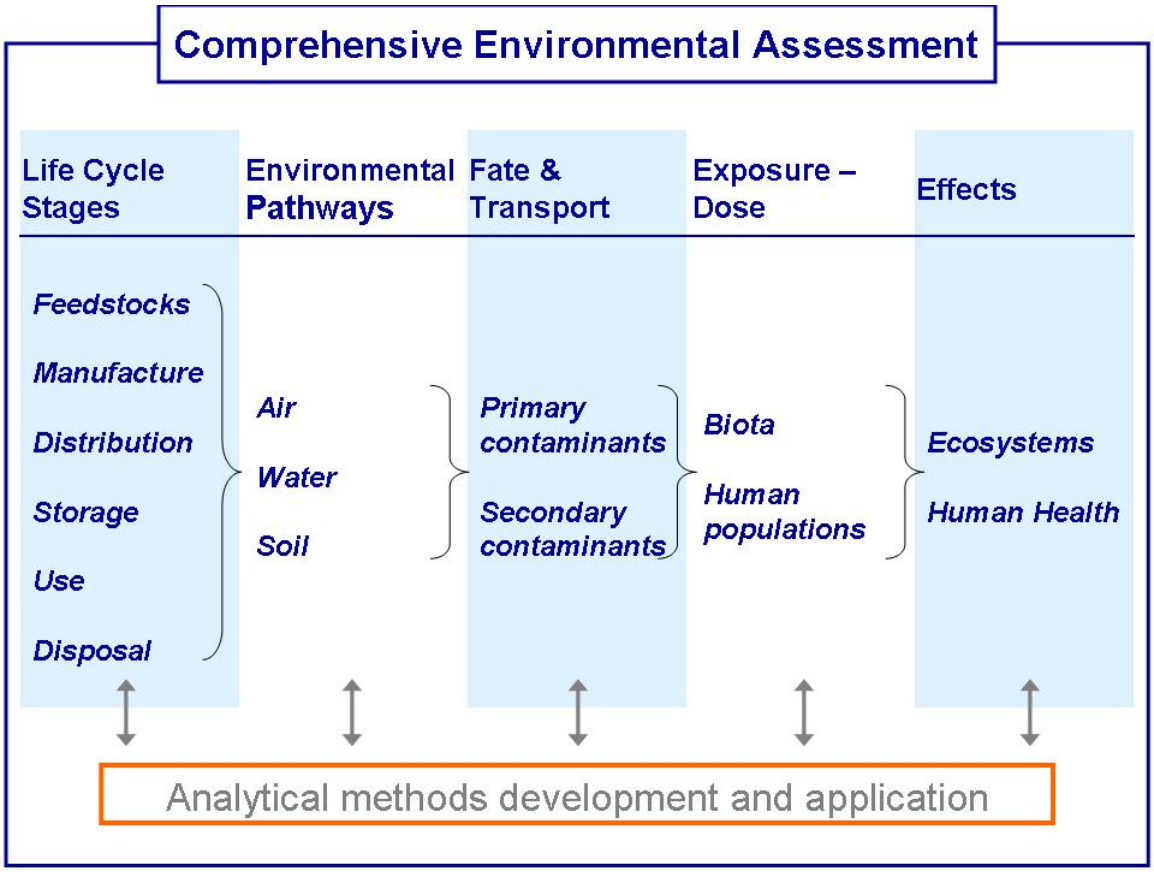
Chapter 1. Introduction

1.1. Background

Engineered nanoscale materials (nanomaterials) have been described in part as having at least one dimension on the order of approximately 1 to 100 nanometers (nm) and unique or novel properties that arise from their small size (National Nanotechnology Initiative, 2006). Like all technological developments, nanomaterials offer the potential for both benefits and risks. The assessment of such risks and benefits requires information, and given the nascent state of nanotechnology, much remains to be learned about the characteristics and impacts of nanomaterials before such assessments can be completed. This document is a starting point to identify what is known and, more importantly, what *needs* to be known about selected nanomaterial applications – in this case, for nanoscale titanium dioxide (nano-TiO_2) – to assess their potential ecological and health implications.

The complex properties of various nanomaterials make evaluating them in the abstract or with generalizations difficult if not impossible. Thus, this document focuses on two specific uses of nano-TiO_2, as a drinking water treatment and as topical sunscreen. These "case studies" do not represent completed or even preliminary assessments; rather, they present the structure for identifying and prioritizing research needed to support future assessments of nano-TiO_2 and an approach to study other nanomaterials.

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

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

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

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

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

1.2. How to Read this Document

The intent of this document is to identify systematically what is known and what needs to be known about nano-TiO_2 to conduct an adequate assessment of such nanomaterials in the future. The goal is not to provide an actual comprehensive environmental assessment or to state conclusions regarding possible ecological or health risks related to nano-TiO_2.

This document is organized around two case studies of nano-TiO_2 using the CEA approach as a basic framework. Although the differences between the applications of nano-TiO_2 as a water treatment agent versus a topical sunscreen are important, the information currently available does not allow complete differentiation between the two. For example, the ecological and health effects of nano-TiO_2 are described in a single chapter without regard to whether the source of nano-TiO_2 is water treatment or sunscreen. However, where distinctions are possible or seem likely (e.g., in life-cycle stages such as manufacturing and use), the discussion of water treatment is presented first, followed by discussion of sunscreen. In some sections, the discussions are not strictly parallel, reflecting the availability of data.

Also important to note is that these case studies have been developed without a specific regulatory objective in mind. Although the topics selected for consideration, water treatment and sunscreen, might be of interest in various policy and regulatory contexts, this document is not intended to serve as a basis for risk management decisions in the near term on these specific uses of nano-TiO_2. Rather, the intent is

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

Focusing on only two examples of nano-TiO_2 applications obviously does not represent all the possible ways in which this nanomaterial could be used or all the issues that different applications could raise. Rather, by considering the commonalities and differences between two applications of nano-TiO_2, research needs can be identified that apply not only to these specific applications but generally to nano-TiO_2 and perhaps even more broadly to other nanomaterials. Also, additional case studies will be developed for other applications and nanomaterials so that this process can continue and research strategies to support assessment efforts can be further refined.

When implemented, a CEA is intended to be comparative, examining the relative risks and benefits of different technological options, for example. Ultimately, a CEA of nano-TiO_2 for water treatment or for topical sunscreen would seek to compare these options against current water treatment practices or sunscreen ingredients. However, it is beyond the scope of this document to describe the various alternatives to nano-TiO_2 for these applications, given that the immediate objective is to identify and prioritize research needs related to nano-TiO_2 as illustrated by the two cases under consideration. Readers seeking comparative assessments of topical sunscreen products, with or without nano-TiO_2, may wish to consult evaluations by the Scientific Committee on Consumer Products (SCCP) (2007) and the Environmental Working Group (EWG) (2009). The EWG analysis in particular takes a broad view that is consistent with the CEA approach in referring to the product life cycle and noting potential ecological as well as human health considerations.

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

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

1.3. Terminology

This document focuses on nano-TiO_2 particles primarily in the size range of 1 to 100 nm. Where information is not specific to nanoscale particles, TiO_2 may be referred to without the "nano" prefix. To make an explicit distinction between the nanoscale material and other forms of TiO_2 not having the

special characteristics of nano-TiO_2, the term "conventional" is used.[2] Even so, conventional materials will often contain a range of particle sizes, including a fraction with nanoscale dimensions. Conversely, as discussed in more detail below, in many circumstances primary nanoscale particles can aggregate or agglomerate into secondary particles with dimensions greater than 100 nm. However, it is not clear that once a cluster of primary nano-TiO_2 particles exceeds 100 nm their properties become like those of conventional TiO_2. For example, inhalation of nano-TiO_2 (20 nm diameter) induced more pulmonary inflammation in the rat than inhalation of fine TiO_2 (about 250 nm diameter) at a similar mass concentration, even though particles in both groups had similarly sized agglomerates (0.71 micrometer [μm] mass median aerodynamic diameter [MMAD] nano; 0.78 μm MMAD fine) (Oberdörster et al., 1994; Oberdörster, 2000). Additional analysis revealed that effects were similar when expressed on the basis of surface area. Whether the constituent primary particles necessarily remain agglomerated or aggregated if conditions change also is not clear. As will be discussed under Fate and Transport (Chapter 3), disaggregation can occur under some conditions. Given these considerations, this document does not use 100 nm as a definitional hard line in considering what might be relevant to an evaluation of nano-TiO_2. This view is consistent with a statement by the European Commission (2008) that extends the term nanomaterial to encompass "nanostructured materials," defined by the International Organization for Standardization (ISO) (Technical Specification [TS] 27687) as "[a]ggregates and agglomerates, often existing at a micro size, [that] may have some of the behaviour and effects of their smaller sub units, e.g., due to an increased surface area."

Degussa AEROXIDE® P25 (hereafter referred to as P25) is a commercial-grade, uncoated nano-TiO_2 product that has been studied extensively and referenced in the literature and is therefore often mentioned in later sections of this document. As discussed below, however, P25 does not represent all nano-TiO_2 preparations and should not be equated with the generic term nano-TiO_2.

1.4. Conventional TiO_2

Although this document focuses on nano-TiO_2, highlighting some facts about conventional titanium dioxide (TiO_2) first is instructional. Also known as titania, TiO_2 has been used commercially since the early 1900s in numerous consumer and industrial applications, particularly coatings and pigments. TiO_2 is a naturally occurring mineral that can exist in three crystalline forms, known as rutile, anatase, and brookite, and in amorphous form. Rutile is the most common form of TiO_2 found in nature.

[2] The terms "bulk" and "pigmentary" are also often used to distinguish conventional from nanoscale TiO_2. 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.

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

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

1.5. Nano-TiO_2

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

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

Such properties have led to the development or use of nano-TiO_2 for a wide variety of applications, including self-cleaning surface coatings, light-emitting diodes, solar cells, disinfectant sprays, sporting goods, and the subjects of this document, water treatment agents and topical sunscreens. Before considering specific applications of nano-TiO_2, some fundamental issues related to characterization of this material should be noted.

Not all nano-TiO_2 is the same. Commercially available brands of nano-TiO_2 can vary in particle size, surface area, purity (e.g., due to doping, coating, or quality control), surface characteristics, crystalline form, chemical reactivity, and other properties (see Table 1-1). Nano-TiO_2 is available in pure anatase, pure rutile, and mixtures of anatase and rutile. In general, anatase nano-TiO_2 is more photocatalytic than the rutile form, and nanoscale rutile is less photoreactive than either anatase and rutile mixtures or anatase alone (Sayes et al., 2006). However, a mixture of 79% anatase and 21% rutile nano-TiO_2 (P25) was found to be more photocatalytic than 100% anatase nano-TiO_2 in some instances (Coleman et al., 2005; Uchino et al., 2002), but less effective in others (Nagaveni et al., 2004). Such contrasts point to the role of other factors in accounting for the behavior and effects of nano-TiO_2. For example, surface treatment of nano-TiO_2 can change nano-TiO_2 activity, including photoreactivity. Aeroxide T805, which is nano-TiO_2 that has been treated with trialkoxygoctyl silane on the surface, has very low surface reactivity (Degussa, 2003). Similarly, surface coatings of silicone and other compounds are used to decrease nano-TiO_2 photoreactivity so that nano-TiO_2 can be used to protect human skin, plastic, and other objects from UV radiation.

Table 1-1. Examples of nano-TiO_2 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).

External factors can also influence photoreactivity. Krishna and coauthors (2006), for example, found that the presence of fullerenes, which scavenge photogenerated electrons, enhances the photocatalytic efficacy of nano-TiO_2. Likewise, Komaguchi and colleagues (2006) saw significant increases in photocatalytic efficiency of P25 after exposure to an oxidizing environment.

Photocatalytic nano-TiO_2 is preferred for water treatment, and photostable nano-TiO_2 is preferred for sunscreen use. Some sunscreens, however, contain photoreactive nano-TiO_2. Although pure uncoated and undoped anatase TiO_2 is photocatalytic, and uncoated and undoped rutile TiO_2 is generally photostable, there is no quick way to identify the photoreactivity of other nano-TiO_2. For example, although doped rutile nano-TiO_2 can be extremely photostable (Reisch, 2005), rutile nano-TiO_2 produced by a certain specific powder-preparation method can be highly photocatalytic (Kim et al., 2003b). Similarly, not all coatings decrease nano-TiO_2 photoreactivity.

Due to various degrees of porosity, nano-TiO_2 particles with the same diameter can differ in surface area. Because nano-TiO_2 reactivity and consequently behavior and effects are influenced by many nano-TiO_2 physicochemical properties, two nano-TiO_2 products with the same reported (but limited) parameters should not be assumed in fact to be equivalent. For instance, a manufacturer might use the same core nano-TiO_2 for surface-treated and untreated nano-TiO_2, and both might have the same particle size and surface area, but differ in reactivity, as in the case of P25 and Aeroxide T805.

Another characteristic of significance is the aggregation or agglomeration of nano-TiO_2 particles.[3] According to one industrial manufacturer of nanoscale titania produced through flame hydrolysis (see Section 2.2 for a description of this manufacturing technique and others), "tests and calculations have shown that free primary particles with dimensions of less than 100 nm only exist in [flame] reactors for a few milliseconds" (Degussa, 2009). Aggregates of nano-TiO_2, sometimes referred to as "colloidal," are often roughly an order of magnitude greater in size than primary particles (Dunphy Guzman et al., 2006; Kormann et al., 1988; Lecoanet et al., 2004). The mean aggregated particle diameter of P25 is about 3.6 µm, with the smallest 4% of particles having an average diameter of 160 nm (Klaessig, 2006). After being subjected to sonication for 10 minutes, the smallest 15% of P25 particles averaged an agglomerate diameter of 160 nm, while the 50th percentile diameter was 1.6 µm, roughly two orders of magnitude larger than the reported primary particle size of P25, which is 21 nm (Degussa, 2007; Wahi et al., 2006). Ridley et al. (2006) observed that a suspension of uncoated nano-TiO_2 anatase from Ishihara Techno 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- to 30-μm diameter range, and that these size ranges persisted even under sonication and other conditions that would favor disaggregation.

Despite the presence, and sometimes the predominance, of such large particles, several researchers investigating laboratory-synthesized anatase and commercial nano-TiO_2 products such as P25 have also found free particles or aggregates with diameters less than100 nm in varying amounts, depending on synthesis method, temperature, solution pH, and the presence of buffers (Jiang et al., 2009). Moreover, some preparations are specifically designed to generate dispersed particles (e.g., Seok et al., 2006), which would be important in using nano-TiO_2 as a catalyst.

The pHpzc of a nanoparticle (the pH at the "point of zero charge," where the net electric charge at the particle surface is zero) has important ramifications for aggregation, because at that pH particles will fail to electrostatically repel each other. In laboratory studies, the size range of aggregates and the presence of free nano-TiO_2 particles (synthesized on-site, ranging from 5 to 50 nm) were found to be pH-dependent: when the solution pH differed from the pHpzc of the particles, the aggregates tended to be smaller (Dunphy Guzman, pers. comm., 2007; Dunphy Guzman et al., 2006). Sampled aggregates ranged up to150 nm in size, and contained an estimated 8 to 4,000 nanoparticles (Dunphy Guzman et al., 2006). The pHpzc also depends at least in part on the crystallinity of the nano-TiO_2 particles: Finnegan et al. (2007) reported pHpzc values of ~5.9 for rutile and ~6.3 for anatase.

Coatings and surface treatments also affect particle aggregation/agglomeration behavior. A preliminary report by Wiench and colleagues indicated that coated nano-TiO_2 particles (rutile, size 50 x 10 nm, surface area of 100 square meters per gram [m^2/g]; coatings included combinations of aluminum hydroxide, hydrated silica, and various polymers) had slower agglomeration and sedimentation rates and a larger fraction of primary nanoparticles remaining in the sample compared with uncoated particles (20 to 30 nm, anatase/rutile 80/20, surface area 48.6 m^2/g) (Wiench et al., 2007).

The complexity of nano-TiO_2 characterization is illustrated in Table 1-2, from Warheit et al. (2007a). The chemical composition of three different types of ultrafine TiO_2 manufactured by DuPont was determined by X-ray fluorescence. The cores of all three types of nano-TiO_2 were TiO_2, but the crystalline form and the surface coating of alumina or silica differed. Each type of particle was said to exhibit a mean diameter of approximately 140 nm but with (unspecified) fractions of the size distributions below 100 nm. The chloride ions on the surface of the particles were neutralized during production. (Other effects these materials cause are described in Chapter 5.) As shown in Table 1-2, the surface area, crystallinity, chemical reactivity, surface coating, particle size distribution, and pH varied for the materials, all three of which were nominally nano-TiO_2.

Table 1-2. Characterization of three nano-TiO_2 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).

The characteristics of a nano-TiO_2 product might change over time. Using a custom-made anatase nano-TiO_2 formulation (uncoated) with a range of particle sizes, Kolář et al. (2006) found that average particle sizes increased over time, due to both agglomeration and re-crystallization (smaller particles dissolving in the aqueous medium and their constituent molecules then adding to the mass of the larger particles). Over the course of 8 years, average (mode) particle size increased from about 10 nm to about 14 nm. The investigators also observed that over time relative surface area decreased, light energy absorbance characteristics changed, and perhaps most surprisingly, photocatalytic performance improved, even as relative surface area decreased.

As discussed in greater detail in Chapter 5 (Section 5.1.1), these and other issues have been noted in various recommendations for improving the characterization of nanomaterials in exposure and ecological as well as health effects studies. In general, however, reports of toxicity and exposure studies of nano-TiO_2, especially those conducted prior to the year 2000, have not been sufficiently attentive to the issues described above. Manufacturers' literature often has been accepted as having described their products under all conditions – an oversimplification at best. Additionally, attempts to characterize nanoscale particle sizes and size distributions in relation to toxicity and exposure evaluations have been prone to errors involving non-representative sampling, agglomeration during sample preparation, contamination and degradation during product storage, measurement methods, and conditions under which the study was conducted (Powers et al., 2007). Further, some particle characterization techniques can affect measurement accuracy, suggesting that more than one technique might be necessary to describe particle sizes accurately. Accurate characterization is clearly important if the behavior and effects of nano-TiO_2 are to be understood, predicted, and related to other materials (both nanoscale and conventional).

1.5.1. Water Treatment

This document assumes that nano-TiO_2 would be used specifically for arsenic removal in a drinking water treatment facility. In addition to arsenic removal (Li et al., 2009), however, nano-TiO_2 could be used for disinfection of pathogens (Alrousan et al., 2009; Coleman et al., 2005; Li et al., 2008a; Rincon and Pulgarin, 2003) or for remediation of ground water or waste water contaminated with various organic and inorganic pollutants (Adams et al., 2004; Chen and Ray, 2001; Han et al., 2009; Kim et al., 2003a; Lee et al., 2008; Lin and Valsaraj, 2003; Ryu and Choi, 2008; Xu et al., 2009b). The latter use would pose rather different scenarios of environmental releases and fate and transport, and would add considerably to the complexity of this document. Therefore, the case study of nano-TiO_2 for water treatment has been limited to the consideration to arsenic removal in water treatment facilities.

Most of the relevant literature to date has reported laboratory tests of nano-TiO_2 as a photocatalytic treatment for conversion of arsenite [As(III)] to arsenate [As(V)], a species that is more easily removed in water treatment because of its lower solubility in typical drinking water treatment conditions (e.g., Dutta et al., 2004; Ferguson et al., 2005; Pena et al., 2006). Although neither conventional TiO_2 nor nano-TiO_2 is known to have been used in a full-scale drinking water treatment plant, both conventional TiO_2 and nano-TiO_2 as photocatalytic agents have been pilot-tested in dinking water treatment plants (Dionysiou, pers. comm., 2009; Pichat, 2003; Purifics Solutions, 2008; Richardson et al., 1996).

For arsenic removal from water, both conventional and nanoscale TiO_2 have been developed to photocatalytically oxidize arsenic and absorb arsenic. Studies have shown that TiO_2 can oxidize As(III) to As(V) and adsorb inorganic arsenic (Dutta et al., 2004; Fostier et al., 2008; Hristovski et al., 2007). The mechanism for TiO_2 photocatalytic oxidation of As(III) has been suggested to be through the generation of superoxide ions, and the major oxidant species might be hydroxyl radicals (·OH) (Sharma and Sohn, 2009). Recently, nano-TiO_2 was shown to mineralize methylated arsenic and to adsorb methylated arsenic (Xu et al., 2007; Xu et al., 2008). Both dimethylarsinic acid [DMA(V)] and monomethylarsonic acid [MMA(V)] were readily mineralized to As(V) by transforming the methyl group into organic compounds such as methanol, formaldehyde, and formic acid. Dimethylarsinic acid was photocatalytically oxidized into MMA(V), which was subsequently oxidized into As(V). Hydroxyl radicals could be the primary oxidant (Xu et al., 2007; Xu et al., 2008).

The mechanism of arsenic adsorption onto TiO_2 surfaces was through the formation of bidentate inner sphere complexes for As(V), As(III), and MMA(V), and forming monodentate inner sphere complexes for DMA(V) (Jing et al., 2004; Jing et al., 2005a; Jing et al., 2005b; Pena et al., 2006). In ground water containing As(III), As(V), MMA(V), and DMA(V), nano-TiO_2 adsorbs As(III) and As(V) most, followed by MMA(V), but almost no DMA(V) (Jing et al., 2009). The difference in competitive

adsorption could be due to lower stability of the monodentate surface structure formed between TiO_2 and DMA(V) than that of the bidentate structure formed between TiO_2 and other arsenicals.

Photocatalytic oxidation is also the mechanism for TiO_2 degradation of organic pollutants in waste water. Photocatalytic degradation is based on the formation of radicals (hydroxyl radicals, superoxide radical anions [O_2^-], and hydroperoxyl radicals [·OOH]), which serve as oxidizing species in the photocatalytic oxidation process (Lu et al., 2009). Hydroxyl radicals, the most powerful oxidants TiO_2 produces in the photocatalysis, can act on organic contaminants present at or near the surface of TiO_2 (Bianco Prevot et al., 1999).

One generally accepted mechanism of nano-TiO_2 antimicrobial property is the generation of reactive oxygen species (ROS), which can cause cell wall or cell membrane damage (Kühn et al., 2003; Neal, 2008), such as lipid peroxidation (Maness et al., 1999). Although UV illumination increases photocatalytic nano-TiO_2 toxicity to bacteria and fungi, photocatalytic nano-TiO_2 is also toxic in the dark (Adams et al., 2006; Coleman et al., 2005). Because TiO_2 generates ROS (mainly highly reactive hydroxyl radicals) in the presence of UV and oxygen (Reeves et al., 2008), mechanisms other than oxidative stress might also contribute to nano-TiO_2 toxicity in the dark (and possibly also under UV), as suggested by a recent study indicating that anatase nano-TiO_2 can generate carbon-centered free radicals in the dark in the presence of dissolved oxygen (Fenoglio et al., 2009).

1.5.2. Sunscreen

Nano-TiO_2 formulations of sunscreen have proven popular because they appear transparent on the skin; formulations using conventional TiO_2 or other inorganics such as zinc oxide (ZnO) (Schlossman et al., 2006) create a milky white appearance. Nano-TiO_2 serves as a sunscreen in two ways, by absorption and scattering, depending on the wavelength of UV light. UV-B wavelengths are in the range of 290–320 nm, and are primarily absorbed by nano-TiO_2; UV-A wavelengths are in the range of 320–400 nm, and are primarily scattered by nano-TiO_2 (Shao and Schlossman, 1999). Optimal scattering is thought to occur when the diameter of the particles is approximately half the wavelength of the light to be scattered (Fairhurst and Mitchnick, 1997; Klaessig, 2009); also see Appendix A for more information on how nano-TiO_2 particle size relates to UV-A and UV-B protection). Information on chemical and other properties of topical sunscreens containing nano-TiO_2 can be found in Appendix A.

Conventional TiO_2 absorbs and scatters UV radiation, making it an effective active ingredient in sunscreens. Like ZnO, TiO_2 is a "physical blocker" of UV radiation, as opposed to many chemically active ingredients that serve as "chemical filters," such as avobenzone and benzophenone, which in some individuals can cause adverse skin reactions, including blisters, itching, and rash (U.S. EPA, 2006d).

Thus, sunscreens containing physical blockers have long been an attractive option to those with sensitive skin. Apart from this niche market, the use of TiO_2 in sunscreen was historically limited because of aesthetic considerations. Because conventional TiO_2 scatters visible light, it remains visible as a white film when applied on skin. With the advance of technology to produce transparent nanoscale TiO_2 particles, which scatter very little visible light and therefore appear transparent when applied on skin, nano-TiO_2 has entered the mainstream as an active ingredient in sunscreens and has also been added to numerous other cosmetic products to provide UV protection. With exposure to UV radiation (wavelengths less than ~ 400 nm), pure anatase nano-TiO_2 is photocatalytic. In sunscreen, however, photocatalysis is an undesirable property that can be addressed by applying surface treatments to the crystals, selecting a less photoreactive form (rutile), or adding antioxidant ingredients to the formula.

The maximum concentration of TiO_2 in sunscreen that the U.S. Food and Drug Administration (FDA) allows is 25% (FDA, 1999), but this limit does not distinguish between conventional and nano-scale TiO_2, between anatase and rutile, or between coated and uncoated particles. The concentrations actually used, according to product labels, typically range from 2% to 15% (see Table A-1, Appendix A). Europe, Australia, Canada, and South Korea also have approved the use of TiO_2 as a UV filter in sunscreen with a maximum concentration of 25%. Japan does not regulate TiO_2 as a UV filter in sunscreen (Oxonica, 2005; Risk & Policy Analysts Limited, 2004; Steinberg, 2007).

Some TiO_2-bearing sunscreens are explicitly labeled as containing nanoparticles. Others are labeled as containing "micronized" TiO_2, a grade commonly used in cosmetics. "Micronized" implies a particle size of about 1 micron (or micrometer, which is one order of magnitude larger than 100 nm), but how precisely manufacturers use the term is unclear. Sometimes "micronized" is taken to imply a nano size range (e.g., Shao and Schlossman, 1999), and sometimes it is considered distinct from nano (e.g., Environmental Working Group, 2008). In the latter case, TiO_2 with a mean particle size of several micrometers is still very likely to include a significant fraction of particles in the nano size range. Even sunscreens using pigment-grade TiO_2 likely contain a proportion of nano-sized particles. When Consumer Reports tested seven leading national sunscreens labeled as containing ZnO or TiO_2 or both, but with no indication on the container regarding the presence of nanoparticles, they found nanoparticles in all seven products (Anonymous, 2007; La Farge, 2007). (They also confirmed the presence of nanoparticles in an eighth brand labeled as containing nanoparticles.) No information was available, however, on the quantities or sizes of the nanoparticles detected in any of these sunscreens (La Farge, 2007). Due to concerns over consumer acceptance of nanotechnology, some nano-TiO_2 sunscreens might simply be labeled as containing "titanium dioxide."

1.6. Analytical Methods

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

Section 1.4 addressed the aspects of characterization generally needed for nanomaterials, particularly nano-TiO_2. This section provides a brief review of analytical methods that could be suitable for nano-TiO_2, with a focus on currently available methods. Because nano-TiO_2 is not radio-labeled and does not fluoresce, analytical methods based on these two attributes are not relevant. Additionally, the importance of chemical analysis of nanomaterials is acknowledged (such as for identifying their molecular components and for characterizing certain surface properties), but these methods also are not discussed in this section. Some of the chemical analysis methods suitable for nanomaterials are discussed in (Powers et al., 2006; U.S. EPA, 2008c). For detailed comparison of various methods, readers are referred to review articles by Maynard and Aitken (2007), Powers et al. (2006; 2007), and Domingos et al. (2009b).

1.6.1. Methods for Laboratory Research

The physicochemical properties of nano-TiO_2 can change over time (Kolář et al., 2006) and in various milieux; therefore, the characteristics of engineered nanomaterials at the point of production could be vastly different after transport, storage, and preparation for testing. Nanomaterials used in toxicological testing ideally would be characterized by analyzing the raw material (as received from the manufacturer or supplier); nanomaterials in the testing media for the duration of the experiment; and nanomaterials (and possibly degraded products or biotransformed products) in the biological samples being tested, such as in urine, organs, and cells.

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

inductively coupled plasma atomic emission spectroscopy (ICP-AES) for elemental composition and quantitative nanomaterial uptake; video-enhanced differential interference contrast (VEDIC) microscopy for uptake and localization (Marquis et al., 2009); and scanning probe microscopy (SPM) for size and three-dimensional images (Gwinn, accepted for publication). ICP, X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) can be used to determine chemical composition (Gwinn, accepted for publication). The combination of flow field flow fraction (FIFFF) and ICP-AES has been used to detect nano-TiO_2 in the tested commercial sunscreen, with information on mass-size distribution and Ti content of extracted nano-TiO_2 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 impacter 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

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

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

In a study comparing six analytical methods for determining nanomaterial sizes [TEM, atomic force microscopy (AFM), DLS, fluorescence correlation spectroscopy, nanoparticle tracking analysis, and flow field flow traction], Domingos et al. (2009b) concluded that the two most commonly used techniques reported in the literature [electron microscopy (EM) on air-dried samples and DLS] were also the two techniques that appear to be most prone to artifacts. Using multiple analytical techniques or 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)	
	Calculation from transmission electron microscopy (TEM) (length and width) and atomic force microscopy (AFM) (height) measurements, and particle nanocrystalline geometrics	Only nanomaterials with a regular or pseudo-regular geometry and without significant porosity
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

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

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

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

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

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

Questions about Characterizing Nanoscale Titanium Dioxide

1-1. To evaluate nano-TiO_2 (in these or other applications) or to compare products containing nano-TiO_2, 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-TiO_2 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-TiO_2?

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-TiO_2 (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-TiO_2?

1-6. What factors determine whether and to what extent aggregation or agglomeration of nano-TiO_2 occurs?

1-7. Are data available that indicate the level of agglomeration/aggregation/dispersion of nano-TiO_2 in specific products? If so, what do the data show?

1-8. Is there a difference between the opacity of nano-TiO_2 aggregates and conventional TiO_2 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

This chapter discusses the life cycle of nanoscale titanium dioxide (nano-TiO_2) as either a water treatment agent or an ingredient in topical sunscreen. Each stage in the life cycles of the respective applications is considered from the standpoint of potential releases to the environment.

2.1. Feedstocks

Two ores, ilmenite ($FeTiO_3$) and rutile (TiO_2), predominate as feedstock materials for TiO_2 production (nano and otherwise) (Haridasan et al., 2008). Ilmenite and rutile are often found together, but ilmenite is found and mined in far greater quantities (at a ratio of more than 10:1 by weight) (Gambogi, 2008) and supplies ~ 90% of titanium minerals worldwide. For rutile-based manufacturing processes, the most common manufacturing pathway for producing TiO_2 of all kinds is via the chloride route using titanium tetrachloride ($TiCl_4$), a liquid that accounts for about 60% of current manufacturing (Hext et al., 2005). Creating synthetic rutile from ilmenite is often more economical than eliminating impurities from natural rutile.

World ilmenite production in 2007 was around 5.6 million metric tons, and world rutile production was around 0.5 million metric tons. The nations that produce the greatest quantities of ilmenite are Australia, South Africa, Canada, China, Norway, India, the United States, and Ukraine. Significant producers of rutile include Australia, Ukraine, South Africa, India, and the United States (Gambogi, 2008). An estimated 1 billion tons of TiO_2 could be produced from existing world ilmenite resources, with another 230 million tons from rutile deposits (Mineral Information Institute, 2009).

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

Low levels of radioactive materials are present in ilmenite and natural rutile (Collier et al., 2001; Haridasan et al., 2008). A study in India found that those who work with ilmenite could be exposed to an annual dose of 1 millisievert (mSv) of gamma radiation and another 0.7 mSv of radioactivity via particle inhalation, mostly due to thorium. Thorium radioactivity in ilmenite was about 60% of the regulatory exemption limit established in the International Atomic Energy Agency (IAEA) Basic Safety Standards. Levels of radioactivity in natural rutile, ilmenite-derived synthetic rutile, and TiO_2 pigment (produced by

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

Another common feedstock is titanium sulfate solution, which can be hydrolyzed to form TiO_2. 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

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

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

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

According to one industrial manufacturer of nanoscale titania, flame hydrolysis can generate high-purity nano-TiO_2 using $TiCl_4$ as a feedstock (Degussa, 2004). Like CVD, flame hydrolysis can be used to deposit a thin film on a surface, a process known as flame hydrolysis deposition (FHD). In FHD, an inert gas carries $TiCl_4$ into a flame that produces hydrogen chloride and the metal oxide (Tok et al., 2009). Flame hydrolysis is used for manufacturing P25 and yields agglomerated particles with a mean diameter of about 3.6 μm, with the smallest 4% of particles having an average diameter of 160 nm (Klaessig, 2006).

Anticipated by-products of the chloride method of TiO_2 production include those resulting from chlorine contamination of the TiO_2 (from the $TiCl_4$ precursor). Warheit et al. (2007b) have suggested that solutions of P25 in water are acidic (pH = 3.28) because of chloride ions on the particle surface. Other information, however, indicates that a steam washing step during the manufacturing process removes hydrochloric acid adsorbed on the surface of P25 (Vormberg, 2004).

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

Another method of TiO_2 production, which could be the preferred method of nano-TiO_2 production in commercial settings, is the sulfate process (Medley, 2008). Details on this and other processes used in producing nano-TiO_2 can be found in Appendix B.

2.2.1. Water Treatment

No information was found on processes specific to preparing or formulating nano-TiO_2 for use in drinking water treatment. P25 is used in a commercial water treatment system (Photo-Cat from Purifics) that can be used for drinking water, ground water, and waste water treatment (NSF International, 2009; Pichat, 2003; Purifics Solutions, 2008);. For this treatment system, P25 is neither specially prepared nor coated (Powell, pers. comm., 2009).

2.2.2. Sunscreen

Unlike for water treatment agents, information on the manufacture of topical sunscreens that incorporate nano-TiO_2 is relatively abundant. Although specific details of manufacturing protocols are

typically proprietary, general information on manufacturing processes and materials is available. The choice of nano-TiO_2 crystalline form is a key issue in manufacturing sunscreens because forms differ in photostability. In particular, rutile is much more photostable than anatase (Chaudhuri and Majewski, 1998; Maynard, 2008). Although less photostable, anatase appears to be in common use: Barker and Branch studied five TiO_2 sunscreens purchased over the counter and found that one was pure rutile, while the other four were anatase/rutile mixes in which anatase predominated (Barker and Branch, 2008).

To increase nano-TiO_2 photostability, the particles are commonly given a surface coating such as silica, alumina, simethicone, or a variety of other compounds (see Appendix B for more information on coatings). Another technique for increasing photostability is "doping" nano-TiO_2 particles by embedding within them minute amounts of metals such as manganese, vanadium, chromium, and iron (Park et al., 2006).

Another important consideration in the manufacture of most topical sunscreens is the use of a liquid medium, or dispersion, to ensure that nano-TiO_2 will be distributed evenly, thereby reducing aggregation and agglomeration (which could negatively impact ultraviolet (UV) scattering performance and transparency by increasing the effective particle size). Sunscreen manufacturers can purchase nano-TiO_2 powder and formulate their own dispersion, or they can purchase ready-made "predispersions."

Surface coatings influence the interaction of nano-TiO_2 with the dispersion medium, which can be water-based (aqueous), oil-based, or silicone-based. These and many other factors figure into the manufacture of sunscreens, including pH; emulsifiers; emollients; other physical UV blockers (e.g., ZnO, which can also be micronized); chemical UV filters; and various inert ingredients to achieve the desired viscosity/liquidity, spray-ability, color/transparency, water resistance, and spreadability. More detailed information on manufacturing processes is presented in Appendix B.

Questions about Manufacturing

2.2-1. How do various manufacturing processes for nano-TiO_2 affect their physicochemical properties?

2.2-2. How are manufacturing processes likely to evolve with increasing demand for nano-TiO_2?

2.2-3. Are certain manufacturing processes used specifically for nano-TiO_2 as a water treatment agent or as topical sunscreen?

2.2-4. What waste products or other by-products, both nanoscale and larger, might be released, and in what quantities, for nano-TiO_2 manufacturing processes?

2.2-5. Where is nano-TiO_2 manufactured? What is the potential for general population exposure to nano-TiO_2 in these areas?

2.3. Distribution and Storage

Limited information about nano-TiO_2 distribution and storage was located. P25 is shipped as a powder in 10-kilogram (kg) "multilayer ventilated paper bags, equipped with an additional polyethylene lining when required" (Degussa, 2007). Another brand of photocatalytic nano-TiO_2 (KRONOS vlp 7000, 7001, and 7500) is also shipped in 10-kg paper bags (KRONOS International, 2006). Nano-TiO_2 powders from Sigma, on the other hand, are shipped in amber glass bottles enclosed in foil or plastic bags, which are shrink-wrapped before being placed in cardboard boxes with shipping cushion peanuts. P25 presumably could be stored as a powder in a chemical storage facility in the original 10-kg shipping bags. Degussa recommends storing it in closed containers under dry conditions (Degussa, 2007). Releases could occur if bags were damaged during shipping or storage. Standard good management practices would be expected to reduce the occurrence of accidental releases, but to what extent is unknown.

As a dispersion, nano-TiO_2 is shipped in pails, drums, or totes (Klaessig, 2008). Sigma ships its nano-TiO_2 dispersion in essentially the same way nano-TiO_2 powders are shipped. Dispersion-formulated nano-TiO_2 presumably would require protection from freezing. Depending on where accidental releases of such dispersions occurred, nano-TiO_2 could be released into water or soil during shipment or discharged into industrial or municipal waste water treatment systems during storage.

2.3.1. Water Treatment

No information pertaining specifically to the distribution and storage of nano-TiO_2 water treatment agents was located.

2.3.2. Sunscreen

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

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

At any point in the distribution-to-storage chain, accidental releases could occur. For example, a 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-TiO_2 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-TiO_2 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-TiO_2 characteristics and behavior?
2.3-7.	How much nano-TiO_2 could be released under various routine and accidental scenarios of distribution and storage?

2.4. Use

2.4.1. Water Treatment

Nano-TiO_2 could be used in various ways to treat drinking water, as discussed in Section 1.5.1. This discussion, however, assumes that nano-TiO_2 would be used in water treatment facilities only for removing arsenic.

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

churches, nursing homes, and factories (i.e., about 1,100 systems serving 2 million people) have also taken some action to comply with the arsenic MCL (U.S. EPA, 2007a). Altogether, about 13 million people use water that is treated to remove arsenic. Although it is unknown to what extent nano-TiO_2 might be used in any of these systems in the future, these numbers provide perspective on its potential usage for drinking water treatment.

Depending on the type of water treatment system, nano-TiO_2 might be used as powder (e.g., in a slurry) or fixed on a supporting material. Each approach has its potential advantages and disadvantages. Powdered nano-TiO_2 has a large surface area and offers highly efficient photocatalytic oxidation, but a means to filter or recycle all of the photocatalyst is required (Dionysiou, pers. comm., 2009; Pichat, 2003). This suggests the possibility that some amount of nano-TiO_2 suspended in water might pass through filters, including microfilters. Also, if nano-TiO_2 builds up on the filter matrix (i.e., if it is not removed by filter backwashing and hydraulic cleaning of sand), it could saturate the filtration medium, and small quantities might be released with filtered water into subsequent steps of the treatment sequence. Fixed nano-TiO_2 has a smaller surface area and thus is less efficient. Although the attachment to the supporting material should allow no leaching, a fixed photocatalyst might not require filters or recycling systems to remove nano-TiO_2 from the final product (Dionysiou, pers. comm., 2009).

Zhang et al. (2008) investigated the removal of nano-TiO_2 in a simulated conventional water treatment procedure, which included coagulation, flocculation, sedimentation, filtration, and disinfection. Two types of nano-TiO_2 (crystal form unspecified, primary particle sizes of 15 and 40 nm, and aggregates 200 and 500 nm, respectively) in 2-L jars were subjected to the treatment procedure. Adding magnesium chloride ($MgCl_2$) or alum ($Al_2(SO_4)_3 \cdot 16H_2O$), followed by coagulation, flocculation, and sedimentation, still left more than 20% of an initial 10-mg/L concentration of nano-TiO_2 in the settled water. Furthermore, the removal efficiency was lower in tap water than in buffered nanopure water (pH 5.6) due to the presence of organic matter in the tap water. Membrane filtration with a pore size of 0.45 μm (450 nm) after sedimentation removed nano-TiO_2 aggregates larger than 500 nm, leaving only 1-8% of the initial TiO_2 in the treated water. Although most, but not all, of the nano-TiO_2 in the initial water was removed, this level of filtration is not typical in water treatment plants (Flummer, 2008; Kline, 2008), nor is it available in most whole-house filtration systems (Johnson, 2005).

At least two commercially available water treatment systems can employ nano-TiO_2, although to date they are not known to be routinely used in this manner. One uses nano-TiO_2 in a fixed membrane and the other uses nano-TiO_2 in a slurry. A system from Matrix Photocatalytic Inc. uses a tube covered with fiberglass mesh in which nano-TiO_2 is embedded; the tube contains water that circulates and ultraviolet (UV) lamps illuminate the outside (Dionysiou, pers. comm., 2009; Pichat, 2003). In the Photo-Cat system by Purifics, nano-TiO_2 (P25) circulates in a slurry inside a narrow annulus surrounded by a UV lamp (Pichat, 2003). A ceramic membrane filters out nano-TiO_2 (Purifics Solutions, 2008). No

empirical data are available on the life expectancy of either system or whether they can release nano-TiO_2 into treated water.

The Purifics system was pilot-tested for two months in a community drinking water treatment facility (Purifics Solutions, 2008). The ceramic membrane used to filter nano-TiO_2 (particles as small as 12 nm) from the finished product was reported to require no servicing or cleaning during the 2-month period because the nano-TiO_2 particles collected in the membrane were removed by bursts of high-pressure air (Pichat, 2003; Purifics Solutions, 2008). Although the purpose of this pilot test was not to remove arsenic, several studies have bench-tested nano-TiO_2 in slurry systems for removal of arsenic from water (Dutta et al., 2004; Ferguson et al., 2005; Lee and Choi, 2002; Li et al., 2003; Meridian Institute, 2006). Higher arsenic oxidation rates occurred using a slurry that was continuously stirred (compared to immobilized nano-TiO_2) (Li et al., 2003). In actual use, steps likely would be taken to keep nano-TiO_2 dispersed during treatment, which could affect solubility and particle agglomeration. Surface modification could affect dispersion and could also improve the material's photocatalytic properties as described (Ryu and Choi, 2004). Additionally, numerous chemicals can be added for drinking water treatment (NSF International, 2007), any or some combination of which could affect the solubility, particle size, and behavior of the nano-TiO_2.

2.4.2. Sunscreen

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

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

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

The total amount of sunscreen, and more particularly the total amount of nano-TiO_2 in sunscreen, used in the United States is unknown. Furthermore, the available survey data does not differentiate between sunscreen products with or without nano-TiO_2, although the percentage of sunscreen with nano-TiO_2 is thought to be substantial. In 2006, the Australian Therapeutic Goods Administration (TGA) estimated that 70% of sunscreens containing titanium and 30% of sunscreens containing zinc in Australia were formulated with nanoparticles (TGA, 2006).

As noted in Section 2.2, annual global production of nano-TiO_2 was estimated at 2000 metric tons around 2005, with about 65%, or 1300 metric tons, used in "personal care" products such as topical sunscreens and cosmetics (Dransfield, 2005; Osterwalder et al., 2006).

A recent report by Barker and Branch (2008) has noted that the surface coatings on nano-TiO_2 in many sunscreens might not be stable or effective. The investigators studied the weathering of paint in contact with sunscreen. Of five nano-TiO_2 sunscreens tested, four released photocatalytically generated hydroxyl radicals that accelerated the weathering of the paint. All four of those sunscreens used an anatase/rutile mix. The one nano-TiO_2 sunscreen formulation that showed no appreciable effect on paint weathering used 100% rutile doped with manganese rather than surface coating (Barker and Branch, 2008).

Questions about Use

2.4-1. To what extent is nano-TiO_2 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 TiO_2 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-TiO_2 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-TiO_2 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-TiO_2 influence the bacterial biofilm community or the occurrence of corrosion?

2.4-5. What is the total quantity of nano-TiO_2 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-TiO_2 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-TiO_2 be released from the sunscreen matrix?

2.5. Disposal

2.5.1. Water Treatment

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

After nano-TiO_2 is used in water treatment, a sludge material (floc) containing nano-TiO_2 would likely be created. In one scenario, the sludge might be taken to a landfill. Whether TiO_2 could diffuse (and thus be released) from a solid matrix such as sludge is unknown. Nano-TiO_2 and other contaminants such as residual arsenic could become suspended in leachate and enter ground water, or they could pass through a solid waste facility liner into the subsurface.

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

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

If nano-TiO_2 is present in finished drinking water that reaches the tap, it would eventually enter the ambient environment or be captured by a waste water stream, after which it could enter sewage treatment facilities.

2.5.2. Sunscreen

Sunscreen containers likely would be disposed of primarily as municipal solid waste and thus end up in landfills or incinerators. The potential for leaching of nano-TiO_2 from landfill disposal of containers would depend on many factors, including the integrity of liners and leachate collection systems, if present. Incineration of sunscreen containers raises the question of whether nano-TiO_2 could enter the stack and be released to air, or become a trace contaminant in fly or bottom ash.

Depending on the packaging, sunscreen containers might be recycled, suggesting the possibility that nano-TiO_2 could be incorporated into recycled materials.

Questions about Disposal

2.5-1.	How much residual nano-TiO_2 is present in packaging of the primary material or derived products? How is such packaging disposed of?
2.5-2.	If nano-TiO_2 were to become much more widely used and produced at a much higher volume, would packaging and shipping methods of nano-TiO_2 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-TiO_2 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-TiO_2 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

Chapter 3 explores what might happen to nanoscale titanium dioxide (nano-TiO_2) after it is released to the environment at various stages of the product life cycles for water treatment agents or topical sunscreens. Nano-TiO_2 could be released to air, water, or soil and then transported or transformed through chemical or biological processes. The lack of data on the fate and transport of nano-TiO_2 by-products and waste produced during the manufacturing process precludes a comprehensive discussion in this chapter. This chapter does, however, summarize what is known about the environmental pathways and transport and transformation processes of nano-TiO_2 related to the various life-cycle stages described in Chapter 2.

Although most studies cited in this chapter consider nano-TiO_2 in aggregate or agglomerate form (as discussed in Chapter 1), whether all constituent primary particles remain in clusters if conditions change is unclear. Disaggregation, for example, can occur at certain pH_{pzc} levels. The pH_{pzc} of a nanoparticle is defined as the pH at the "point of zero charge," which occurs when the net electric charge at the particle surface is zero. At the pH_{pzc} particles fail to electrostatically repel each other. In laboratory studies, the size range of aggregates and the presence of free nano-TiO_2 particles (ranging from 5 to 50 nm in size) were found to be pH-dependent: when the solution pH differed from the pH_{pzc} of the particles, the aggregates tended to be smaller (Dunphy Guzman, pers. comm., 2007; Dunphy Guzman et al., 2006). Sampled aggregates ranged up to150 nm in size, and contained an estimated 8 to 4,000 nanoparticles (Dunphy Guzman et al., 2006). The pH_{pzc} also depends at least in part on the crystallinity of the nano-TiO_2 particles: Finnegan et al. (2007) reported pH_{pzc} values of ~5.9 for rutile and ~6.3 for anatase.

The pH_{pzc} depends in part on the crystal form of the nano-TiO_2 particles. Finnegan et al. (2007) reported pH_{pzc} values of ~5.9 for rutile and ~6.3 for anatase. The degree of aggregation generally increases with the presence of salt or increases in ionic strength, minerals, and organic matter in water (Domingos et al., 2009a; French et al., 2009).

Despite the presence, and sometimes the predominance, of large particles, several researchers investigating laboratory-synthesized and commercial nano-TiO_2 products have found free particles or aggregates with diameters less than 100 nm in varying amounts, depending on synthesis method, temperature, solution pH, and the presence of buffers (Kormann et al., 1988; Li et al., 2003; Nagaveni et al., 2004; Pena et al., 2006; Ryu and Choi, 2006; Sun et al., 2007; Wahi et al., 2006). Moreover, some preparations are specifically designed to generate dispersed particles (e.g., Seok et al., 2006) to increase

the efficacy of nano-TiO_2 as a catalyst, increasing the potential for the presence of disaggregated nano-TiO_2 to occur in the environment. However, no studies of nano-TiO_2 aggregation/disaggregation behavior under "real-world" ambient environmental conditions, irrespective of medium, were located.

3.1. Water

Although numerous studies characterize nano-TiO_2 particles in aqueous solution under laboratory conditions, the fate and behavior of the particles in the environment have received less attention. One report indicates that nano-TiO_2 was detected in river water in Montana, but the source (natural or engineered) and the concentration of nano-TiO_2 were not determined (Wigginton et al., 2007).

Several physicochemical properties of nano-TiO_2 can contribute directly to its environmental fate and transport in water. Long et al. (2006) reported that P25 rapidly aggregated in both Hank's Basic Salt Solution (HBSS) and Dulbecco's Modified Eagle's Medium (DMEM) buffer solutions, both of which are high-osmolarity fluids that contain high concentrations of the monovalent cations Na^+ and K^+ [160 millimolar (mM)] and the divalent cations Ca^{2+} and Mg^{2+} (2 mM). The ionic strengths of these two solutions are approximately 155 mM and 166 mM, respectively. After 1 minute of sonication, aggregation continued for 20–45 minutes until a steady-state, stable aggregate size formed. The steady-state aggregate sizes ranged from 826 to 2,368 nm and the concentration of P25 ranged from 2.5 to 120 parts per million (ppm).

Ridley et al. (2006) found that results were reproducible for classical titration procedures (with modification) to characterize the surface charging properties of a commercially available, uncoated anatase nano-TiO_2 product (from Ishihara Techno Corporation, Osaka, Japan) in suspension. These findings demonstrate that water treatment pH can affect the surface charging properties, and thus the aggregation/agglomeration, potential bioavailability, and reactivity of nano-TiO_2.

Schmidt and Vogelsberger (2006) studied the solubility of four types of nano-TiO_2 (P25 from Degussa, DT51D and G5 from Millennium Chemicals, and an original formulation – presumably all uncoated particles) in various aqueous solutions, particularly focusing on the kinetics of the dissolution process. At the beginning of the process, solubility increased rapidly over time and then reached a steady-state value. The maximum solubility value (i.e., saturation concentration) was observed to depend on the morphology of the TiO_2, the crystalline form of the nano-TiO_2, and on the size of the nanoparticles exposed to dissolution. The saturation concentrations were higher in hydrolysis-generated nano-TiO_2 than in precipitation-generated nano-TiO_2, and higher in smaller particles than larger particles.

Sager et al. (2007b) attempted to disperse nano-TiO_2, and other types of nano-sized particles in several suspension media, including phosphate-buffered saline (PBS), rat and mouse bronchoalveolar

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

Although many studies have demonstrated the potential to use nano-TiO_2 for waste water treatment (Chen and Ray, 2001; Han et al., 2009; Khataee et al., 2009; Rincon and Pulgarin, 2003; Wang et al., 2008c; Watlington, 2005; Xu et al., 2009b), data on the fate of nano-TiO_2 in waste water treatment are scarce. Westerhoff et al., (2008) however, have reported the occurrence of nano-TiO_2 at full-scale waste water treatment plants (in both raw and finished waters) in a conference proceeding abstract. The authors predicted nominal nanomaterial concentrations on the order of one part per billion in liquid discharges from waste water treatment systems, with higher concentrations in waste water biosolids (which may subsequently be applied to land, landfilled, or incinerated).

Other types of nanoparticles also have been studied in waste water treatment plants. Limbach et al. (2008) studied the fate of cerium oxide nanoparticles (20–50 nm diameter) in a model waste water treatment plant under a variety of conditions (e.g., with different surfactants to stabilize dispersions, and in media with different ionic strengths and pH values). They found that surfactants stabilized dispersions under a wide range of test pH values even at high ionic strength. The model sewage treatment plant consistently reduced the cerium oxide nanoparticle concentration in the waste water from 100 ppm to 2–5 ppm. Most nanoparticles were removed via agglomeration with microorganisms in the sedimentation sludge. Comparing the physical properties and behavior of various oxides, the investigators speculated that TiO_2 and other insoluble oxides would behave similarly to cerium oxide, while more soluble or reactive oxides like zinc oxide (ZnO) would be even more likely to aggregate and be more amenable to removal by sedimentation. The investigators cautioned, however, that the high nanoparticle concentration (100 ppm) used in the study favors aggregation, and that at more realistic initial concentrations, a greater percentage of nanoparticles are likely to break through.

Although no field studies on the behavior of nano-TiO_2 in the environment were identified, that conventional TiO_2 can photogenerate fairly long-lived reactive oxygen species such as hydrogen peroxide in aqueous environments has long been recognized (Harbour et al., 1985). Similar behavior would be anticipated for nano-TiO_2.

The interaction between nano-TiO_2 and natural organic matter, which is ubiquitous in the environment, has been investigated in controlled conditions in the laboratory. Yang et al. (2009) found that humic acid, a common type of natural organic matter, is easily adsorbed onto nano-TiO_2 in aqueous

media (Yang et al., 2009). Because humic acid adsorption decreased the ξ (Chi) potential (i.e., increased electrostatic repulsion) of nano-TiO_2 particles, humic acid-coated nano-TiO_2 could be more easily dispersed and suspended and thus more stable in an aqueous medium than uncoated nano-TiO_2 (Yang et al., 2009).

3.1.1. Drinking Water Treatment-specific

Although the processes for using nano-TiO_2 for commercial water treatment are not yet well established and therefore a definitive understanding of nano-TiO_2 fate is not possible, nano-TiO_2 is not expected to be destroyed. One might anticipate that, given the size of nano-TiO_2, it would remain suspended in solution; alternatively, it could adsorb to other particles and become part of the sedimentation (floc). Some evidence suggests that nano-TiO_2 suspended in water could pass though various stages of conventional treatment and filtration, perhaps even microfiltration (Zhang et al., 2007).

Various fate pathway scenarios could be anticipated for nano-TiO_2 post-treatment. For example, nano-TiO_2 might remain in solution as colloidal particles in the water and enter water tanks or reservoirs. If some water were lost from the distribution system via leaks or spills, nano-TiO_2 could end up in surface waters or the subsurface environment. If nano-TiO_2 were to enter ground water aquifers, nano-TiO_2 would presumably persist as a particle, given that other inorganic compounds are not readily broken down in that environment; however, particle size and other characteristics could change. Conceivably, nano-TiO_2 could release, or modify the bioavailability of, other water contaminants of concern.

In another scenario, nano-TiO_2 might settle with floc in the sedimentation step, where it presumably could become part of the sediment sludge and be partially removed from the water with the sludge (AWWA, 2003). The discarded sediment could be transported off-site for disposal or reuse. For example, sludge could be used as cover in municipal solid waste landfills or applied to agricultural or recreational land.

Alternatively, nano-TiO_2 might become part of the filter matrix. Conventional water treatment processes apply filtration following flocculation and sedimentation. U.S. EPA's Filter Backwash Recycling Rule (U.S. EPA, 2001) requires that, when the filter is backwashed, the water be recycled back into the coagulation process. This could reintroduce nano-TiO_2 into the treatment process, but the implications for levels of nano-TiO_2 in finished water are not clear.

If nano-TiO_2 is present in the final drinking water product that reaches the tap, it eventually might enter the ambient environment or be captured by a waste water stream, after which it could reach a waste water treatment plant. If the particular waste water treatment method employed does not remove nano-TiO_2, it is likely to enter downstream water sources.

3.1.2. Sunscreen-Specific

The environmental fate of nano-TiO_2 in topical sunscreens could be affected by the surface treatments and doping applied to nano-TiO_2 particles, by the sunscreen vehicle, or by any number of other constituents in such products (see Appendix B). Nano-TiO_2 in emulsion, dispersion, and possibly powdered form could be present in waste water (e.g., from equipment and site cleaning) and solid waste from sunscreen manufacturing facilities, depending on the trapping and filtration processes the facility uses. In the powdered form, nano-TiO_2 could escape the facility through air venting and filtration systems.

Nano-TiO_2 also could be released to natural water bodies or waste water through bathing or laundry following sunscreen use. Swimming in artificial pools could result in an accumulation of sunscreen material in the water and potential release into the environment as untreated waste water. If nano-TiO_2 remains mobile in water, it could enter downstream water sources in a manner similar to that of the nano-TiO_2 used for drinking water treatment.

Parallels are suggested by recent studies that have detected topical sunscreen constituents in untreated waste water, treated waste water, surface water (lakes and rivers), fish from lakes and rivers, and biosolids (Balmer et al., 2005; Fent et al., 2008; Rodil and Moeder, 2008). The organic compounds detected in these studies were UV filter compounds such as 4-MBC (4-methylbenzylidene camphor) and OC (octocrylene), which generally biodegrade slowly and can bioaccumulate. Some evidence also indicates that nano-TiO_2 can bioaccumulate (Zhang et al., 2006). Although nano-TiO_2 is unlikely to behave exactly the same way as other components of sunscreen, the observed nano-TiO_2 bioaccumulation in fish (Zhang et al., 2006) suggests the possibility of persistent presence of nano-TiO_2. However, no studies to date have documented the occurrence of nano-TiO_2 specifically from sunscreens in waste water or natural water bodies.

3.2. Soil

Three studies were located that address the fate and transport of nano-TiO_2 in soil. Dunphy Guzman et al. (2006) studied the effect of pH on nano-TiO_2 mobility in a model soil column. They found that both surface potential and aggregate size influence transport. In the pH region where electrostatic forces between nano-TiO_2 aggregates and the experimental Pyrex surface should have been strong (pH 2.5 to 5.9), nano-TiO_2 was highly mobile. The calculated interaction energy was expected to be greatest for the largest aggregates at pH 12, but these were the particles that most strongly attached to microchannel surfaces. At pH 3, where conditions were predicted to be favorable for negative/positive

interaction, 84% of the particles were transported. The authors concluded that current transport theory does not adequately predict nanoparticle and aggregated nanoparticle transport. The results suggest that nano-TiO_2 particles and aggregates of nanoparticles in a stable dispersion might be highly mobile in the subsurface over a wide range of conditions. This also raises the possibility that colloid transport mechanisms might be more relevant than particle transport.

Lecoanet et al. (2004) showed that the mobility of aqueous anatase nano-TiO_2 particles in a porous medium was comparable to that of other types of nanoparticles when compared on the basis of particle size. Primary particles of 40-nm diameter were found to be aggregated to a diameter of 198 nm. About 55% was recovered after three pore volumes passed through the column, roughly twice the quantity of ferroxane particles with mean diameter of 303 nm and just more than half the quantity of silica particles with a diameter of 57 nm. After three pore volumes, approximately 95% of the 57-nm silica particles were recovered, compared with 60% of the 135-nm silica particles. Although the results are specific to the experimental protocol, they suggest that particle size affects mobility of nanoparticles and that anatase might be mobile in ground water (Lecoanet et al., 2004).

A recent study using soil samples from 11 sites found that nano-TiO_2 could remain suspended in soil suspensions for 10 days (Fang et al., 2009). Furthermore, the calculated maximum travel distance for some soil samples was more than 30 cm, which suggested that nano-TiO_2 might be transferred to deep soil layers or even to ground water. In general, large soil particles and low ionic strength conditions favor nano-TiO_2 movement, while high clay content, dissolved organic carbon, and salinity conditions favor soil retention of nano-TiO_2.

If nano-TiO_2 enters municipal sewage systems, the plants would separate liquid waste from solid waste and nano-TiO_2 would likely be present in both waste streams. The solid waste, or sludge, could present a route by which nano-TiO_2 could enter soil media, and could be dealt with in a number of ways. In one scenario, the sludge might be sent for land disposal. The ability of TiO_2 to diffuse (and thus be released) from a solid matrix such as sludge is unknown. Nano-TiO_2 and other contaminants such as residual arsenic could become suspended in leachate and enter ground water, or they could pass through a solid waste facility liner into the subsurface.

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

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

Nano-TiO_2 in sludge could be broadly distributed to land used for crops or grazing, where it could enter the food chain, or to high-use areas such as parks, where people and pets could contact nano-TiO_2 in soil or inhale wind-blown material. The nanomaterial could enter runoff and storm water during wet weather events, returning to the aquatic medium. Ecological receptors also could also be exposed to nano-TiO_2 in soil by direct contact with soils or via the food web, including uptake by plants. Because it is an inorganic compound, nano-TiO_2 in soil could be expected to persist, in the same way that conventional TiO_2 is very thermodynamically stable and is unlikely to undergo significant transformation in the environment. Reactivity of nano-sized TiO_2, however, might differ (and is largely unknown at this time) due to its greater surface area-to-volume ratio.

3.2.1. Drinking Water Treatment-specific

One scenario by which nano-TiO_2 specifically used in drinking water treatment could enter soils would be through land application of sludge. In addition to the sludge produced in waste water treatment described above, a sludge material (floc) containing nano-TiO_2 would likely be created in the process of using nano-TiO_2 to treat drinking water. If nano-TiO_2 settles with floc in the sedimentation step, it would likely become part of the sediment sludge. Similarly, as described above, if nano-TiO_2 is present in finished drinking water, it will eventually enter sewage treatment facilities where any residual nano-TiO_2 could also enter the sediment sludge. The discarded sediment would be transported off-site and could be used as cover in a municipal solid waste landfill or used for land application. Either use would result in direct application of nano-TiO_2-contaminated waste to soils. Alternatively, nano-TiO_2 could enter soils if treated water were used to irrigate residential or agricultural plants. These scenarios could have implications for soil microbes and could also be noteworthy in relation to uptake by edible vegetation.

3.2.2. Sunscreen-specific

As described above, nano-TiO_2 in topical sunscreens could end up in the sludge produced at a waste water treatment plant. The disposal of this sludge on land seems likely to represent the primary pathway by which nano-TiO_2 in sunscreen could enter soil.

3.3. Air

Nano-TiO_2 manufacturing facilities could emit such particles to the ambient atmosphere. An occupational exposure study by Berges et al. found that "outside the plant," the airborne TiO_2 particle concentration was approximately 13,000 particles per cubic meter, with nearly 94% of particles 100 nm or less in size, and approximately 52% at 40–60 nm (Berges, 2007, 2008).

Some potential for environmental or occupational atmospheric emissions and releases of nano-TiO_2 presumably exists if the transport or storage containers were to be compromised (e.g., due to a forklift error, train derailment, or truck accident). Also, land application of sludge from either drinking-water or waste-water treatment might also contribute nano-TiO_2 to the atmosphere if dried material were to be re-entrained.

The large surface area of nano-TiO_2 presents an opportunity for other co-occurring contaminants to adsorb onto the surface, potentially changing the physicochemistry of the particle and the behavior and effects of the other contaminant(s). Such interactions have been well documented for particulate matter 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-TiO_2 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-TiO_2 in various environmental media?

3-3. Are available fate and transport models applicable to nano-TiO_2? 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-TiO_2?

3-5. If nano-TiO_2 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-TiO_2?

3-6. How might nano-TiO_2 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-TiO_2 in land-applied sludge to both terrestrial and aquatic organisms? Is bioavailability likely to change when nano-TiO_2 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-TiO_2 cause other unintended substances to form, for example, degradation products, in various environmental media?

3-10. Will nano-TiO_2 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-TiO_2?

3-12. Irradiated photocatalytic nano-TiO_2 is potentially biocidal and antimicrobial. What is the potential for interactions of nano-TiO_2 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-TiO_2 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 TiO_2?

3-14. What is the impact to nutrient and metals cycling and microbial diversity when sludge with nano-TiO_2 is applied to soils?

3-15. How do sunscreen ingredients affect nano-TiO_2 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-TiO_2 in biosolids will become part of the food web and ground water contamination?

3-18. What is the potential for plant uptake of nano-TiO_2 from contaminated soil and irrigation water?

Chapter 4. Exposure–Dose Characterization

This chapter examines the potential for biota and humans to be exposed to nanoscale titanium dioxide (nano-TiO_2) and associated pollutants through various environmental pathways tracing back to the life cycle of two types of applications of nano-TiO_2, water treatment agents and topical sunscreens. Exposure is more than the occurrence of a substance in the environment; actual contact between the substance and an organism must occur. Exposure characterization entails much more than simply identifying the concentration of a substance in the environment. It also involves, for example, various temporal and spatial dimensions, including activity patterns and other complex variables. For nano-TiO_2, even characterizing the primary material of interest, as discussed in Chapter 1, is not a simple matter. Further complications arise when considering the potential for aggregate exposure across multiple routes (e.g., inhalation, ingestion, dermal absorption) and for cumulative exposure to multiple contaminants that derive, either directly or indirectly, from the life cycle of the products in question.

Dose[4] refers to the amount of a substance that enters an organism by crossing a biological barrier such as the skin, the respiratory tract, the gastrointestinal tract, or the eyes. Dose can vary for individuals exposed to the same concentration of a substance. For example, an adult and a child in a room breathing the same air containing a contaminant would both inhale the same contaminant concentration, but the inhaled contaminant quantity and absorbed dose would differ due to differences in physiology (e.g., respiration rates), morphology (e.g., lung volume and surface area), and other variables such as clearance. Dose can also reflect the integration of aggregate exposures across different routes of uptake.

Organisms might be exposed to nano-TiO_2 in the environment at any stage of the product life cycle. In the feedstock and manufacturing process, nano-TiO_2 could be present in the air exhaust, waste-water effluent, and solid waste, if appropriate control technologies are not in use. Nano-TiO_2 in the air can lead to inhalation exposure to organisms in the area. The material could agglomerate or attach to other pollutants and deposit on soil and water surfaces, as well as on animals, whose grooming habits could then result in ingestion of nanomaterials. Nano-TiO_2 in soil could become airborne when the soil is dry 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.

During distribution and storage, nano-TiO_2 could be released accidentally into the environment, and cleaning the contaminated site with water could lead to nano-TiO_2 exposure to both aquatic and terrestrial organisms. The use of nano-TiO_2 in drinking water treatment could result in some level of nano-TiO_2 in water, as described in Chapter 3, and thus potential exposure to human populations as well as biota. The use of sunscreens containing nano-TiO_2 is expected to lead to nano-TiO_2 in waste water after users bathe or shower to remove residual sunscreen on the skin and launder clothes containing traces of sunscreen. Because typical waste water treatment plants currently do not monitor for or specifically target nanomaterials, nano-TiO_2 might not be completely removed by treatment. Therefore, nano-TiO_2 might be present in the effluent and lead to exposure to aquatic species. In the disposal stage, wastes from factories and research facilities containing nanomaterials are often incinerated, possibly releasing nano-TiO_2 into the air. Household wastes containing consumer products with nano-TiO_2 might be incinerated or landfilled; landfilling might lead to nano-TiO_2 leaching into ground water.

Occupational exposure to nano-TiO_2 and associated contaminants (e.g., waste by-products) could occur even with appropriate safety and protective practices. (See Appendix C for a more thorough discussion of occupational exposure control measures.) Such occupational exposures could differ from those of the general public in various ways. For example, workers might more likely be exposed to free nano-TiO_2, whereas the public might more commonly encounter nano-TiO_2 embedded in a product. Exposure durations and concentrations are also likely to be different in occupational settings. Likewise, dose levels could differ between workers and the general population or even between workers in different occupations, depending on factors such as respiration rates in relation to sedentary or strenuous activity in the presence of airborne nano-TiO_2.

4.1. Aggregate Exposure to Nano-TiO_2 from Multiple Sources and Pathways

Nano-TiO_2 is used in various products, raising the possibility that biota and humans could be exposed to nano-TiO_2 from more than one source. Such sources might include water treatment agents, topical sunscreens, cosmeceuticals (traditional cosmetics such as moisturizers and color cosmetics that incorporate active sunscreen ingredients with nano-TiO_2), sun-protective clothing, cleaning agents, air purifiers, coatings, and food packaging, among many others (Woodrow Wilson International Center for Scholars, 2006). Kaegi et al. (2008), for example, reported nano-TiO_2 in water runoff from both new and naturally aged building façades painted with paint containing nano-TiO_2. Hsu and Chein (2007) found that nano-TiO_2 powder-coated materials (wood, polymer, and tiles) under various conditions emitted nanoparticles to the air. Of course, merely the presence of nano-TiO_2 in a product does not mean that

exposure will occur. For example, if nano-TiO_2 is firmly embedded in a product and the product remains intact, little or no exposure to nano-TiO_2 might actually occur.

A hypothetical scenario for aggregate exposure to nano-TiO_2 in both water and sunscreen could involve a person's ingesting the water (oral route), bathing (dermal) or showering (dermal and inhalation) in it, applying sunscreen lotion to the skin (dermal), ingestion of sunscreen through hand-to-mouth contact (oral), or uptake from hand-to-eye (ocular) contact. The latter two exposures pathways are particularly relevant for young children. Biota also could be subject to aggregate exposures. A fish, for example, could take up nano-TiO_2 that originated from a waste water treatment facility and could also ingest prey whose contamination originated from ambient water, sediment, or other biota containing sunscreen constituents. The seemingly widespread occurrence of nanoparticles of various types in aquatic media reported by Wigginton et al. (2007) lends plausibility to these scenarios.

4.2. Cumulative Exposure to Nano-TiO_2 and Other Contaminants

Nano-TiO_2 is not the only substance relative to the life cycle of products containing nano-TiO_2 to which biota and humans could be exposed. As noted in Chapter 2, releases of other contaminants might also occur during various stages of the product life cycle, particularly waste materials during feedstock processing and during manufacturing of the primary product. Such waste materials are not necessarily nanoscale in size. As described in Chapter 3, if wastes are released into the environment, they could undergo transformation, potentially resulting in even more types of contaminants; they might also be transported to other locations, e.g., downstream or downwind.

The creation of secondary contaminants through transformation processes in various environmental media also raises the possibility of exposure to substances indirectly related to nano-TiO_2. Many nanoparticles, including nano-TiO_2, tend to bind transitional metals and organic chemical pollutants (Nagaveni et al., 2004; Pena et al., 2006). With a tendency to adsorb pollutants and an ability to penetrate the body and cells (see sections 4.6.1 Inhalation, 4.6.3 Ingestion, and 4.6.4 Blood-Brain Barrier and Placental Transfer), nano-TiO_2 could carry toxic pollutants to sites where the pollutants would not normally go (Moore, 2006). This type of "Trojan horse" effect could result in increased uptake of other pollutants or interactive effects that would otherwise not occur if these substances were only present individually.

Increased uptake of other pollutants in the presence of nano-TiO_2 has been reported by Sun et al. (2007) and Zhang et al. (2007; 2006) (see Table 4-1). Sun et al. (2007) demonstrated that arsenic as arsenate [As(V)] strongly binds to AEROXIDE® P25 (P25) in water and that carp exposed to water containing 10 milligrams per liter (mg/L) of this photocatalytic nano-TiO_2 and 200 micrograms per liter

(μg/L) arsenate accumulated more arsenic than fish exposed to either nano-TiO_2 or arsenic alone. The bioconcentration factor of arsenic[5] was more than twice as high when nano-TiO_2 was present than when it was not (Sun et al., 2007). The tested arsenate concentration, 200 μg/L, is environmentally relevant, given that higher total arsenic concentrations (mainly inorganic arsenic in the forms of arsenite and arsenate) in drinking water have been reported in many countries, including Bangladesh, China, Chile, and India (Basu et al., 2004; Feng et al., 2001; Moore et al., 1997; Tian et al., 2001). The presence of nano-TiO_2 did not alter the distribution of arsenic within fish tissues. Over various time intervals, arsenic and TiO_2 accumulated significantly in the intestine, stomach, and gills, and to a lesser degree in liver, skin, and scales; the least accumulation occurred in muscle. Because the accumulation of arsenic was much greater in the presence of nano-TiO_2, Sun et al. (2007) concluded that adsorption to nano-TiO_2 facilitated arsenic transport and uptake.

Table 4-1. Tissue concentrations of various pollutants in fish after exposures to nano-TiO_2 in water.

Test Species	Material	Protocol (no UV illumination, unless specified)	Study Outcome	Reference
Fish (carp, *Cyprinus carpio*)	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, *Cyprinus carpio*)	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 μg/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, *Cyprinus carpio*)	21-nm primary particle, BET 50 m^2/g (P25) (photocatalytic)	Up to 25-day exposure to ~97 μg/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, *Oncorhynchus mykiss*)	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 (μg/g dry weight) / arsenic concentration in water (μg/L).

Zhang et al. (2007) showed that nano-TiO_2 (21 nm) also enhanced cadmium uptake in carp. After 20 days of exposure, the bioconcentration factor for whole-body cadmium was 64.4 in carp exposed to cadmium alone, but reached 606 in carp exposed to both cadmium and nano-TiO_2. Natural sediment particles (19 μm) did not increase cadmium uptake. Both nano-TiO_2 and sediment particles adsorb cadmium and reach equilibrium within 30 minutes, but nano-TiO_2 adsorbed more than 5 times as much cadmium as the sediment particles. Based on the facts that nano-TiO_2 can adsorb cadmium and that concentrations of cadmium and nano-TiO_2 are positively correlated, the authors suggested that increased cadmium uptake in the presence of nano-TiO_2 may have been due to accumulation of cadmium adsorbed on nano-TiO_2 (i.e., facilitated transport).

Zhang et al. (2007) also found that carp exposed to cadmium in water (at approximately 97 μg/L) along with 10 mg/L photocatalytic nano-TiO_2 accumulated more cadmium than fish exposed to either nano-TiO_2 or cadmium alone (Table 4-1). After 25 days of exposure, cadmium concentration in the whole fish was 9.07 μg/g in the cadmium-only group and 22.3 μg/g in the cadmium-plus-nano-TiO_2 group, indicating a 146% increase in the cadmium bioconcentration factor in the presence of nano-TiO_2. When carp were analyzed after 20 days of exposure, cadmium concentrations in all groups were higher in internal organs than in gills, muscle, and skin and scale (Zhang et al., 2007). Unlike nano-TiO_2, natural sediment particles (at equivalent concentrations) did not affect cadmium bioaccumulation. The authors also reported a positive correlation between nano-TiO_2 concentration and cadmium concentration in the carp, and found high nano-TiO_2 concentrations in the gills. The increases in cadmium bioaccumulation could be due to increased transport of cadmium into carp via adsorption to nano-TiO_2. The transport routes could be from water onto the gill surfaces or from consumed food into internal organs. Toxicity was not measured in this study.

The fact that organic disinfection by-products can be formed by the photocatalytic oxidation of conventional TiO_2 in treating drinking water (Richardson et al., 1996) suggests the possibility that nano-TiO_2 could have the same effect. Richardson et al. (1996) compared the organic disinfection by-products detected after using (1) chlorine as the sole disinfectant and (2) TiO_2/ultraviolet (UV) light treatment followed by chlorination. The authors reported detecting an additional by-product (tentatively identified as dihyro-4,5-dichloro-2(3H)furanone) after the combined TiO_2/UV and chlorine treatment compared to chlorine treatment alone. Overall, however, the numbers and concentrations of chlorinated disinfection by-products were lower after combined TiO_2/UV and chlorine treatment than after chlorination alone.

Cumulative exposure to nanomaterials could also occur. Some consumer products contain more than one type of nanomaterials, e.g., nano-TiO_2 and nano-silver have been used together in multiple products (The Project on Emerging Nanotechnologies, 2009).

4.3. Models to Estimate Exposure

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

Although empirical data on nano-TiO_2 concentrations in the environment are currently lacking, a recent study used computer modeling to predict nano-TiO_2 concentrations in different environmental media. Using limited data from published literature and various assumptions, researchers in Switzerland developed models to estimate predicted environmental concentrations (PEC) and predicted no-effect concentrations (PNEC). PEC values were calculated for "realistic exposure scenarios" (based on nano-TiO_2 use, estimated as 25 tons per year in Switzerland) and for "high exposure scenarios" (based on 500 tons per year). The authors estimated that more than 60% of nano-TiO_2 is used in cosmetics, including sunscreen, and that most of it is discharged into wastewater. To estimate PNEC, the lowest no-observed-effect concentration [based on a published study on acute toxicity to *Daphnia* by Hund-Rinke and Simon (2006)] was divided by an assessment factor of 1000, in accordance with the Technical Guidance Document on Risk Assessment published by European Chemicals Bureau, because, as the authors noted, the "accuracy of the data was low" (European Chemicals Bureau, 2003; Mueller and Nowack, 2008). The PEC of nano-TiO_2 in water was 0.7 µg/L ("realistic scenario") or 16 µg/L ("high scenario"), compared to a PNEC of <1 µg/L (for daphnia). The authors (Mueller and Nowack, 2008) stated that, given that the PEC is close to or greater than the PNEC, European Union authorities would consider the substance "of concern" and call for more data to validate the result (Umwelt Bundes Amt, 2009).

4.4. Biota

Various scenarios and ways in which nano-TiO_2 from water treatment agents and topical sunscreens could enter different environmental media were described in Chapters 2 and 3. In this section, some of

these scenarios are explored further in relation to exposure of aquatic and terrestrial organisms to nano-TiO_2 under various conditions. Also discussed are the potential for bioaccumulation and entry of nano-TiO_2 into the food web.

4.4.1. Aquatic

Data on sediment concentrations of nano-TiO_2, whether in a laboratory or a natural environment, are limited. Nano-TiO_2 concentrations could be higher at the sediment surface than in the water (Handy et al., 2008b). Settling of nano-TiO_2 aggregates (with nano-TiO_2 or with organic matter) would increase nano-TiO_2 exposure to benthic and benthopelagic species, such as mussels, sea cucumbers, marine worms, flatfish, and other species that sometimes feed at the bottom of water bodies. At the same time, settling decreases nano-TiO_2 concentrations in the water column and would be expected to decrease exposure to suspension feeders (such as *Daphnia*) and animals that live in or drink the water.

Nanoparticles can also deposit or aggregate on the surfaces of aquatic organisms. Surface aggregation can be caused by the slower flow near the interface between liquids and solids or by the viscous properties of the surface of an organism (Handy et al., 2008b). Surface deposition or aggregation can result in a higher concentration of nano-TiO_2 on the organism's surface than in the water, and might cause toxicity even if the nano-TiO_2 does not enter the cells. Surface-acting metal toxicity of nano-TiO_2 has been suggested as a cause of gill damage in rainbow trout where the titanium concentration in gill tissue was not increased (Federici et al., 2007).

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

4.4.1.1. Bioaccumulation

Zhang et al. (2006) found that nano-TiO_2 can accumulate internally in carp (Table 4-1). The authors exposed carp to photocatalytic nano-TiO_2, or P25 for up to 25 days. Before dissection and TiO_2 analysis, carp were rinsed and wiped. The nominal concentrations of nano-TiO_2 in the water were 3 and 10 mg/L (based on the amount of stock nano-TiO_2 suspension added to the fish tank), and the authors reported that nano-TiO_2 concentrations were 2 and 7 mg/L after 24 hours, with most of the decreases occurring within 4 hours after the addition of stock solution. The TiO_2 concentration in carp tissue increased rapidly over the first 10 days and then more gradually between day 10 and day 25. TiO_2 concentrations were highest in visceral organs, distantly followed by gills, and then closely followed by

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

In contrast to the finding of bioaccumulation of nano-TiO_2 in carp that Zhang et al. (2006) reported, Federici et al. (2007) detected no accumulation in trout exposed to up to 1 mg/L nano-TiO_2 for 14 days. Although the findings appear contradictory, each study might simply reflect the results of the specific test conditions. For instance, the rainbow trout were exposed to lower concentrations of nano-TiO_2 than were the carp. The Federici et al. (2007) study used photocatalytic nano-TiO_2 (P25), and 80% of the water in the fish tank was changed every 12 hours. Similar to Zhang et al. (2006), Federici et al. (2007) reported that more than 85% of the initial nano-TiO_2 concentrations in the tank water remained after 12 hours. Other environmental factors, such as water temperature at 14 °C for trout and at 23 °C for carp, could influence the behavior or effects of nano-TiO_2 and contribute to the difference between these two studies. Furthermore, carp feed mainly by grubbing in sediments, and therefore also could be exposed to settled nano-TiO_2 aggregates, to which rainbow trout might not be exposed.

Although nano-TiO_2 can bioaccumulate in fish, the uptake mechanism is not clear. Substances in water can enter fish through waterborne exposure (through gills and then into blood through absorption), dietary uptake, or cutaneous absorption. Handy et al. (2008a) suggested that the absorption of nano-TiO_2 on the gill surface into the blood might be slow or uncertain, but that nano-TiO_2 on the gut surface might be taken into cells by endocytosis. Although intact fish skin is unlikely to be permeable to nano-TiO_2, these authors proposed that cutaneous uptake of nano-TiO_2 might be possible if the skin is infected or inflamed (Handy et al., 2008a). Handy et al. (2008a) did not provide experimental data to support nano-TiO_2 uptake through endocytosis, but a recent in vitro study indicated that an endocytosis inhibitor, Nystatin, decreased the mutation frequencies induced by exposures to 5-nm and 40-nm nano-TiO_2, but not 325-nm TiO_2, in mouse embryo fibroblasts, implying that endocytosis is involved in modulating cellar response to nano-TiO_2 exposure (Xu et al., 2009a). The concentration of nano-TiO_2 or Ti in cells was not measured (Xu et al., 2009a).

4.4.2. Terrestrial

Terrestrial organisms could be exposed to nano-TiO_2 under various scenarios. For example, spillage during shipping or storage, including breaching of containers by vermin, could result in contact by microbial, invertebrate, and vertebrate species. Plants could be exposed by taking up water containing nano-TiO_2 or by growing in soil that contains nano-TiO_2, for example, as a result of application of sludge from water treatment facilities. No empirical data on the potential for such exposures to terrestrial organisms have been located.

4.4.3. Food Web

Nano-TiO_2 could enter the food web at various levels, depending on the point and extent of its release to the environment. If nano-TiO_2 were dispersed in water, for example, it could be taken up by algae, which are primary producers in ecosystems. Many invertebrates, which are primary consumers in aquatic systems, eat algae and in turn are consumed by larger animals such as fish. A common aquatic invertebrate is the water flea (genus *Daphnia*), which is a small crustacean filter feeder (also known as suspension feeder). Daphnids use their legs to generate water flow and use the comb-like setae on their thoracic limbs to strain or catch smaller organisms (such as algae) for consumption. Because daphnids have been reported to filter up to 120–160 mL each per day (Vanoverbeke, 2008), they could be exposed to quite high numbers of nanoparticles in water (Griffitt et al., 2008). Even if nano-TiO_2 is not absorbed into tissues, nano-TiO_2 in the digestive tract of daphnids could still contribute to bioaccumulation in the food web.

4.5. Humans

As noted at the beginning of this chapter, exposure is a complex function of not only the amount of a substance in the environment but also various temporal and spatial dimensions of contact with the substance. At this early stage of investigation and understanding of human exposure to nano-TiO_2, however, even basic information on the potential for and amount of human contact with this material is limited. Moreover, exposure characterization encompasses not just the primary material but the secondary waste and transformation products related to the entire life cycle of nano-TiO_2 in various applications. These indirect and secondary aspects of exposure are even less well understood and therefore not discussed here. Their potential significance, however, should not be discounted.

The potential for human exposure to nano-TiO_2 depends first on the production and use of this material in the applications under consideration here. Generally, exposure related to life-cycle stages leading up to actual use appears more likely to occur in occupational situations, whereas exposure related to the use and disposal stages of the life cycle could occur in either occupational or non-occupational settings. Although not absolute, this distinction provides a basis for discussing exposure with reference to either the general population or the occupational population, both of which are essential in examining the broad implications of nano-TiO_2 in water treatment and topical sunscreens.

4.5.1. General Population

4.5.1.1. Water Treatment-specific

Although the actual use of nano-TiO_2 in water treatment facilities appears to be limited at present to pilot testing (see Section 2.4), the potential for general population exposure to nano-TiO_2 *if it were to be used widely* could involve sizeable numbers of people, given the number of U.S. community water suppliers that currently treat drinking water to reduce arsenic levels. As discussed in Section 2.4.1, such water suppliers serve roughly 13 million people in the United States alone.

If nano-TiO_2 were present in potable water, exposure could involve more than just ingesting the water. Such water could be used for bathing, including showering, which could imply exposure not only by dermal contact but by inhalation of water droplets and even contact through the eyes. Also, the general population includes infants and other individuals who could have relatively greater exposure to water and thus possible vulnerability if the water were contaminated. For example, on a body weight basis, 1- to 3-month-old infants consume far more water directly and indirectly than 18- to 21-year olds. The 90th percentile consumption rate is 151 milliliters per kilogram per day (mL/kg/day) for these infants versus 17 mL/kg/day for the older age group [see Table 3-9 in (U.S. EPA, 2008a)]. Children also have a greater water intake while swimming, so they may be more vulnerable to contaminated water in that respect as well (U.S. EPA, 2008a).

4.5.1.2. Sunscreen-specific

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

Topical sunscreens are available as traditional lotions, in spray-on form, and as wipes (Jeffries, 2007). Nano-TiO_2 sunscreen powders are also available, according to the Woodrow Wilson Center's nanotechnology consumer product inventory (Woodrow Wilson International Center for Scholars, 2006). Another sun protection option available to consumers is "cosmeceuticals," cosmetics that incorporate active sunscreen ingredients (Davis, 1994). In the mid-1990s, up to 30% of lipsticks and 20% of makeup were estimated to have sunburn protection factor (SPF) ratings, sunscreen claims, or both (Davis, 1994). Other products with active sunscreen ingredients include hair care products (e.g., hair spray, gel, mousse,

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

For the general population, the principal exposure route to nano-TiO_2 in sunscreen is through the skin. When sunscreen is applied by spray, inhalation presents another route, although it is not clear that the primary nanoparticles as such would be inhaled. Ingestion is also conceivable through hand-to-mouth contact and mucociliary clearance of inhaled nano-TiO_2.

Dermal Exposure

Potential nano-TiO_2 dermal exposure from sunscreen use can be estimated by the amount of applied sunscreen. Although the recommended sunscreen application rate is 2 milligrams per square centimeter (mg/cm^2) of skin (roughly 1.5 ounces or 3 tablespoons for the entire body of an average adult), most consumers use 0.5 to 1.5 mg/cm^2 skin (Srinivas et al., 2006). Assuming sunscreen is applied to all areas of skin exposed to sun on a day at the beach or exposed to water while swimming, an adult would use an estimated 10–46 g sunscreen per application, and a 3-year old would use an estimated 3–15 g sunscreen per application (Table 4-2). Assuming that a sunscreen contains 5% nano-TiO_2 (the mass percent concentrations of nano-TiO_2 in sunscreens range from 2% to 15%, see Table A-1 in Appendix A), the amounts of nano-TiO_2 applied on the skin could range from 0.5 to 2.3 g per person per application for an adult, and 0.17 to 0.76 g per person per application for a 3-year old (Table 4-2). Sunscreens, including the water-resistant or water-proof types, should be reapplied every 2 hours, regardless of the SPF values. Exposure to nano-TiO_2 from sunscreen could range from 1.0 to 4.6 g for an adult and 0.33 to 1.5 g for a 3-year old for a half day at the beach (2 applications in 4 hours). As shown in Table 4-2, the ranges of applied nano-TiO_2 would be 12–55 mg per kg of body weight per application for a 3-year old and 8.0–37 mg per kg of body weight per application for an adult. This relatively higher exposure in young children could be noteworthy in relation to indications that the skin of infants and young children might have less barrier function than matured skin (Hostynek, 2003), although this contrasts with another report indicating that human skin is mature both structurally and functionally at 2–3 weeks of age (Makri et al., 2004). Although not everyone applies sunscreen at the recommended dose and frequency in real life, parents reported greater use of sunscreen on their children than on themselves (Weinstein et al., 2001).

Table 4-2. Estimated dermal exposure to nano-TiO_2 from sunscreen containing 5% nano-TiO_2 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 (50th 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 (95th 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 (50th 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 (95th 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-TiO_2 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

Consumers could inhale water aerosol while showering or from nebulizing room humidifiers. Spray sunscreen products also present an inhalation exposure scenario. For such products and for water containing nano-TiO_2, the characteristics of the resulting aerosol have not been documented in the published literature. Section 4.5.2 discusses inhalation exposure to nano-TiO_2 for several occupational scenarios.

Oral Exposure

Nano-TiO_2 from sunscreen could be ingested by accident or as a result of routine hand-to-mouth contact (from residual sunscreen on hands), particularly for young children. If nano-TiO_2 were inhaled, mucociliary clearance could lead to uptake through the gastrointestinal tract. Although no estimates of this type of nano-TiO_2 exposure are available, dietary intake of all sizes of TiO_2 from all sources (food, pharmaceuticals, etc.) has been estimated. The estimation was based on 7-day food diaries and records of pharmaceutical, dietary supplement, and toothpaste use of 182 people in the United Kingdom. The amounts of TiO_2 were calculated or estimated from product labels (the listing of food-additive TiO_2 is required by British law in most foods), manufacturer reports, and laboratory testing. The total median

dietary intake of nano-TiO_2 and micro-TiO_2 (0.1–3 μm) was estimated to be 2.5 mg per individual per day (Lomer et al., 2004). Food was the main source of dietary TiO_2, followed by pharmaceuticals, dietary supplements, and toothpaste. Individual TiO_2 intake varied widely (0–112 mg per individual per day), and no particle size information was provided.

4.5.2. Occupational

Nearly every stage of the life cycle for the applications considered here presents some potential for occupational exposure to nano-TiO_2. Moreover, no exposure route can be ruled irrelevant to these workers. Thus, assessing occupational exposure is essential to completing a CEA of nano-TiO_2 in either water treatment agents or topical sunscreens. As a frame of reference, NIOSH (2005) proposed a draft occupational exposure limit of 1.5 milligrams per cubic meter (mg/m^3) for fine TiO_2 (less than 2.5 μm in size) and 0.1 mg/m^3 for ultrafine TiO_2 (less than 0.1 μm [100 nm]).

Most information on workplace TiO_2 exposure relates to the production of conventional TiO_2, not nano-TiO_2 specifically. Additionally, given that nano-TiO_2 tends to agglomerate or aggregate, occupational exposure conditions for nano-TiO_2 could involve both nanoscale and larger than nanoscale TiO_2 particles. The manufacturing stage of the life cycle comprises multiple processes that might vary in exposure characteristics. An epidemiologic study conducted in four U.S. TiO_2 manufacturing factories indicated that occupational exposure to TiO_2 is greatest during bagging, milling/micronizing, and internal recycling (shoveling spilled material from the floor into the processing bins) stages (Fryzek et al., 2003).

The manufacturer of P25 has stated on its Web site that workplace inhalation exposures to TiO_2 are typically less than 0.5 mg/m^3 (Degussa, 2007). The Web site also indicated that photocatalytic P25 production occurs in a closed reactor, which presumably limits exposure. The highest exposures the manufacturer reported were less than 0.5 mg/m^3 and occurred during the packaging step, which is also enclosed. This manufacturer is said to require the use of personal protective equipment during any repair work that could lead to dust exposure (Maier, 2007). Such information suggests limited potential for inhalation exposure during P25 manufacturing, but it does not address other routes such as dermal exposure or incidental ingestion from hand-to-mouth contact.

Another manufacturer of nano-TiO_2 products reported that air concentrations in production areas for DuPont™ Light Stabilizer 210 and 220 (which protects plastic from UV damage) were less than 2 mg/m^3, and in most cases were lower than the detection limit of 0.3 mg/m^3 (size not specified) (DuPont, 2007). No exposure data were available for the material incorporation, packing, and product fabrication areas. Although occupational exposure was stated to be low (DuPont, 2007), the detection limit (0.3 mg/m^3) is above the draft NIOSH recommended limit for ultrafine or nano-TiO_2, 0.1 mg/m^3 (NIOSH, 2005).

Preliminary estimates of workplace exposure in a factory that produces rutile nano-TiO_2 for sunscreen and cosmetics were reported by Berges (2007, 2008). Measurements were made in 2006, and then in 2007, when improvements to local exhaust systems were in operation (Berges, 2007, 2008). In 2007, the TiO_2 in the "inhalable" dust mass concentration at the bin filling station was 0.014 mg/m^3, and the TiO_2 in the "respirable" dust mass concentration was 0.004 mg/m^3. [*Inhalable* refers to all particles that can enter the respiratory tract through the nose or mouth (e.g., up to about 100 μm); *respirable* refers to particles that penetrate to the alveolar (pulmonary) region with a mass median aerosol diameter (MMAD) of about 4 μm (European Committee for Standardization, 1993).] In the bag filling area in 2007, the TiO_2 inhalable fraction was 0.028 mg/m^3, and the respirable fraction was 0.022–0.042 mg/m^3. Personal sampling in 2007 over a 4.87-hour period measured 0.010 mg/m^3 TiO_2 in the respirable fraction.

Liao et al. (2009) further reported and analyzed the Berges (2007, 2008) data, as well as data from several other sources to model the occupational exposure and characterize risk. In the bin filling area of the facility studied by Berges (2007, 2008), the total airborne TiO_2 particle number concentrations ranged from 15,000 to 156,000 particles/cm^3, with a measured size range of 14–673 nm. More than 97% of the particles were 100 nm or less in size, and 60% were 20–30 nm. After a leak was sealed, the high-end concentration decreased to less than 29,000 particles/cm^3. Near the leak, the particle surface area concentrations reached 200 square micrometers per cubic centimeters ($\mu m^2/cm^3$) for "alveolar deposited" particles and 50 $\mu m^2/cm^3$ for "tracheobronchial deposited" particles. Under normal operating conditions, the particle surface area concentrations were 50 $\mu m^2/cm^3$ for the alveolar deposited particles and 13 $\mu m^2/cm^3$ for the tracheobronchial deposited particles. Outside the plant, the airborne TiO_2 particle concentration was approximately 13,000 particles/cm^3. Among other things, their model indicated that the highest TiO_2 burdens in terms of lung surface area of packers were 0.174 m^2 (anatase) and 0.122 m^2 (rutile) for particles sized 10–20 nm. For particle sizes 80–300 nm, the burdens were 0.002 m^2 (anatase) and 0.0017 m^2 (rutile). So-called surface treatment workers (involved in drying, packing, and blending operations) had a higher TiO_2 burden in the lung surface area. For particles 10–20 nm, the burdens were 0.40 m^2 (anatase) and 0.28 m^2 (rutile).

Using exposure data specific to particle size in the workplace from the Berges (2007, 2008) reports as well as conventional TiO_2 studies (Boffetta et al., 2004; Fryzek et al., 2003), Liao et al. (2009) used computer modeling to calculate that exposures to nano-TiO_2 (expressed as particle surface area concentrations) were 0.1685 m^2 TiO_2 per 300 m^3 air (working space volume) for packers and 0.387 m^2 TiO_2 per 300 m^3 air for surface treatment workers. For nano-TiO_2 in the 10- to 50-nm size range, the airborne concentrations (expressed as particle surface area concentrations) were higher in anatase nano-TiO_2 than in rutile nano-TiO_2 for both packers and surface treatment workers. The highest airborne concentration was anatase for surface treatment workers, followed in order by rutile for surface treatment workers, anatase for packers, and rutile for packers.

Liao et al. (2009) also modeled the dose-response relationships from in vitro cytotoxicity studies of human dermal fibroblasts and inflammatory responses of human lung epithelial cells. They then compared exposure levels to the dose-response functions and concluded that packers and surface treatment workers at the studied location were "unlikely to [be at] substantial risk [of] lung inflammatory response, [but they] have significant risk [of] cytotoxicity response at relatively high airborne TiO_2 anatase NP [nanoparticle] concentrations at size 10-30 nm" (Liao et al., 2009).

In a presentation at a professional conference, Li et al. (2008b) displayed photographs of a factory that mixed, but did not manufacture, nano-TiO_2. The photographs appeared to show that nano-TiO_2 was stored in shipping bags piled on pallets. White powder was visible on the facility floor, but its composition is unclear as the factory also handled conventional "pigmentary grade" and "food grade" TiO_2 (Ichihara, 2009). Li et al. (2008b) reported that workers had been given masks and shirt-like protective clothing but that the masks were not always worn. The authors also noted that shirt-like protective clothing provided no protection for the forearms and legs of the workers, many of whom wore short-sleeved tops and shorts. Although this factory may not be representative, it illustrates how inhalation and dermal exposure might occur during the manufacturing or mixing process.

As noted in Section 2.3, nano-TiO_2 is routinely shipped in paper bags, which could be a source of exposure if they were to be ruptured, punctured, or otherwise compromised during distribution or storage. Nano-TiO_2 in dispersion form shipped in pails, drums or totes (Klaessig, 2008) could be subject to accidents resulting from forklift errors, train derailments, and truck accidents, but no empirical data on such incidents specifically related to nano-TiO_2 are available.

The above information suggests that inhalation and dermal exposure could occur during manufacturing, packaging, shipping, and storage of nano-TiO_2. Without incidence and concentration data, however, the potential for and nature of worker exposure cannot be characterized.

4.6. Dose

Dose is defined as the amount of a substance that actually enters an organism by crossing a biological barrier. Uptake of nano-TiO_2 by different routes has been investigated in various species. Table 4-1 in Section 4.2 summarizes several studies that measured tissue concentrations in fish that had been exposed to nano-TiO_2 in water. The exposures included, but were not necessarily limited to, nano-TiO_2, appropriately reflecting the multiple substances to which fish can be exposed in the natural environment. For terrestrial organisms, including laboratory animals used for toxicological studies and as models for human health effects, the route of exposure is important in determining the dose that actually enters the body, hence information on uptake of nano-TiO_2 is presented here according to the route of

uptake, i.e., inhalation, ingestion, or dermal. Additionally, this section discusses special biological barriers (blood brain barrier and placenta), and issues related to dose-metrics for nano-TiO_2.

4.6.1. Respiratory (Inhalation and Instillation)

Animal studies have shown that inhaled or instilled nano-TiO_2 can translocate into the interstitium of the lung, lymph nodes (Ma-Hock et al., 2009; Oberdörster et al., 1992; Oberdörster et al., 1994), blood (Geiser et al., 2005), and the brain (Wang et al., 2005; Wang et al., 2008b; Wang et al., 2007a).

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

Intranasal instillation of three sizes of nano-TiO_2 particles (approximately 20, 70, and 155 nm) resulted in increased titanium concentrations in the olfactory bulb of mice (Wang et al., 2005, 2007a). Also, two forms of nano-TiO_2 particles (80-nm rutile and 155-nm anatase) were found to increase Ti concentrations in the hippocampus, central cortex, and cerebrum, in addition to olfactory bulb, in mice after repeated intranasal instillation (Wang et al., 2008b). The authors noted that the fact that brain tissue Ti concentrations were higher than lung tissue concentrations suggested that the olfactory nerve was the path of transport in this study.

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

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

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

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

Inhaled nano-TiO_2 persisted in the lung longer than fine TiO_2 in rats (Oberdörster et al., 1994). After 12 weeks of inhalation (6 hours/day, 5 days/week) of approximately equivalent mass concentrations of fine TiO_2 (22.3 ± 4.2 mg/m^3) and nano-TiO_2 (23.5 ± 2.9 mg/m^3), the total retained lung burdens were 6.62 ± 1.22 mg for fine TiO_2 and 5.22 ± 0.75 mg for nano-TiO_2. The estimated retention half-times were 174 days for fine TiO_2 and 501 days for nano-TiO_2 (Oberdörster et al., 1994).

In animal studies of nano-TiO_2 disposition (Table 4-3), 13 weeks of inhalation exposure to nano-TiO_2 increased TiO_2 burden in lymph nodes in rats (2 and 10 mg/m^3), mice (10 mg/m^3), but not in hamsters (at up to 10 mg/m^3) (Bermudez et al., 2004).

Table 4-3. Nano-TiO_2 disposition in animals after inhalation or intratracheal instillation of nano-TiO_2.

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-TiO_2 disposition in animals after inhalation or intratracheal instillation of nano-TiO_2 (continued).[a]

Species/strain	Aerosol	Study Protocol	Observations	Reference
Wistar rats, 20 adult males, 250±10 g	TiO_2 (22-nm CMD, σ_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, σ_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, σ_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

Because sunscreen is used on the skin, human skin penetration of nano-TiO_2 (as particles in vehicles or in sunscreens) has been discussed in several reports and reviews (NANODERM, 2007; Nohynek et al., 2007; TGA, 2006). Most dermal exposure studies reviewed used human skin and pig skin; several were in vivo studies in humans. Compared to other routes of exposure, dermal exposure may be more directly relevant in assessing potential health effects associated with its use in sunscreens, at least for unflexed skin from healthy adults.

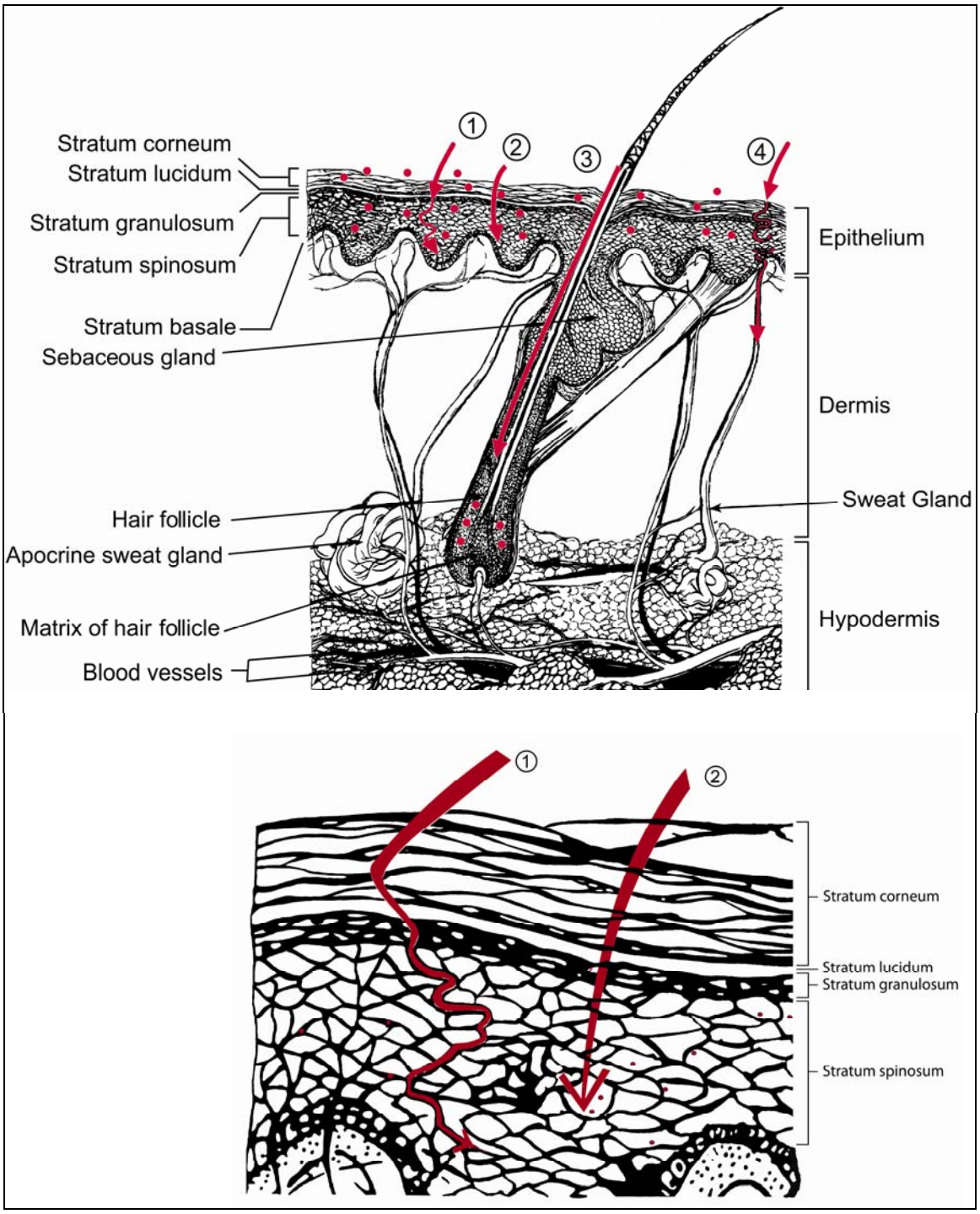
Because of the relatively noninvasive nature of skin penetration testing, several laboratory studies have focused on skin absorption in humans, rather than animals. Human skin regulates the penetration of contaminants primarily through the stratum corneum layer, which contains keratinized cells and has no blood vessels. The thickness of the layer varies, ranging from hundreds of micrometers to several micrometers in different parts of the body. Published studies indicate the stratum corneum of full-term infants and babies is comparable to that of adults (Fairley and Rasmussen, 1983); such is not the case with pre-term infants (Kalia et al., 1998). Skin studies include a range of experimental conditions, including in vivo and ex vivo / in vitro. With few exceptions discussed below (Kertész et al., 2005; Menzel et al., 2004; Sadrieh et al., 2008), most of these studies (Table 4-4) found clear evidence that nano-TiO_2 does not

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

In healthy human skin, topically applied nano-TiO_2 penetrates only into the upper layers of the stratum corneum (Table 4-4). The pathways of skin penetration can include intracellular penetration, intercellular penetration, and penetration through hair follicles (Figure 4-1) (Nohynek et al., 2007). Penetration through sweat glands has not been reported, according to one source (page 29 of (NANODERM, 2007)). Although increased skin penetration of other nanomaterials has been reported in flexed skin (Zhang and Monteiro-Riviere, 2008) and in UV-exposed skin (Mortensen et al., 2008), studies of skin penetration in healthy flexed skin or damaged skin are still underway for nano-TiO_2.

Nano-TiO_2 was observed in some hair follicles (Lekki et al., 2007), but did not reach the living follicle cells. The presence of nano-TiO_2 in hair follicles is most likely due to mechanical force, such as the movement of the hair during sunscreen application. Nano-TiO_2 in hair follicles might contribute to increased Ti levels in the dermis (Sadrieh et al., 2008) because parts of hair follicles are in the dermis. Nanoparticle loss from hair follicles is expected to be slow because the elimination occurs only by its flowing out with sebum or by its being pushed out with sebum. In a study using a hydrogel formulation containing fluorescence-labeled nanoparticles (Resomer RG 50.50 H, poly(lactide-co-glycolide) on human skin (Mittal and Ravi Kumar, 2009), approximately 15% of total nanoparticles detected in hair follicles 30 minutes after application remained in the hair follicle for 10 days, which is at least 10 times longer than particles remain in the stratum corneum (Lademann et al., 2006).

In human skin that is diseased, nano-TiO_2 might penetrate more deeply. The only available study of nano-TiO_2 on skin with dermal lesions was completed on psoriatic skin. Psoriatic skin is a symptom of a chronic, and possibly immune-mediated or genetic, disease called psoriasis. Unlike normal skin cells, which mature and are shed in 28 to 30 days, psoriatic skin cells mature in 3 to 4 days, accumulate on the skin surface (instead of shedding, because new skin develops faster than dead skin sheds), and develop into patches of dead skin (National Psoriasis Foundation, 2006; Pinheiro et al., 2007). Psoriatic skin has a looser corneocyte organization than healthy skin due to the loss of stratum corneum cohesion (Pinheiro et al., 2007). In the Pinheiro et al. (2007) study, nano-TiO_2 in a sunscreen formulation penetrated into deeper areas of the stratum corneum in psoriatic skin than in healthy skin, but not into living cells in 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-TiO_2 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-TiO_2 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 TiO_2 skin absorption/penetration studies.[a]

Test Material		Skin Model [b] (Sampling Technique)	Results	Reference
Sunscreen Formulations Containing Nano-TiO_2				
Nano-TiO_2 in a sunscreen formulation	Primary particle 17 nm (Kemira, 2000), rutile, Al_2O_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 TiO_2 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-TiO_2 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_2O_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 TiO_2 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_2O_3-, Al_2O_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 TiO_2 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 caster 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)
TiO_2/Nano-TiO_2 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.

Mortensen et al. (2008), working with quantum dots rather than TiO_2, reported greater skin penetration following UV exposure and suggested that even mildly sunburned skin might be more susceptible to penetration by nanoparticles of similar size and chemistry to the quantum dots used in their study.

Using "microfine" TiO_2, Tan et al. (1996) compared uptake in skin samples from 13 elderly persons (age 59–82 years) with samples from 6 control cadavers (used to determine background exposure). The authors reported some dermal uptake, although they suggested caution when interpreting their results, citing the advanced age of their participants, the fact that skin samples were taken from different locations, and the fact that TiO_2 concentrations were close to analytical detection limits. Kertész et al. (2005) reported penetration of nano-TiO_2 into the stratum granulosum of grafted human foreskin in two samples (of an unknown total number).

Penetration of nano-TiO_2 into dermis of minipigs was suggested in a meeting abstract, but the abstract contained insufficient information to determine whether Ti was detected inside the hair follicles or in the living cells of the dermis (Sadrieh et al., 2008). Several other studies that evaluated absorption using pig skin suggest little or no absorption beyond the stratum corneum. In a study using nano-TiO_2 in four formulations on pig skin (Menzel et al., 2004), the authors stated that nano-TiO_2 penetrated through the stratum corneum into the underlying stratum granulosum (but not into stratum spinosum) via intercellular space. The presence of Ti in the dermis, however, was deemed to be an artifact of the preparation process. Other studies using pig skin did not find nano-TiO_2 penetration beyond the stratum corneum (Gamer et al., 2006; Lekki et al., 2007; Pflücker et al., 2001).

Some nanomaterials have been shown to penetrate deeper in damaged skin than in intact skin [quantum dots in human skin (Mortensen et al., 2008); nano-silver in murine skin (Larese et al., 2009)], but no experimental data on nano-TiO_2 dermal penetration in damaged skin were found. Preliminary (not yet peer reviewed) data showed that two types of coated nano-TiO_2 topically applied on either dermabraded or intact skin of SKH-1 hairless mice did not increase Ti concentrations in blood, lymph nodes, liver, spleen, or kidney (Gopee et al., 2009). The depth of nano-TiO_2 penetration in either damaged or intact skin was not reported. Hairless mice data, however, do not exclude the possibility that nano-TiO_2 might penetrate deeper into damaged human skin than intact human skin because relative penetration of chemicals between hairless mice and humans varies and could be chemical specific (Benavides et al., 2009; Simon and Maibach, 1998).

4.6.3. Ingestion

Currently only three toxicological studies of nano-TiO_2 through oral exposure have been reported (see Section 5.3.1.2.2), and of these, only one (Wang et al., 2007a) reported tissue concentrations of nano-

TiO_2. In the Wang et al. (2007a) study, male and female mice received a single oral gavage of 5 g/kg TiO_2 as 25-nm rutile spindles, 80-nm rutile spindles, or 155-nm anatase octahedrons (10 male and 10 female mice for each type of TiO_2, and negative controls) (Table 4-5). The organs with elevated TiO_2 concentrations (measured only in female mice) were liver, spleen, kidney, lung, and brain. Although the liver is expected to receive most of the TiO_2 absorbed from the gastrointestinal tract through the portal vein, elevated TiO_2 levels in the liver were observed only in the 80-nm group. The reason for this size-specific elevation in hepatic TiO_2 concentration is unknown.

4.6.4. Blood Brain Barrier and Placental Transfer

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

Table 4-5. Animal studies that measured Ti concentrations in brain after nano-TiO_2 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-TiO_2 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 tetranutoxide.	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-Alderich)	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

Increased Ti concentrations in the brain were observed in mice 2 weeks after they were exposed to fine and nano-TiO_2 through a single oral gavage (Wang et al., 2007a), and in mice at the end of exposure to nano-TiO_2 through once-daily intravenous injections for 14 days (Liu et al., 2009) (Table 4-5). No increase in Ti concentration in the brain was observed in rats or mice exposed to nano-TiO_2 through a single intravenous injection (Fabian et al., 2008; Sugibayashi et al., 2008). Due to the variations in tested nano-TiO_2, treatment regimen, and other experimental design elements, no specific characteristic of nano-TiO_2 or its administration has been identified as determining factors for BBB penetration.

A recent study showed TiO_2 particles and pathological changes in the brain of 6-week-old mice from nano-TiO_2 exposed dams (Takeda et al., 2009) (Table 4-5), suggesting that nano-TiO_2 might be passed through undeveloped or developing BBB in embryos or young mice. Because the dams were exposed to nano-TiO_2 during pregnancy and the offspring were tested at 4 days and 6 weeks of age, the nano-TiO_2 exposure to the offspring could have been in utero (i.e., nano-TiO_2 could penetrate the placental barrier) or through milk, which was not tested in this study. In addition to the brain, nano-TiO_2 particles and pathological changes were also observed in the reproductive system of male offspring of nano-TiO_2-exposed dams (female offspring were not studied) (Takeda et al., 2009). Although no data on humans for nano-TiO_2 and placental barrier were located, an ex vivo study using perfused human placentas showed that nano-gold (PEGylated gold nanoparticles at 15 and 30 nm) did not cross the placenta into the fetal circulation at the tested condition (Myllynen et al., 2008). Nano-gold might behave

differently from nano-TiO_2, given that uncoated nano-gold does not penetrate either the BBB or placental barrier in mice (Sadauskas et al., 2007), whereas nano-TiO_2 does pass to BBB in mice (Liu et al., 2009; Wang et al., 2007a).

4.6.5. Dose-Metrics

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

Total particle surface area, which is closely related to primary particle size, has been suggested as a suitable dose metric for inhalation and instillation studies (Faux et al., 2003; Liao et al., 2008; Oberdörster et al., 2005). Although two distinctive dose-response curves for fine TiO_2 and nano-TiO_2 can be drawn based on mass concentration, certain observed respiratory effects of fine TiO_2 and nano-TiO_2 have been shown to fit well with a single linear dose-response curve based on primary particle surface area, even where both types of particles agglomerated to about 0.7 μm in diameter (Oberdörster et al., 1994). Hext et al. (2005) found that, compared to gravimetric lung burden (particle mass per lung mass), administered primary particle surface area correlated better with lung burdens, clearance half-lives, and certain biological responses in rats, mice, and hamsters. However, the evidence on this issue is somewhat mixed. For instance, biological responses after exposure to similarly-sized agglomerates of fine TiO_2 and nano-TiO_2 were similar in severity according to Warheit et al. (2007c; 2006); by contrast, Sager and Castranova (2009) found that well-dispersed nano-TiO_2 yielded greater effects than well-dispersed fine TiO_2

As mentioned previously, any one or more of various characteristics, including particle number, size (including agglomerations or aggregations), shape, crystalline form, mass, surface area, and surface modifications, could play a role in nano-TiO_2 toxicity. Including one or more of these factors in the dose metric could be a better choice than surface area alone. For instance, based on administered primary particle surface area, the data used in the Hext et al. study (2005) – the increases in the numbers of pulmonary polymorphonuclear neutrophil (PMN) due to exposure to anatase fine and nano-TiO_2 (Oberdörster et al., 1994) and rutile fine TiO_2 (Tran et al., 1999) – would better fit two dose-response

curves (one each for anatase TiO_2 and rutile TiO_2), instead of one dose-response curve. Similarly, a recent study of pulmonary effects of intra-tracheal instilled rutile fine TiO_2 and 80% anatase/20% rutile nano-TiO_2 (Sager et al., 2008) showed that when dose was normalized to surface area of the particles administered, the dose-response curves for inflammogenic responses were not statistically different between fine and nano-TiO_2, but the anatase-rutile nano-TiO_2 always yielded greater (1.3- to 2-fold) responses than the rutile fine TiO_2.

Due to limited toxicological data from oral or dermal exposure to nano-TiO_2, the choice of dose metric for these exposure routes has not been widely discussed. For in vitro studies, nanoparticle concentration (mass or surface area) is often used to express dose. In vitro cytotoxicity, however, has been reported to be affected by both the concentration and the total mass (or total number or total surface area, since these three are closely related) of nanoparticles (Lison et al., 2008). In the Lison et al. study (2008), when cells were cultured in various volumes of a medium containing the same amount of nano-silica (same mass/number/surface area), higher toxicity occurred in a lower volume of medium, that is, in higher nano-silica concentrations. When the medium contained the same concentrations of nano-silica, higher toxicity occurred in cells cultured with a higher volume of medium than lower volume of medium.

Questions about Exposure–Dose Characterization

4-1. Which sources, pathways, and routes pose the greatest exposure potential to nano-TiO_2 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-TiO_2?

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-TiO_2 via drinking water? …via topical sunscreens?

4-5. Approximately how many workers are involved in nano-TiO_2 production, distribution, and use?

4-6. What concentrations, routes, frequencies, and durations characterize worker exposures to nano-TiO_2 across the life cycle and within certain stages (e.g., manufacturing)?

4-7. What management practices exist to control occupational exposures to nano-TiO_2?

4-8. What personal protective equipment do workers use at the various life cycle stages of nano-TiO_2 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-TiO_2 applications?

4-10. Are available methods adequate to characterize nano-TiO_2 exposure via air, water, and food? What properties of nano-TiO_2 should be included in such exposure characterizations?

4-11. Given the potential for greater uptake of certain substances in the presence of nano-TiO_2, should monitoring and exposure studies include a suite of substances that might interact with nano-TiO_2?

4-12. What happens when nano-TiO_2 is trapped in the stratum corneum and the dead skin flakes off? Is there a potential for dead-skin nano-TiO_2 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-TiO_2 may increase the uptake of other pollutants, such as arsenic, would nano-TiO_2 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-TiO_2?

4-15. Which physiologically-based pharmacokinetic models are optimal for understanding absorption, distribution, and elimination of nano-TiO_2 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-TiO_2 to transfer to or accumulate in the food web and cause adverse effects on ecological receptors?

4-18. Nano-TiO_2 has been shown to attach to the surfaces of algae and fish as well as bioaccumulate in fish. Does nano-TiO_2 biomagnify?

Chapter 5. Characterization of Effects

The preceding chapters have laid a foundation for the present chapter by providing an exposure context for characterizing the effects of nanoscale titanium dioxide (nano-TiO_2) used for water treatment and in topical sunscreens. This chapter provides information on the factors that influence nano-TiO_2 ecological and health effects (Section 5.1), the ecological effects of nano-TiO_2 (Section 5.2), and the toxicological and human health effects of nano-TiO_2 (Section 5.3). Whether there are specific by-products (e.g., waste and transformation products) or interactions with other substances that should or can be evaluated has not yet been determined. For this reason, the focus of this chapter is on nano-TiO_2.

Although literature exists on the effects of conventional TiO_2 on humans and laboratory animals [for a review, see NIOSH (2005)], comparatively less information is available on the effects of nano-TiO_2. Consistent with studies of other nanomaterials (Ostrowski et al., 2009), most nano-TiO_2 studies have investigated the ecological or health effects of nano-TiO_2 itself, and relatively few have investigated the ecological or health effects of end-use products containing nano-TiO_2 or their life-cycle by-products.

The physicochemical characteristics of nano-TiO_2 could be important to the biological effects of these materials (Section 5.1), yet those characteristics frequently are not evaluated or reported as part of studies of such effects. This observation should serve as a caveat in examining and interpreting the results described throughout this chapter.

The following sections are not meant to be an exhaustive review of the ecological and human health effects literature for nano-TiO_2. Instead, this chapter is intended to highlight recent work on the effects of nano-TiO_2 and to identify current knowledge status and gaps in information needed for assessing potential risks of nano-TiO_2 in water treatment and sunscreen.

5.1. Factors that Influence Ecological and Health Effects of Nano-TiO_2

The large number of variables associated with nano-TiO_2 material itself and its ecological and health effects makes it extremely difficult to identify the primary characteristic(s) of nano-TiO_2 contributing to an effect or to compare the importance of different characteristics to such effects. A common statement from early studies is the announcement of size effects (or the lack of size effects) from nano-TiO_2 of different crystalline forms or anatase/rutile ratios. That size alone does not account for the effects of nano-TiO_2, however, is now generally accepted; other factors, such as shape, surface chemistry,

photoreactivity, and other characteristics, could also play a role in these effects (Gonzalez et al., 2008; Hassellöv et al., 2008; Powers et al., 2006). With the advance of nanoparticle synthesis, the influence of different physicochemical characteristics of nano-TiO_2 has been investigated using well-characterized nano-TiO_2 and better control of variables in recent studies (Jiang et al., 2008).

Three categories of factors (nano-TiO_2 physicochemical characteristics, experimental conditions, and environmental conditions) that could influence the ecological and toxicological or health effects of nano-TiO_2 are discussed here in Section 5.1. These are not the only factors of potential importance. As noted previously, exposure route can play a major role in the effects of nano-TiO_2, and the importance of this is reflected in the fact that much of the information in this chapter is organized around environmental media and routes of exposure. Host effects, particularly species differences, can also play an important role in the effects of nano-TiO_2. For example, skin penetration is greatest in rabbits, followed by rats, pigs, monkeys, and humans (Nohynek et al., 2007). However, little information is available on these species differences or on differences in susceptibility of different cell types to nano-TiO_2 effects (Kiss et al., 2008). The phenomenon of pulmonary particle clearance "overload" and subsequent effects in rats and mice are much more understood and are discussed in Section 5.3.1.2.3. In the following sections, the order in which factors are presented does not imply relative importance. This section focuses on factors that have been shown to be important for nano-TiO_2, but findings related to other types of nanomaterials are noted where relevant.

5.1.1. Nano-TiO_2 Physicochemical Characteristics

Size, crystal structure, and surface chemistry (such as coating) are among the factors that influence nano-TiO_2 effects. Other physicochemical properties, such as shape (Warheit et al., 2006; Yamamoto et al., 2004), manufacturing process, doping, and purity (or impurities) could also play a role in nano-TiO_2 toxicity, but such information is usually not reported in ecological and toxicological studies. Contributing to this lack of reported characteristics are limitations in the availability of analytic methods for characterizing such nanomaterials. Databases describing detailed nanoparticle properties and health effects are being developed (Miller et al., 2007a).

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

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

5.1.1.1. Size

Size is a main determining factor for the distribution of (inhaled or instilled) nano-TiO_2 in and outside of the respiratory tract (Oberdörster et al., 2004). For particles with a diameter less than 100 nm, the smaller the particles are, the more total particle deposition in the respiratory tract and deposition in nasopharyngolaryngeal regions (Oberdörster, 2000). Smaller sizes, however, do not always result in more deposition in other regions of the respiratory tract. For example, the highest percentages of alveolar deposition have been observed in nanoparticles of about 20 nm in size, and the highest percentages of tracheaobronchial deposition were observed in nanoparticles 1–10 nm in size (Oberdörster, 2000). Furthermore, particles less than 200 nm in size can be transported from olfactory mucosa to the olfactory bulb of the brain via the olfactory nerve (Elder et al., 2006). Exposures to nano-TiO_2 (with mean diameters of 21.05 ± 5.08 nm, 71.43 ± 23.53 nm, and 154.98 ± 32.98 nm) through intranasal instillation increased titanium concentrations in the olfactory bulb in mice (Wang et al., 2005; Wang et al., 2007a), and two types of nano-TiO_2 particles (80-nm rutile and 155-nm anatase) were found to increase Ti concentrations in hippocampus, central cortex, and cerebrum, in addition to olfactory bulb, in mice after repeated intranasal instillation (Wang et al., 2008b).

Jiang et al. (2008) investigated the size effects of nano-TiO_2 on reactive oxygen species (ROS) generation per unit of particle surface area. Using nine different sizes (4–195 nm) of anatase nano-TiO_2, the investigators found that the highest levels of ROS generation per unit surface area were generated by 30-nm and larger particles. For nano-TiO_2 less than 30 nm, the ROS generation per surface area decreased with decreasing particle diameter down to 10 nm, below which it was constant (Jiang et al., 2008).

5.1.1.2. Crystallinity

TiO_2 crystalline forms also influence TiO_2 and nano-TiO_2 photoreactivity, reactive species generation, and toxicity. Nano-TiO_2 containing more anatase tends to generate more free radicals and induce more toxicity (e.g., cytotoxicity, inflammatory response) than nano-TiO_2 containing more rutile (Hidaka et al., 2005; Sayes et al., 2006; Uchino et al., 2002). The influence of crystal forms of nano-TiO_2 on ROS generation was investigated using a fixed total surface area by Jiang et al. (2008), who tested 13 nano-TiO_2 particles of varying crystallinity, all within the size range of 42–102 nm. Size was found not to affect ROS generation per unit surface area in this study. The researchers found that the ROS generation

per unit surface area was highest in amorphous nano-TiO_2, followed by anatase and then nano-TiO_2 containing both anatase and rutile, and was lowest in rutile nano-TiO_2 (Jiang et al., 2008). This finding is consistent with those of a study investigating unusually fast weathering (loss of gloss) or degradation of surface coating on steel roofing, associated with sunscreens left by workers during installation (Barker and Branch, 2008). Nano-TiO_2 in the coating-damaging sunscreens was an anatase/rutile mixture, whereas nano-TiO_2 in the one sunscreen that did not accelerate loss of gloss was pure rutile (Barker and Branch, 2008).

The cytotoxicity of anatase and anatase-mixtures was further increased by UV illumination. Anatase nano-TiO_2 can be 100 times more cytotoxic under UV than rutile of similar size (Sayes et al., 2006). The hydroxyl (·OH) radical production by nano-TiO_2 in cultured cells was found to depend on the crystalline form and size, but differences in OH radical production were not explained by the differences in UV-A absorption between anatase and rutile (Uchino et al., 2002). Smaller particles that contain more anatase, however, are not always more toxic either in vitro (Sayes et al., 2006) or in vivo (Warheit et al., 2006) than larger particles containing more rutile.

5.1.1.3. Surface Chemistry

Although coatings have been used to decrease the photoreactivity of nano-TiO_2 intended for sunscreen (see Section 2.2.2), coatings affect more than photoreactivity. Coatings for nano-TiO_2 particles can be designed to reduce agglomeration/aggregation, which in turn affects the behavior of the particles in various media, including sedimentation. This also affects the exposure to organisms living in different parts of water bodies or feeding on different sized particles. Particle surface modifications can also change the effects of nano-TiO_2 on living cells, tissues, or organisms. Using in vitro methods, Serpone et al. (2006) reported that a "thermally assisted" modification of the TiO_2 particle surface reduced photocatalytic activity, which in turn decreased (if not eliminated) toxicity to DNA plasmid, human cells, and yeast. In rats intra-tracheally instilled with two types of nano-TiO_2 having the same core material, the nano-TiO_2 with a hydrophobic surface (Aeroxide® T805, silanized) caused a slightly lower bioactivity than hydrophilic P25, although the authors concluded that silanization[6] did not "lead to remarkable 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 SiH_4. 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

As noted in Chapter 1, nanomaterials, and nano-TiO_2 in particular, can be characterized in several ways in terms of physicochemical properties (see Table 1-1). Given that the relationship between such properties and the behavior and effects of nanomaterials, including nano-TiO_2, remains to be fully understood, it might seem desirable for researchers to characterize every possible property of the material they are investigating. In practice, this is not feasible. Consequently, recommendations for characterization of nanomaterials have come forth from time to time.

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

An expert working group convened by the International Life Sciences Institute (ILSI) Research Foundataion/Risk Science Institute recommended measuring mass, size distribution, surface area, and number for exposure characterization in inhalation studies (Table 5-1), and 17 measurements/aspects for 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 strucures). 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).

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

5.1.2. Experimental Conditions

Experimental conditions, particularly the choice of media/vehicle in which to disperse nano-TiO_2, preparation of testing solutions or suspensions, and the formation of aggregates, can influence the behavior and effects of nano-TiO_2 and other nanomaterials. The advantages and disadvantages of various

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

5.1.2.1. Media/Vehicle

Nano-TiO_2 in an oily dispersion penetrates deeper into skin than nano-TiO_2 in an aqueous dispersion, as shown in an ex vivo study using healthy adult skin (intact samples of tissue removed from the body, and manipulated in vitro) (Bennat and Muller-Goymann, 2000). Furthermore, when the dispersal of nano-TiO_2 was made in the aqueous phase of an oil-in-water emulsion, nano-TiO_2 did not penetrate into skin, but the emulsion was not stable (Bennat and Muller-Goymann, 2000). Although the stability could be improved by encapsuling the nano-TiO_2 into liposomes, liposome formulation increases nano-TiO_2 skin penetration. An in vivo study by Lansdown and Taylor (1997) in rabbits also demonstrated that uptake of TiO_2 particles in sizes ranging from 2 to 20 μm was affected by the vehicle: uptake was greatest in castor oil, followed by water, and then polyethylene glycol. According to Bennat and Muller-Goymann (2000), the ideal sunscreen formulation, which is stable and does not allow nano-TiO_2 penetration into skin, has yet to be developed.

Different levels of radical production in cultured cells were observed in similar nano-TiO_2 within different formulae of suspensions (Uchino et al., 2002). Although nano-TiO_2 F-1R (a formula containing nano-TiO_2 that is 3% anatase and 97% rutile, with an average size of 93 nm and a surface area of 17 m^2/g) produced OH radicals after UV-A exposure, no OH radical production was detected after UV-A exposure in nano-TiO_2 in a different formula, St-C n (sunscreen standard C from the Japan Cosmetic Industry Association containing nano-TiO_2 that is 2% anatase, 98% rutile, with an average size of 85 nm and a surface area of 19 m^2/g). Most rutile nano-TiO_2 is relatively inefficient in radical production, and the F-1R used in this study produced more OH radicals than all four other, mainly rutile nano-TiO_2 forms and one of the anatase forms tested (Uchino et al., 2002). Although nano-TiO_2 has been reported to generate ROS in cell-free conditions but not in cells (a murine macrophage cell line, RAW 264.7) (Xia et al., 2006), whether nano-TiO_2 in different formulae also causes different levels of ROS production in cells has not been verified.

The purity of water affects the degree of aggregation, which in turn may affect exposure-dose and toxicity. The degree of aggregation generally increases with the presence of salt or with an increase in ionic strength, minerals, and organic matter in water (i.e., decreased purity as compared to pure water) (Domingos et al., 2009a; French et al., 2009). Aggregation was more severe in tap water than in nanopure water (Zhang et al., 2008), and is likely to be more severe in fish tank water or pond water than in tap water. Because nano-TiO_2 in the environment is more likely to be present in aggregated form, results from nano-TiO_2 suspensions with aggregates can be informative, and as noted earlier, might even be more

relevant than results from a perfectly dispersed suspension with nano-TiO_2 in primary particle form. The lack of accurate measurement of nano-TiO_2 (e.g., size distribution, mass concentrations, numbers, and surface area) and a generally-agreed-upon choice of dose metrics, however, impede the establishment of a reliable dose-response relationship.

In respiratory exposure studies, intra-tracheal instillation exposure typically uses saline as a vehicle for TiO_2 delivery while inhalation exposure uses air. The behavior of nano-TiO_2 (such as agglomeration) is expected to be different in air than in solution. Furthermore, the vehicle alone can affect respiratory system responses, at least for a short time. Transient inflammation in the respiratory tract occurs in rats given saline alone through instillation (Driscoll et al., 1990; Henderson et al., 1995). Sager et al. (2007a) tried to disperse several types of nano-sized particles, including TiO_2, in several suspension media, including: phosphate buffered saline (PBS); rat and mouse BAL fluid; and PBS containing dipalmitoyl phosphatidylcholine (DPPC) or mouse serum albumin or both. Although the dispersion in PBS was not satisfactory, BAL fluid was an excellent vehicle for dispersing the particles. The dispersion was also unsatisfactory in saline containing albumin alone or DPPC alone, in concentrations found in BAL fluid. Adding protein plus DPPC in PBS, however, produced satisfactory, albeit slightly less effective, substitutes for BAL fluid. The Sager et al. (2007a) experiment demonstrates the importance of the suspension medium and suggests that the immediate milieu (such as the BAL fluid and protein and DPPC in lung) affects not only the agglomeration of nano-TiO_2, but also the consequent effects on nano-TiO_2 behavior and effects.

5.1.2.2. Dispersion Preparation

The potential importance of dispersion preparation for nanomaterial ecotoxicity is illustrated by fullerene (C_{60}) studies. C_{60} toxicity in daphnids and fishes was higher when the C_{60} suspension was prepared with the organic solvent tetrahydrofuran (THF) than when the suspension was prepared by stirring and sonication (Henry et al., 2007; Zhu et al., 2006). Entrapped or residual THF in the C_{60} and THF degradation products were suspected to have contributed to toxicity (Henry et al., 2007). Nevertheless, no difference in toxicity to daphnids was observed between nano-TiO_2 suspensions prepared with and without THF (Klaper, 2008; Lovern and Klaper, 2006). Regardless of dispersion method, aggregation of nano-TiO_2 might be unavoidable. Several studies reported that nano-TiO_2 formed aggregates in water, and that these aggregates could not be disaggregated into primary particles by ultrasound or chemical dispersants (Griffitt et al., 2008; Jeng and Swanson, 2006; Zhang et al., 2008). Furthermore, an unfiltered nano-TiO_2 suspension with aggregates has been reported to be less toxic to daphnia than a filtered nano-TiO_2 suspension, which has a much smaller mean secondary particle size than filtered suspension (Lovern and Klaper, 2006). In contrast to the reported difficulty of

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

In addition to the medium itself, the dispersion method can affect not only the nanoparticle agglomeration or aggregation (such as the degree and size of agglomerates) but also the effects of nanoparticles (Bihari et al., 2008). For example, sonication with ultrasound has been used to decrease nano-TiO_2 agglomeration (Bihari et al., 2008) and has been shown to generate particles or agglomerates in the nanoparticle range (Maier et al., 2006). However, sonication alone could increase the size of nano-TiO_2 agglomerates, as reported by Porter et al. (2008) who found that the mean agglomerate size of P25 in PBS increased from 1930 nm before sonication to 2849 nm immediately after sonication, while the same sonication procedure decreased the sizes of agglomerates of P25 dispersed in BAL fluid and in a mimic BAL fluid that contained Ca^{2+}- and Mg^{2+}- free PBS, serum albumin, and DPPC. No explanation was provided. Furthermore, ultrasound sonication has been reported to increase nano-TiO_2 catalytic activity in breaking down an organic dye (acid red B) (Wang et al., 2009b), but also to decrease changes in enzyme activity caused by ingested nano-TiO_2 in isopods (Jemec et al., 2008). Post-preparation analysis of particle size is important when comparing laboratory studies and formulations with sunscreen preparations. Although studies of nano-TiO_2 particle and agglomerate sizes are available (Delrieu et al., unknown), very few health effects studies have characterized nano-TiO_2 in sunscreen formulations and only a few studies characterized nano-TiO_2 in other experimental media. Most health effects studies have reported characteristics of only dry nano-TiO_2 primary particles, which are important but not representative of all exposure scenarios.

Finally, without a special hydrophilic coating, nano-TiO_2 forms a suspension in water (rather than a solution). Standard ecotoxicological test methods are intended for soluble or poorly soluble substances, and not designed for testing suspensions (German Federal Institute for Occupational Safety and Health (BAuA) et al., 2007).

5.1.3. Environmental Conditions

Once nano-TiO_2 is released into the environment, its fate depends on abiotic and biotic conditions, which are likely to be more complex and diverse than standard ecological testing conditions. Of the many environmental factors that might be relevant to nano-TiO_2 ecotoxicity, ultraviolet (UV) exposure, purity of water (Zhang et al., 2008), and presence of organic matter (Domingos et al., 2009a) have been investigated. Factors that affect nano-TiO_2 aggregation, such as pH value, ionic strength, and cation valence (Domingos et al., 2009a; Dunphy Guzman et al., 2006; French et al., 2009), would influence not

only nano-TiO_2 fate and transport (see Chapter 3), but also potential exposure and possibly ecological effects. Only environmental factors that have been shown to affect toxicity in organisms used for ecological effects testing are discussed here.

UV is well known to increase the cytotoxicity of nano-TiO_2, particularly photocatalytic nano-TiO_2 such as anatase or anatase/rutile mix, to cultured mammalians cells (Sayes et al., 2006) and fish cells (Reeves et al., 2008; Vevers and Jha, 2008) as well as microorganisms (Adams et al., 2006). Genotoxicity (Nakagawa et al., 1997) and clastogenicity (Nakagawa et al., 1997; Theogaraj et al., 2007) of nano-TiO_2 to cultured mammalians cells were also increased by UV. This UV-increased toxicity is at least partially due to the greater number of hydroxyl radicals (·OH) generated by anatase than by rutile under UV exposure (Sayes et al., 2006; Uchino et al., 2002). UV exposure may influence the effects of nano-TiO_2 in sunscreen indirectly by causing sunburn, which can make skin more permeable (Mortensen et al., 2008). In addition to UV, visible light was shown to increase the cytoxocity of nano-TiO_2 (carbon-doped TiO_2 and TiO_2 modified with platinum [IV] chloride complexes) in bacteria and fungi (Mitoraj et al., 2007).

Nano-TiO_2 was found to aggregate more in pond water than in pure water (Milli-Q water), although no nano-TiO_2 toxicity to soil bacteria, green algae, or water fleas was detected in either pond water or pure water at up to 100 mg/L (Velzeboer et al., 2008). The adsorption of nano-TiO_2 onto certified reference material sediment did not increase the toxicity of the sediment (Blaise et al., 2008).

Additional environmental factors that might indirectly influence the effects of TiO_2 nanoparticles in sunscreen include moisture; pH and water chemistry; and temperature. High humidity in the environment could increase the hydration level of the stratum corneum, and could lead to increases in skin permeability and penetration of both hydrophilic and lipophilic components (Benson, 2005; Zimmerer et al., 1986). For example, the level of penetration of nano-TiO_2 on soaked skin, which is likely to occur after swimming or other water activities, has not been investigated. Similar to media and vehicle effects on nano-TiO_2, the pH and chemistry of water with which sunscreen may be mixed might also modulate nano-TiO_2 effects, e.g., in a pool versus a lake or an ocean. Finally, sunscreen is often used at much higher temperatures than typical ambient laboratory temperatures. Although nano-TiO_2 itself is not expected to change in the temperature range tolerable for human beings, increased body temperature and sweat may affect nano-TiO_2 dermal penetration and thus its effects (Lu et al., 2008).

The influence of the immediate milieu on nano-TiO_2 behavior and effects is also evident when nano-TiO_2 is inside an organism. For instance, in vitro studies showed that in rat BAL, nano-TiO_2 formed smaller aggregates and the aggregates remained small longer than nano-TiO_2 in PBS (Porter et al., 2008; Sager et al., 2007a, b). Because pH affects the charge of nano-TiO_2, it is plausible that nano-TiO_2 would

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

5.1.4. Summary

Nano-TiO_2 physicochemical properties, experimental conditions, and the immediate environment or milieu, all can influence nano-TiO_2 ecological and health effects. For example, nano-TiO_2 size, crystalline form, and surface characteristics all influence nano-TiO_2 behavior, including distribution, exposure potential, and effects. Although the influences of media and vehicle and dispersion methods on particle aggregation and distribution have been reported, information on these influences on health effects is very scarce (Jemec et al., 2008). The presence of UV and visible light often increase photocatalytic nano-TiO_2 activity and toxicity; other environmental factors, such as pH, ironic strength, and presence of organic matter of the aquatic environment, could also affect nano-TiO_2 behavior and effects.

5.2. Ecological Effects

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

Section 5.2.1 features a review of the ecological effects of nano-TiO_2 exposure. Effects on bacteria and fungi are discussed in Section 5.2.1.1, effects on aquatic organisms are discussed in Section 5.2.1.2, effects on terrestrial organisms are discussed in Section 5.2.1.3, and indirect and interactive toxicity are 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-TiO_2 Exposure

Most of the nano-TiO_2 ecological effect studies surveyed in this report (Table 5-3) used photocatalytic nano-TiO_2, some of which could be suitable for water treatment purposes. Two of the studies used photostable nano-TiO_2 intended for topical sunscreen (Wiench et al., 2007) or for protecting plastic from UV degradation (Warheit et al., 2007a). Current FDA regulation of TiO_2 in topical sunscreen does not specify crystalline form and does not require proof of photostability (or lack of photoreactivity). Pure anatase nano-TiO_2 is much more photoreactive than pure rutile nano-TiO_2, but it is possible to have photostable anatase or an anatase/rutile mix of nano-TiO_2 by using doping or surface treatments, such as coating with silica. The coating of photostable nano-TiO_2 is designed to endure the manufacturing process and consumer use (Lademann et al., 2000), but the long-term stability of coated TiO_2 in sunscreen remains unclear. Once nano-TiO_2 is released into the environment, various environmental factors, such as high ionic strength in sea water and high acidity in landfill leachate, could compromise some nano-TiO_2 coatings. Therefore, the ecological effects of photocatalytic nano-TiO_2 might be relevant not only for nano-TiO_2 used in drinking water treatment but also for nano-TiO_2 in sunscreen, because photoreactive nano-TiO_2 can be used as the core material of photostable nano-TiO_2 in sunscreen. For example, the core of Aeroxide T805 is P25, a photocatalyst, and has been used as a UV filter in some sunscreens (Barker and Branch, 2008; Evonik, 2007).

Because mass concentration is reported for all studies reviewed, this dose metric is presented in Table 5-3 and in all subsequent discussion referring to the literature. Whenever information on surface area of the particles (to calculate particle surface area concentration) or the measured nano-TiO_2 concentration (versus calculated based on added mass) in the final test suspension is available, it is also provided in Table 5-3. It should be noted that several studies reported visible turbidity in nano-TiO_2 stock suspension (Velzeboer et al., 2008; Zhang et al., 2006; Zhang et al., 2008). Because turbidity is likely caused by large aggregates of nano-TiO_2, which can settle out of the liquid phase by gravity, actual concentrations of nano-TiO_2 in the liquid phase might be lower than concentrations calculated based on mass of nano-TiO_2 added.

Table 5-3. Summary of nano-TiO_2 ecological effects.[a]

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Microorganisms			
Bacteria (*Escherichia coli* and *Bacillus subtilis*) (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, *B. subtilis*: 0% and 75% growth inhibition at 500 and 1000 ppm,[b] respectively *E. coli*: 0%, 15% and 44% inhibition at 100, 500, 1000 ppm, respectively
Bacterium (*Vibrio fischeri*) (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, *Vibrio fischeri* (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 (*Vibrio fischeri*) (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 Measure the reduction of light output from *Vibrio fischeri* (Flash assay) as an indicator of growth inhibition	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)		The highest concentration tested: 20 g/L conventional TiO_2 (30 min and 8 hr exposure) did not decrease bacterial growth
Bacterium (*Vibrio fischeri*) (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-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms			
Alga (green alga, *Desmodesmus subpicatus*) (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 *D. subspicatus* growth Algal growth (without preillumiaton): 0, 3.1, 6.2, 12.5, 25, 50 mg/L (producs 1 and 2) Shading effect:: 0, 12.5, 25, 50 mg/L Algal growth (with preillumiaton): 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 (Mehrvar et al., 2002)		
Alga (green alga, *Pseudokirchneriella subcapitata*) (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, *Pseudokirchneriella subcapitata*) (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, *Pseudokirchneriella subcapitata*) (Blaise et al., 2008)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	72-hr growth inhibition, tested concentraions not specified	IC_{25} >100 mg/L

Table 5-3. Summary of nano-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (water flea, *Daphnia magna*) (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 (Mehrvar 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, *Daphnia magna*) (Lovern and Klaper, 2006)	Primary particle <25-nm (smallest 5-nm), anatase, uncoated (photocatalytic) (Klaper, 2008); filtered through a 0.22-µm nylaflo 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, *Daphnia magna*) (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-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (water flea, *Daphnia magna*) (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, *Daphnia magna*) (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, *Daphnia pulex* and *Ceriodaphnia dubia*) (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 *D. pulex* adults and *C. dubia* 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 *D. pulex* and *C. dubia*
Invertebrate (water flea, *Chydorus sphaericus*) (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, *Daphnia magna*; fairy shrimp, *Thamnocephalus platyurus*) (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 *D. magna* 24-hr immobilization for *T. platyurus* Up to 20000 mg/L for both nano- and conventraional TiO_2	NOEC >20,000 mg/L for *T. platyurus*; not tested in *D. magna*
	Conventional TiO_2: size and crystal form not disclosed (Sigma product 14027, Estonia; a former Riedel-de Haën product) (Heinlaan, 2008)		NOEC >20,000 mg/L for *T. platyurus*; 60% mortality at 20,000 mg/L for *D. magna*
Invertebrate (fairy shrimp, *Thamnocephalus platyurus*) (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-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Aquatic Organisms (continued)			
Invertebrate (freshwater hydra, *Hydra attenuata*) (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, *Danio rerio*), 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, Jiangshu 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, *Danio rerio*) (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, *Oncorhynchus mykiss*) (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, *Daphnia magna*) (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, *Daphnia magna*) (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-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Chronic Exposure to Aquatic Organisms (continued)			
Fish (rainbow trout, *Oncorhynchus mykiss*) (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 phytosynthetic 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, *Spinacia oleracea*) (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 Conventrional TiO_2: No significant changes
Plant (spinach, *Spinacia oleracea*) (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, *Porcellio scaber*) (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-TiO_2 ecological effects (continued).

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Acute Exposure to Terrestrial Organisms (continued)			
Invertebrate (nematode, *Caenorhabditis elegans*) (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)
EC_{50} – Effective concentration 50; the concentration at which 50% of subjects showed response
EU – European Union
IC_{20}, IC_{25} – inhibitory concentration at which organisms showed 20%, 25% inhibition in measured endpoints
ISO – International Organization for Standardization
GST – Glutathione-S-transferase
LC_{50} – 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-TiO_2 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-TiO_2 in medium were not reported.

[c] Authors reported cloudy appearance in 100 mg/L TiO_2 suspension. After centrifugation, nano-TiO_2 concentrations were no more than 10% of initial concentrations. For example, 200 µg/L nano-TiO_2 was added into pond water, and nano-TiO_2 was only 1 µg/L after centrifugation.

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

Data for the effects of photostable nano-TiO_2 on bacteria and fungi are lacking. On the other hand, photocatalytic nano-TiO_2 is known for its antibacterial and antifungal properties and has been tested for various applications, including drinking water treatment (Coleman et al., 2005); surface coatings and paints (Kühn et al., 2003; Tsuang et al., 2008); and food packaging (Chawengkijwanich and Hayata, 2007). Examples of recent studies of photocatalytic nano-TiO_2 in bacteria and fungi are provided in Table 5-3.

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

Sensitivity to photocatalytic nano-TiO_2 toxicity varies among species of bacteria. Adams et al. (2006) reported that in the presence of sunlight, gram-negative *Escherichia coli* were more sensitive to nano-TiO_2-induced growth inhibition than gram-positive *Bacillus subtilis*. With 2,000 parts per million (ppm) of nano-TiO_2 in the growth medium, *E. coli*. growth was decreased by 46% while *B. subtilis* growth was inhibited by 99%. At 500 ppm, *E. coli*. growth was decreased by only 15% and *B. subtilis* growth was not inhibited (Adams et al., 2006). The different dose-response relationships of gram-positive and gram-negative bacteria to nano-TiO_2 suggests the potential for nano-TiO_2 to alter microbial population balance (diversity), both in wastewater treatment plants and during various phases of use and disposal of nano-TiO_2. One generally accepted explanation for nano-TiO_2-induced toxicity in bacteria and fungi is the generation of ROS, which can cause cell wall or cell membrane damage (Kühn et al., 2003; Neal, 2008), such as lipid peroxidation (Maness et al., 1999). Although, as discussed above, UV illumination increases photocatalytic nano-TiO_2 toxicity, photocatalytic nano-TiO_2 is also toxic in the dark (Adams et al., 2006; Coleman et al., 2005). Because TiO_2 generates ROS (mainly highly reactive hydroxyl radicals, ·OH) in the presence of UV and oxygen (Reeves et al., 2008), mechanisms other than oxidative stress might also contribute to nano-TiO_2 toxicity in the dark and possibly also under UV. For example, several types of nano-TiO_2 (anatase and a mixture of anatase/rutile) have been shown to adsorb

protein and calcium (Ca^{2+}) in the medium, and cause in vitro cytotoxicity in mammalian cell lines (Horie et al., 2009).

5.2.1.2. Effects on Aquatic Organisms

Data on the effects of nano-TiO_2 in aquatic organisms are available for freshwater algae, freshwater invertebrates (water fleas and fairy shrimp), and freshwater fish (rainbow trout) (Table 5-3). Only two aquatic organism studies in the literature involve photostable nano-TiO_2 (Warheit et al., 2007b; Wiench et al., 2007). For other aspects of U.S. Environmental Protection Agency (EPA) tier 1 aquatic toxicity testing (e.g., estuarine and marine organism acute toxicity, whole sediment acute toxicity, and bio-availability/bio-magnification toxicity) (U.S. EPA, 2008d), studies have not yet been reported.

5.2.1.2.1. Algae

Algae are primary producers in ecosystems. In addition to being the food base in aquatic systems, algae provide much of the earth's oxygen. Effects on algae are measured at the population level, for example, in terms of population growth. In algal tests, 72-hour exposures are considered acute exposure in European Union (EU) regulations, and 96-hour exposures are considered chronic by U.S. EPA (2008d). A limited number of studies on the effects of either photocatalytic or photostable TiO_2 in algae have been completed.

For photostable nano-TiO_2, EC_{50} values determined for 72-hour growth inhibition in green alga (*Pseudokirchneriella subcapitata*) were 21 mg/L (based on decreases in healthy cell numbers) and 87 mg/L (based on inhibition of growth rate) (Warheit et al., 2007a). In contrast, exposure to concentrations of 0.001 to 1 mg/L of photostable nano-TiO_2 increased growth rate by 1–3% (green alga cell numbers increased 6–19%) (Warheit et al., 2007a). U-shaped dose-response relationships are not unique to nanomaterials, and it cannot be ruled out that increased growth at the low dose was a compensatory response to low levels of toxicity (Calabrese and Baldwin, 1998; Davis and Svendsgaard, 1990). Fine (approximately 380-nm) TiO_2 showed almost no inhibition in growth rate (or cell number) at up to 1 mg/L, and inhibition of growth rate was 3% at 10 mg/L and 66% at 100 mg/L (Warheit et al., 2007a).

For photocatalytic nano-TiO_2, the EC_{50} values determined for 72-hr growth inhibition in green algae (*Desmodesmus subpicatus*) ranged from approximately 30 mg/L to more than 50 mg/L (Blaise et al., 2008; Hund-Rinke and Simon, 2006). Hund-Rinke and Simon (2006) also tested the potential for TiO_2 to reduce growth by physically shading algae, and reported that as much as 50 mg/L of photocatalytic nano-TiO_2 physically above the algae did not decrease algal growth, that is, it did not cause a shading effect. When nano-TiO_2 and algae are in the same liquid medium, photocatalytic P25 nano-

TiO_2 was reported to adsorb onto the surfaces of green algae (*Pseudokirchneriella subcapatitata)* and to increase cellular weight by more than 130% (Huang et al., 2005). The concentration of P25 was not reported. If the attached nano-TiO_2 directly blocks sunlight that otherwise could reach the algal cell surface or if this extra weight causes algae to stay in deeper water, the consequent reduction in sunlight could inhibit the algal growth. Because photostable nano-TiO_2 would also block UV penetration, similar effects could occur with photostable nano-TiO_2. Without experimental evidence, predicting the impact of nano-TiO_2 on photosynthesis is difficult because nano-TiO_2 exposure reportedly increases photosynthesis in terrestrial plants, namely spinach, as discussed later in this section. Nano-TiO_2 could affect aquatic and terrestrial plants differently due to exposure routes, doses, and other factors.

Although no marine organisms have been tested for nano-TiO_2 toxicity, the physical attachment of nano-TiO_2 particles on cells could pose a risk to aquatic organisms that reproduce by external fertilization. A wide variety of marine organisms fall into this category. Attached nano-TiO_2 could decrease sperm cell mobility and consequently reproductive success. For comparison, carbon black nanoparticles have been reported to decrease sperm frequency of seaweed (marine macroalgae) and to affect seaweed embryo development (Nielsen et al., 2007). As discussed earlier (Section 5.1.1), the salinity in seawater could influence the behavior and effects of nano-TiO_2, such as more aggregation as compared to pure water.

Nano-TiO_2 was reported to increase algal cell weight 2.3-fold by adsorbing to the algal cell surface, but the tested nano-TiO_2 concentrations in water were not reported (Huang et al., 2005). If an increase in weight forces surface algae into deeper water, photosynthesis could be decreased[7] due to less sunlight available in deeper water than at the surface. Because phytoplankton form the base of the food web and generate half of the oxygen produced by all plants (Ramanujan, 2005), harmful effects on phytoplankton from nano-TiO_2 could have wide-ranging implications.

5.2.1.2.2. Invertebrates

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

[7] On the other hand, nano-TiO_2 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).

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

Acute and chronic toxicity of nano-TiO_2 intended for sunscreen use was studied in *Daphnia magna* and reported in a poster at a scientific meeting by Weinch et al. (2007). In the acute exposure study, EC_{50} values (from 48-hour mortality tests) were above 100 mg/L for all tested forms of TiO_2, which consisted of three photostable forms (uncoated T-Lite™ SF, coated T-Lite™ SF-S, and coated T-Lite™ MAX), a photocatalytic nano-TiO_2, and a pigment-grade TiO_2 (Wiench et al., 2007). In the chronic exposure study, photostable coated T-Lite SF-S was given to *Daphnia magna* at up to 100 mg/L for 21 days, and the LC_0 was 30 mg/L. In this study, death was determined by the lack of swimming ability.

For reproductive effects after 21 days, the no observed effect concentration (NOEC) value for T-Lite SF-S was 3 mg/L, and the lowest observed effect concentration (LOEC) value was 10 mg/L (Wiench et al., 2007). In a different study that used photostable nano-TiO_2 intended to protect plastics against UV-induced degradation, 48-hr exposure to 100 mg/L of the nano-TiO_2 induced 10% immobility in *Daphnia magna* (Warheit et al., 2007a).

The effects of photocatalytic nano-TiO_2 toxicity have been studied by several research teams in four types of water fleas (*Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, and *Chydorus sphaericus*), one type of fairy shrimp (*T. platyurus*), and one type of freshwater hydra (*Hydra attenuata*). For water fleas, the 48-hour mortality or immobility EC_{50} was generally greater than 100 mg/L (Lovern and Klaper, 2006; Velzeboer et al., 2008; Wiench et al., 2007), with two exceptions. One study reported an LC_{50} greater than 10 mg/L, which in this case was the highest concentration tested (Griffitt et al., 2008). Another study reported a 48-hour LC_{50} of 5.5 mg/L, using filtered nano-TiO_2 samples, which have an average particle size of 30 nm after going through a 0.22-mm Nylaflo filter (Lovern and Klaper, 2006). In contrast, unfiltered nano-TiO_2 samples had all sizes of nano-TiO_2 clumps, ranging from 100 to 500 nm in diameter, and the mortalities never exceeded 11% at up to 500 mg/L (Lovern and Klaper, 2006). Chronic exposure for 8 days caused 40% mortality at 20 mg/L in daphnids (Adams et al., 2006). For fairy shrimp, the 24-hr mortality or immobility LC_{50} was higher than 100 mg/L (Blaise et al., 2008; Heinlaan et al., 2008). In the only study of hydra, the EC_{50} of 96-hour morphological changes was less than 100 mg/L (Blaise et al., 2008). The relative sensitivity among these aquatic invertebrates to nano-TiO_2 cannot be determined, due to the variability of tested nano-TiO_2 formulations and experimental designs.

When *Daphnia magna* were exposed to photocatalytic P25 nano-TiO_2 in water, nano-TiO_2 was observed on the exoskeleton and antennae and in the digestive tract (Baun et al., 2008). Baun et al. (2008) noted that the aggregation of nanoparticles on the exoskeleton, at sufficient dose, might impede a

daphnid's mobility. Although not investigated in this study, the aggregation of nanoparticles on the antennae, a chemosensory organ important for feeding and reproductive behaviors, could adversely affect a daphnid's growth and reproduction (Oberdörster et al., 2006). Because nano-TiO_2 primary particles are smaller than the size range of particles daphnids feed on (400–40,000 nm), the presence of nano-TiO_2 in the digestive tract suggests that daphnids feed on nano-TiO_2 aggregates (Baun et al., 2008). Whether nano-TiO_2 is taken up by other tissues, excreted, or transformed in daphnids is unclear (Baun et al., 2008). Even if nano-TiO_2 is not absorbed into tissues, nano-TiO_2 in the digestive tract of daphnids could still contribute to bioaccumulation in the food web (see Section 4.4.3).

The behavior and heart rate of *Daphnia magna* were evaluated in daphnids exposed to photocatalytic nano-TiO_2 at 2.0 mg/L for 1 hour (Lovern et al., 2007). In this study, nano-TiO_2 had an average particle diameter of 30 nm, and tetrahydrofuran, an organic solvent used to prevent aggregation, was not detected in the final nano-TiO_2 suspension. The concentration of 2.0 mg/L was selected because it was the lowest observed effect level (LOEL) of *Daphnia magna* mortality after 48-hour exposure (Lovern and Klaper, 2006). Behavior (e.g., hopping frequency, appendage movement as an indicator of feeding frequency, and postabdominal claw curling) and heart rates were not affected by the 1-hour nano-TiO_2 exposure (Lovern et al., 2007).

5.2.1.2.3. Fish

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

The toxicological studies of photostable nano-TiO_2 in fish are very limited. The 96-hr acute toxicity of photostable nano-TiO_2 (DuPont uf-C) in rainbow trout produced an LC_{50} value of greater than 100 mg/L (Warheit et al., 2007a). However, DuPont uf-C is designed to protect plastics from UV-induced degradation, and is not known to be used in sunscreen; no fish studies of nano-TiO_2 intended for sunscreen use were found.

In contrast, photocatalytic nano-TiO_2, which may be used in drinking water treatment, has been tested in fish for acute effects (Griffitt et al., 2008; Zhu et al., 2008) and chronic effects (Federici et al., 2007) (see following discussion), as well as bioaccumulation (Zhang et al., 2006) and interaction with other heavy metals (see Section 4.2, Exposure). In the acute exposure study, the LC_{50} for a 48-hr

exposure to an anatase/rutile mixture of uncoated nano-TiO_2 was greater than 10 mg/L for zebrafish (in both female adults and <24-hr post-hatch fry) (Griffitt et al., 2008). For zebrafish eggs (blastula stage), acute exposures for 96 hours at up to 500 mg/L of either nano-TiO_2 or conventional TiO_2 (both uncoated anatase) did not cause developmental toxicity, as measured by survival rate of the zebrafish embryos and larvae, hatching rate of embryos, and malformation in embryos and larvae (Zhu et al., 2008). In the Zhu et al. (2008) study, nano-Al_2O_3 and conventional Al_2O_3 at up to 1000 mg/L also did not cause developmental toxicity to zebrafish eggs, but both nano-ZnO and conventional ZnO caused decreases in survival rates and hatching rate as well as increases in tissue ulceration at 1 mg/L or higher concentrations.

Sub-lethal toxicity was observed in juvenile rainbow trout after 14 days of exposure to photocatalytic P25 nano-TiO_2 (Federici et al., 2007). Respiratory toxicity and pathological changes in the gill and intestine were seen after a 14-day exposure at concentrations as low as 0.1 mg/L. Furthermore, there were signs of oxidative stress (increased concentrations of thiobarbituric acid substances, an indicator of lipid peroxidation and oxidative stress, in multiple tissues), and activation of anti-oxidant defenses (increased total glutathione levels in the gill). Na^+K^+-ATPase activity was also increased in the gill and intestine. Disturbances were observed in the metabolism of copper and zinc, but not of Na^+, K^+, Ca^{2+} or Mn. No major hematological disturbances were observed. Worth noting is that these effects occurred without appreciable titanium accumulation in the internal organs, suggesting no nano-TiO_2 accumulation, as discussed earlier in Section 4.4.1. The authors suggested that surface-bound TiO_2 (through surface adsorption) might play a role in toxicity, similar to the case of aluminum, a surface-acting toxicant that can cause systemic toxicity without significant internal accumulation. Federici et al. (2007) concluded that although nano-TiO_2 was not a major hemolytic toxicant or disruptor of ion regulation in this study, respiratory distress, organ pathologies, and oxidative stress were adverse effects.

5.2.1.2.4. Summary of Effects on Aquatic Organisms

Sub-lethal effects of nano-TiO_2 include decreases in daphnid reproduction by photostable nano-TiO_2 (Wiench et al., 2007), as well as respiratory distress, pathological changes in gills and intestine, and behavioral changes in fish (rainbow trout) by photocatalytic nano-TiO_2 (Federici et al., 2007). Several studies reported visible turbidity in nano-TiO_2 stock suspensions, and the actual nano-TiO_2 concentration in the liquid phase might be different from the concentration calculated from added nano-TiO_2 (Velzeboer et al., 2008; Zhang et al., 2006; Zhang et al., 2008). Given that natural organic matter in the environment can induce aggregation and deposition of nanoparticles or modify nanoparticle surface charges (Navarro et al., 2008), the bioavailability and behavior of nano-TiO_2 in the environment are likely to be different

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

5.2.1.3. Effects on Terrestrial Organisms

5.2.1.3.1. Plants

Information on nano-TiO_2 interactions with plants is available only for photocatalytic uncoated nano-TiO_2 in spinach (Table 5-1). Photocatalytic uncoated nano-TiO_2 has been shown to enhance the growth of spinach in several studies (Lei et al., 2008; Linglan et al., 2008; Mingyu et al., 2007a; Mingyu et al., 2007b; Yang et al., 2006; Zheng et al., 2005). When a nano-TiO_2 suspension was used to soak the seeds and was sprayed on the leaves, the germination rate and growth of the plant were enhanced (Zheng et al., 2005). These effects were at least partially due to nano-TiO_2-induced increases in the activity of several enzymes important for photosynthesis (Linglan et al., 2008), adsorption of nitrate, transformation of inorganic into organic nitrogen (Yang et al., 2006), and anti-oxidative stress response (Lei et al., 2008). Conventional TiO_2 suspensions showed either insignificant effects (in comparison with untreated controls) or much smaller effects than nano-TiO_2 did (Linglan et al., 2008; Zheng et al., 2005).

5.2.1.3.2. Invertebrates

The only known studies on the effects of nano-TiO_2 on terrestrial invertebrates include a study on an isopod, *Porcellio scaber* (Jemec et al., 2008), and a study on nematodes, *Caenorhabditis elegans* (Wang et al., 2009a). Living in soil, isopods and nematodes contribute to nutrient cycling and decomposition, and have been used as indicators of soil pollutants.

Jemec et al. (2008) investigated the effects of photocatalytic anatase nano-TiO_2 on the terrestrial isopod *Porcellio scaber,* known as woodlouse. Woodlice, about 16 mm long, live in the upper layer of soil and surface leaf litter. They break down organic matter and contribute to soil health, and are commonly used in ecological studies. In the Jemec et al. (2008) study, woodlice ate dry leaves that had been soaked in nano-TiO_2 dispersions (sonicated or non-sonicated). The sonication process decreased the mean agglomerate size from 780–970 nm in a non-sonicated dispersion to 350–500 nm. The activities of catalase and glutathione-S-transferase (GST), two anti-oxidative stress enzymes in the digestive gland (hepatopancrea) were measured. The activities of both enzymes were decreased at 0.5, 2000, and 3000 µg/g of non-sonicated nano-TiO_2, but not at middle concentrations (1, 10, 100, and 1000 µg/g) of non-sonicated nano-TiO_2 or at any concentration (1000, 2000, and 3000 µg/g) of sonicated nano-TiO_2 (Jemec et al., 2008). No changes in feeding rate, defecation rate, food assimilation efficiency, weight, or mortality were noted at concentrations up to 3000 µg/g of either sonicated or non-sonicated nano-TiO_2 in

the food. This study illustrates the importance of nano-TiO_2 dispersion preparation method on nano-TiO_2 toxicity.

Wang et al. (2009a) investigated the lethality, growth inhibition, and effects on reproduction of nano-TiO_2 and conventional TiO_2 in the nematode, *C. elegans*, a small free-living (i.e., not parasitic) roundworm that inhabits soil in temperate climates around the world and feeds on bacteria and fungi. In the laboratory, *C. elegans* is often cultured on agar plates or in liquid medium in a Petri dish and is often fed *E. coli*. In the Wang et al. (2009a) study, *C. elegans* strain Bristol N2 (wild-type) in L1 stage (larvae before the first molting) was exposed to anatase nano-TiO_2 and anatase conventional TiO_2 in water. In addition to lethality and growth inhibition, decreased reproduction was observed at lower mass concentrations of nano-TiO_2 than conventional TiO_2. The tested reproduction parameters were eggs inside body and the number of offspring per worm, which includes offspring at all stages beyond the egg over the entire brood period. The mechanism of reproductive effects was not investigated. Due to the lack of toxicity of supernatant of nano-TiO_2 (obtained by centrifuging the nano-TiO_2 suspension), dissolution of the particle does not contribute to observed nano-TiO_2 effects on *C. elegans* (Wang et al., 2009a).

5.2.1.4. Indirect and Interactive Ecological Effects

In addition to the direct toxicity of nano-TiO_2, indirect effects of nano-TiO_2 could also be important. Nano-TiO_2 could adsorb pollutants (Nagaveni et al., 2004; Pena et al., 2006), carry the pollutants into areas in an organism that the pollutants alone would not naturally appear (Moore, 2006), and increase the uptake of other pollutants (a "Trojan horse" effect). Consequently, nano-TiO_2 could enhance pollutant toxicity, and even cause toxicities different from those caused by exposure to the pollutant alone due to differences in distribution. Also, as discussed in Section 4.2, co-exposure to nano-TiO_2 in water increased the uptake of arsenic (Sun et al., 2007) and cadmium (Zhang et al., 2007) in carp, but toxicity was not measured in these two studies.

Nano-TiO_2 was found to have no effect on the toxicity of sediment and its elutriate in a study using certified reference material sediment (Blaise et al., 2008). The effects of 11 nanomaterials on sediment toxicity (as measured in two direct contact assays, the Microtox solid phase assay[8] and the Luminotox solid phase assay[9]) and sediment elutriate toxicity (as measured with the MARA assay[10]) 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 (IC_{25}) 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, IC_{20} is calculated after 15 minutes of exposure.

using a mixture of each nanomaterial and the certified reference material sediment at a 1:1 ratio. Photocatalytic nano-TiO_2 was one of only three tested nanomaterials that did not increase the sediment or elutriate toxicity in any of the three assays (Blaise et al., 2008).

5.2.1.5. Summary

Limited ecological toxicity information on nano-TiO_2 is currently available. Most ecotoxicological studies have tested photocatalytic nano-TiO_2 that would be suitable for water treatment, but only a few studies have used photostable nano-TiO_2 intended for sunscreen. Coated photostable nano-TiO_2 in sunscreen could lose its coating through processes such as aging, weathering, chemical alterations (e.g., change in pH), and metabolism or biotransformation in living organisms (e.g., digestion by daphnids). If so, the photocatalytic nano-TiO_2 core could be exposed and thus even photostable nano-TiO_2 could have photocatalytic properties.

Effects of chronic exposure to nano-TiO_2 have been investigated only in water fleas and fish. Although acute exposure effects have been studied in microorganisms and various aquatic macroorganisms, these studies focused on lethality or immobility and provided little insight on modes of action. For terrestrial organisms, only acute exposure to anatase nano-TiO_2 was investigated and only in invertebrates (*P. scaber* and *C. elegans*) and spinach. Photocatalytic nano-TiO_2 decreased reproduction in *C. elegans* without affecting body length. Although increased growth in spinach following acute exposure to anatase nano-TiO_2 could be useful for agricultural purposes, the effects of such growth promotion in an ecological system remain unclear. Photocatalytic nano-TiO_2 enhanced the uptake of arsenic and cadmium in fish, indicating the possibility of interactive effects between nano-TiO_2 and co-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-TiO_2 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-TiO_2 manufacturing?

5.2-3. Could ecological effects of pure nano-TiO_2 be predictive of effects from products containing nano-TiO_2 (e.g., containing stabilizers or surfactants)?

5.2-4. How can contributions of various nano-TiO_2 physicochemical properties to nano-TiO_2 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-TiO_2 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-TiO_2?

5.2-7. How do abiotic factors in the environment, such as UV, pH, oxygen level, and other chemicals, affect nano-TiO_2 and its ecological effects?

5.2-8. How do in vivo biochemical processes alter nano-TiO_2 physicochemical characteristics and toxicity?

5.2-9. What are the ecological effects of long-term exposure to nano-TiO_2?

5.2-10. What are the indirect ecological effects (e.g., on soil or water chemistry) of nano-TiO_2?

5.2-11. Nano-TiO_2 has anti-bacterial and anti-fungal properties. What are the effects of both photocatalytic and photostable nano-TiO_2 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-TiO_2? Are the toxicities of arsenic, cadmium, or other chemicals affected by nano-TiO_2? Conversely, do other compounds affect the uptake and toxicity of nano-TiO_2?

5.2-13. Is the available ecotoxicity evidence adequate to support ecological risk assessment for nano-TiO_2? If not, what is needed?

5.3. Health Effects

This section summarizes and evaluates the evidence of nano-TiO_2-induced health effects from epidemiological studies, laboratory animal studies, and a few selected ex vivo and in vitro studies. For a review of nano-TiO_2 in vitro effects, see Fond and Meyer (2006). Organized by human and laboratory animal studies and route of exposure, non-carcinogenic effects are discussed in Section 5.3.1; carcinogenic effects in Section 5.3.2.

5.3.1. Non-Carcinogenic Effects

This section summarizes in vivo studies of nano-TiO_2 non-carcinogenic effects through dermal, oral, respiratory, and other routes of exposure. The presentation is organized by exposure routes, because exposure routes play a profound role in toxicokinetics, toxicodynamics, and health effects. More studies have been completed on respiratory exposure (inhalation and instillation) than on other exposure routes. Studies investigating solely skin penetration (not health effects) are discussed in Section 4.6.2. Most studies tested photocatalytic nano-TiO_2, which could be suitable as an agent in drinking water treatment. Commercial sunscreens were tested in dermal exposure studies only. Known photostable nano-TiO_2 and rutile nano-TiO_2, which is expected to be photostable, were used in some studies (Chen et al., 2006; Mohr et al., 2006; Nemmar et al., 2008; Oberdörster et al., 1992; Pott and Roller, 2005; Wang et al., 2007a, 2007b; Warheit et al., 2007a, 2007b).

5.3.1.1. Studies in Humans

No epidemiological studies or case reports are available for nano-TiO_2 non-carcinogenic effects. A few case reports described non-carcinogenic effects in the respiratory system of workers exposed to TiO_2 particles of unspecified size. For example, exposure to conventional TiO_2 has been associated with pneumoconiosis (Yamadori et al., 1986), pulmonary fibrosis and bronchopneumonia (Moran et al., 1991), and pulmonary alveolar proteinosis (Keller et al., 1995). TiO_2 or titanium accumulation in the lung, sometimes years after workplace exposures, and titanium-loaded macrophages have also been reported in workers (Keller et al., 1995; Määttä and Arstila, 1975; Yamadori et al., 1986), as have titanium particles in the lymph nodes (Määttä and Arstila, 1975; Moran et al., 1991) and in the liver and spleen (Moran et al., 1991). None of these case reports, however, provided quantitative TiO_2 exposure data or measured potentially confounding variables such as exposures to crystalline silica and tobacco smoke.

One epidemiological study (Chen and Fayerweather, 1988) found no consistent relationship between TiO_2 (size not specified) exposure and chronic respiratory disease or fibrosis, but no conclusions can be drawn because of serious limitations, including restricting subjects to workers eligible for pensions; lack of information on the duration of TiO_2 exposure, asbestos or other chemical exposures; and the lack of detailed information on sampling.

5.3.1.2. Animal Studies

For the most part (except as noted below), laboratory animal toxicity studies have investigated the effects of acute or subchronic exposure to nano-TiO_2. This section presents in vivo studies of nano-TiO_2 (Tables 5-4 to 5-7) by route of exposure: dermal, oral, respiratory, and others. Most animal studies of nano-TiO_2 focus on photocatalytic nano-TiO_2, including P25. Although sunscreen nano-TiO_2 formulations are intended to be photostable, the coatings that impart photostability to anatase or part-anatase nano-TiO_2 in some sunscreen formulations are known to degrade over time (Barker and Branch, 2008; Dunford et al., 1997).

5.3.1.2.1. Toxicity from Dermal Exposure

Toxicity findings from studies of dermal exposure to nano-TiO_2 or sunscreen that contains TiO_2 are presented in Table 5-4. For healthy unflexed skin, adverse health effects are not expected from dermal exposure to photostable nano-TiO_2 in sunscreen (NANODERM, 2007; SCCP, 2007). Photocatalytic nano-TiO_2, however, sometimes is used in sunscreens (Barker and Branch, 2008; Dunford et al., 1997). Photocatalytic nano-TiO_2 can generate ROS when exposed to UV and can cause oxidative stress and cytotoxicity in cells (cultured human fibroblasts) and in cell-free in vitro experiments (Dunford et al., 1997; Lu et al., 2008). To date, the effects of long-term or repeated use of sunscreen containing nano-TiO_2 have not been investigated in vivo, and no case reports of skin damage from such use are currently available. As discussed earlier, most available studies indicate penetration of the outer skin layer and the stratum corneum, but not penetration of living skin cells.

After a single topical application of photocatalytic nano-TiO_2, laboratory animals showed no skin irritation 4 hours after application or sensitization 3 days after application (Warheit et al., 2007a). Furthermore, although some sunscreens containing TiO_2 (size not specified) increased skin absorption of herbicides and pesticides (2,4-D, paraquat, parathion or malathion), TiO_2 alone actually decreased the skin absorption of the tested herbicide, 2,4-D (Brand et al., 2003). The investigators reported that a solvent in the sunscreen caused increased skin absorption of herbicides, and this secondary effect can be avoided by substituting phenyl trimethicone as the solvent (Brand et al., 2003).

Some researchers, such as Nohynek et al. (2007), have noted a discontinuity between in vitro and in vivo testing results, particularly for skin toxicity. Some in vitro cultures or preparations (other than those using intact skin samples) lack the stratum corneum layer, which according to currently available data can block penetration, such that in vitro tests might overstate toxicity of chemicals like TiO_2. Of the investigations reviewed, only three report in vivo studies of health effects after dermal exposure to TiO_2 [(Warheit et al., 2007a); pages 16, 17, 41–43 of (NANODERM, 2007)], and only two of those used nano-TiO_2 intended for sunscreen [pages 16, 17, 41–43 of (NANODERM, 2007)]. [Warheit et al. (2007a) used ultrafine particles, roughly 100 nm in size.] All three studies used a single application, and the longest exposure was only 3 days. The NANODERM (2007) report concluded that "TiO_2 exposure did not modify the viability, proliferation, apoptosis, and differentiation [or] adhesive properties of skin cells." As discussed previously, skin penetration studies have shown that some nano-TiO_2 can stay in hair follicles for 10 days.

With relatively few in vivo dermal exposure studies investigating nano-TiO_2 skin absorption and penetration (Table 4-5) and health effects (Table 5-4), several data gaps on the health effects of dermal exposure to nano-TiO_2 are evident. First, information on the dermal penetration and effects of nano-TiO_2 in flexed skin and structurally compromised skin is lacking. Flexed healthy skin (Rouse et al., 2007; Zhang and Monteiro-Riviere, 2008) and compromised skin (Zhang and Monteiro-Riviere, 2008), including UV-exposed skin (Mortensen et al., 2008), have been shown to allow nanoparticles (other than nano-TiO_2, which was not tested) to penetrate deeper than healthy non-flexed skin. Sunscreen containing nano-TiO_2 is expected to be used on flexed healthy skin and misused on sunburned skin or skin with micro-lesions, such as microscopic cuts due to shaving. Cytotoxicity was seen in cultured skin cells treated with nano-TiO_2 (Kiss et al., 2008), and the authors postulated that, in skin with compromised epidermis structure (e.g., sunburned skin or "soaked" skin), contact could occur between nano-TiO_2 from sunscreen and living cells in the skin and lead to adverse effects. Second, effects from long-term, repeated dermal exposures to nano-TiO_2 in sunscreen, similar to real-life exposure, have not been studied. Finally, the toxicity of the various intermediate forms of nano-TiO_2 in the production process (possible sources of occupational exposure, by the dermal and other routes) has not been studied.

Table 5-4. Summary of health effects of nano-TiO_2 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-TiO_2 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: *N*,*N*-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

5.3.1.2.2. Toxicity from Oral Exposure

Currently only three toxicological studies of nano-TiO_2 through oral exposure are available (Table 5-5). Two of them observed the toxicity for up to 2 weeks after a single oral gavage of nano-TiO_2 (Wang et al., 2007a; Warheit et al., 2007a), and the other investigated genomic instability after nano-TiO_2 exposure through drinking water for 5 or 10 days (Trouiller et al., 2008).

The Warheit et al. study (2007a) was intended to provide basic hazard screening information on well-characterized types of nano-TiO_2 through a "base set" of tests spanning mammalian toxicity, genotoxicity, and aquatic (ecological) toxicity endpoints. The acute oral toxicity aspect of this project involved female rats receiving a single oral gavage of up to 5000-mg/kg photocatalytic nano-TiO_2 (P25) (3 rats per dose). The authors reported "no biologically important body weight loss" and no gross lesions at necropsy 14 days after the gavage. Given that this was a basic screening study, no information on organ weights, histological examinations, or blood tests (hematological or biochemical) was obtained, and thus it was not meant to rule out systemic toxicity or functional changes. However, the study does provide evidence that up to 5000-mg/kg nano-TiO_2 was not lethal as tested.

In the Wang et al. study (2007a), male and female mice received a single oral gavage of 5000-mg/kg TiO_2 as 25-nm rutile spindles, 80-nm rutile spindles, or 155-nm anatase octahedrons (see Table 5-5 for more details). The large dose was selected because of the expected low toxicity and was administrated according to OECD testing procedures. No obvious acute toxicity was evident over a 2-week period. However, liver and kidney toxicity were indicated by biochemical parameters in the serum and by pathological examination. Although no abnormal pathology was observed in the heart, lung, testicle/ovary, and spleen tissues, myocardial damage was suggested by increases in serum lactate dehydrogenase (LDH) and alpha-hydroxybutyrate dehydrogenase (α-HBDH), although such increases might also reflect damage to other organs. Morphological changes in the brain were seen in the hippocampus in both the 80-nm and 155-nm groups. The main organs with elevated TiO_2 concentrations (measured only in female mice) were the liver, spleen, kidneys, lungs, and brain. Although the liver is expected to receive most of the TiO_2 absorbed from the gastrointestinal tract through the portal vein, elevated TiO_2 levels in the liver were observed only in the 80-nm group. The reason for this size-specific elevation in hepatic TiO_2 concentration remains unknown.

The preliminary results of the Trouiller et al. (2008) study showed increased DNA and chromosomal damage in various tissues of mice given 60–600 µg/mL photocatalytic nano-TiO_2 (P25) in drinking water for 5 days. Furthermore, the offspring of mice that were given nano-TiO_2 in drinking water in the second half of the pregnancy showed increases in DNA deletions in the eye-spot assay (Trouiller et al., 2008), which detects reversion of the mouse *pink-eyed unstable* (p^{un}) mutation through

DNA deletions of duplicated *pink-eyed dilution* (*p*) gene in the offspring of C57Bl/6Jp^{un}/p^{un} mice (Reliene and Schiestl, 2003; Schiestl et al., 1997). This study showed not only genotoxicity and clastogenicity, but also multi-generation effects of photocatalytic nano-TiO_2 through oral exposure. Although the concentrations investigated in this study are very high, the suggested modes of action and effects of exposure during pregnancy are noteworthy, particularly for photocatalytic nano-TiO_2. This work is also relevant to discussions of the carcinogenicity of nano-TiO_2 (see Section 5.3.2). The application of genotoxicity data to the question of potential carcinogenicity is based on the premise that genetic alterations are found in all cancers. Mutagenicity/genotoxicity is the ability of chemicals to alter the genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the epigenome including proteins associated with DNA or RNA, can also cause transmissible changes. Genetic alterations can occur via a variety of mechanisms including gene mutations, deletions, translocations, or amplification; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

Table 5-5. Summary of health effects of nano-TiO_2 particles in mammalian animal models: oral route.[a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse [wild-type and C57Bl/6Jp^{un}/p^{un}]	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/6Jpun/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-TiO_2 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)]	Nano-TiO_2 (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, uncoated, 25 nm (measured average size 21.1±5.1 nm), surface area 43.0 m^2/g, column/spindle shape, purity >99% (Chen, 2008) Nano-TiO_2 (Hangzhou Dayang Nanotechnology Co. Ltd.), rutile, uncoated, 80 nm (measured average size 71.4±23.5 nm), surface area 22.7 m^2/g, column/spindle shape, purity >99% (Chen, 2008) Fine TiO_2 (Zhonglian Chemical Medicine Co.), 155 nm (measured average size 155.0±33.0 nm), surface area 10.4 m^2/g, anatase, uncoated, octahedrons, purity >99% (Chen, 2008)	Single oral gavage (acute effects) Dose – 5000 mg/kg 10 female and 10 male mice per TiO_2 size group Necropsy at 2 weeks after the gavage	Hepatic toxicity: 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). Pathological changes: hydropic degeneration around the central vein, spotty necrosis of hepatocytes (males and females in 80 nm and 155 nm groups). Nephrotoxicity: 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] Pathological changes: swelling in renal glomerules and proteinic liquid in renal tubule (males and females in 80 nm group). Possible brain toxicity: 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). Possible myocardial damage: 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. No pathological changes in heart. No pathological changes in heart, lung, testicle/ovary or spleen in male and female mice exposed to either 80 nm or 155 nm TiO_2. No pathological changes in any organs of mice exposed to 25 nm TiO_2. TiO_2 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).	Wang et al. (2007a) Chen (2008)

[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 chromotography
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)
LD_{50} – 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).

5.3.1.2.3. Toxicity from Respiratory Exposure

This section discusses the health effects of nano-TiO_2 exposure through the respiratory tract (Table 5-6). Two methods of exposure commonly employed for studies of respiratory toxicity are inhalation and instillation. Instillation can be performed in various ways, but essentially involves the direct administration of a substance to the lungs rather than allowing the subject to inhale the material. Intratracheal instillation "can be a useful and cost-effective procedure for addressing specific questions regarding the respiratory toxicity of chemicals, as long as certain caveats are clearly understood and certain guidelines are carefully followed" (Driscoll et al., 2000). Among the advantages of instillation are that it permits researchers to control the doses administered into the lung and allows fast administration of test material to the lower respiratory tract. Instillation studies can be useful for identifying most types of effects (other than upper respiratory tract effects, such as nasal effects) and for comparing the relative potency of compounds, and for this reason are of interest for screening different materials for toxicity. Additionally, instillation studies require smaller amounts of test material, and chances of incidental ingestion exposure (as in whole-body chamber inhalation) are lower than in inhalation studies (Driscoll et al., 2000; Osier et al., 1997). On the other hand, instillation exposure involves invasive delivery, bypassing of the upper respiratory tract, confounding effects from the instilled vehicle, and the use of higher doses or dose rates than those tested in inhalation experiments. Confounding effects are also a concern from anesthesia (needed for instillation, but not inhalation), which could affect the retention and clearance of the test material (Driscoll et al., 2000). Furthermore, studies have shown that exposure to the same particle through intra-tracheal instillation and inhalation can yield different responses. For example, compared to inhalation, instillation caused more particles to be deposited in the basal regions of the lung and caused particles to be distributed less homogenously (Osier et al., 1997). Also, results from instillation cannot be extrapolated quantitatively for estimating inhalation results (Driscoll et al., 2000).

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

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

The tendency of nano-TiO_2 to agglomerate raises an important issue for interpreting experimental toxicology studies when the respiratory tract is the portal of entry. Upon inhalation, insoluble particles will deposit in the lung according to the aerodynamic diameter of the particulate unit (i.e., the agglomerate) and the physiological/morphometric characteristics of the subject. Once deposited as a result of inhalation or intratracheal instillation, additional factors (e.g., physicochemistry of the particles, biochemistry of the fluid lining of the lung, and other pharmacokinetic factors of the subject) may impact particle size and composition and determine the ultimate dose to the target cell/molecule. The influence of the lung milieu on agglomeration is discussed in more detail below.

It should be noted that the concentrations in available respiratory toxicity studies of nano-TiO_2 are presumably much higher than likely ambient or occupational exposure levels. High concentrations of fine-mode particles are known to cause the phenomenon of "particle overload." In its simplest terms, at sufficiently high concentrations, the body's ability to clear inhaled particles is severely compromised to the point that effects occur that would not occur at high-end "real-world" exposures [see ILSI Risk Science Institute Workshop Participants (2000) for summary]. Thus, under particle overload conditions, exposure-response relationships and even the type of responses produced can be unreliable. However, the nanoparticle exposures evoking particle overload have not been fully described.

Effects in Respiratory Tract

As discussed below and summarized in Table 5-6, pulmonary effects studied through inhalation or instillation of nano-TiO_2 include pulmonary inflammation, recruitment of neutrophils and macrophages, nano-TiO_2 aggregate-loaded macrophages, disruption of alveolar spaces, alveoli enlargement, proliferation of alveolar type II pneumocytes, and increases in alveolar epithelial thickness. Selected instillation studies are highlighted here primarily for effects not investigated in inhalation studies (i.e., effects outside the respiratory tract and interactions with other factors).

Some of the factors that affect nano-TiO_2 respiratory tract toxicity were investigated by Oberdörster et al. (2000). Toxicity of nano-TiO_2 could be decreased by cross-tolerance to oxidative stress, because nano-TiO_2 given through an intra-tracheal instillation caused less inflammation in rats previously exposed (and adapted) to Teflon fumes than in rats that were not adapted. Furthermore, nano-TiO_2 induced more severe pulmonary inflammation in compromised rats, which had been given an endotoxin to mimic gram-negative bacterial infections, than in healthy rats.

Inhalation and Instillation in the Same Study

Grassian et al. (2007a) exposed mice to nano-TiO_2 through either inhalation or intranasal instillation. After instillation exposures to similar surface area doses (based on primary particle surface areas) of 5-nm anatase nano-TiO_2 and 21-nm anatase/rutile nano-TiO_2, mice showed a more severe inflammation response to 21-nm nano-TiO_2 than to 5-nm TiO_2. This example shows that surface area alone is not a sufficient dose metric in all studies (Grassian et al., 2007a; Warheit et al., 2007a), especially when the crystal form and other factors are not the same. In the Grassian et al. (2007a) study, the aggregates of 21-nm and 5-nm nano-TiO_2 differed in both size and density, either of which could affect the surface area that would interact with the tissues. Although the same nano-TiO_2 was used in both inhalation and intranasal instillation, direct comparisons of exposure routes effects were not feasible for two reasons. First, the exposure doses were not the same, whether the doses were expressed as particle concentrations in air or solution, estimated particle mass per mouse, or estimated particle surface area per mouse. Second, different vehicles (water for inhalation and saline for instillation) were used and the sizes of agglomerates were larger in inhalation aerosols than in instillation.

In a study by Osier et al. (1997), acute intra-tracheal inhalation of high levels (125 mg/m^3) of fine and nano-TiO_2 caused less severe pulmonary response than intra-tracheal instillation. Intra-tracheal inhalation involved delivering aerosols to the trachea of anesthetized rats.

Inhalation Studies

The effects in the respiratory tract after inhalation of nano-TiO_2 were consistent among studies. With increases in exposure duration, pulmonary lesions in rodents evolve from reversible pulmonary inflammation (in rats, mice, and hamsters) to impaired particle clearance or overload (in rats and mice, but not hamsters) and cellular proliferation (in rats and mice, but not hamsters). In rats, but not in mice or hamsters, chronic exposure leads to pulmonary alveolar fibrosis, metaplasia, and eventually lung tumors.

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

In subchronic studies of nano-TiO_2 exposure for 12 or 13 weeks, pulmonary inflammation, pathological changes in the lung (including fibrosis), and impairment of alveolar macrophage-mediated test particle clearance were reported (Baggs et al., 1997; Bermudez et al., 2002; Bermudez et al., 2004; Hext et al., 2002; Hext et al., 2005; Oberdörster et al., 1994). Similar to pulmonary lesions after acute and subacute exposure, pulmonary lesions after subchronic inhalation exposure were also decreased with recovery time, but some lesions, such as fibrotic reactions in the lung, were not completely reversed even after 1 year of recovery.

Species differences to nano-TiO_2 effects were observed among rats, mice, and hamsters (Baggs et al., 1997; Bermudez et al., 2002; Bermudez et al., 2004; Hext et al., 2002; Hext et al., 2005; Oberdörster et al., 1994). Pulmonary responses after 13 weeks of exposure were generally most severe in rats, followed by mice, and least severe in hamsters. Rats and mice, but not hamsters, experienced overload at 10 mg/m^2 nano-TiO_2. Furthermore, only rats had fibroproliferative lesions and alveolar epithelial bronchiolization (a type of metaplasia).

In chronic studies of nano-TiO_2 inhalation in rats (Creutzenberg et al., 1990; Gallagher et al., 1994; Heinrich et al., 1995) and mice (Heinrich et al., 1995), lung tumors occurred in rats, but not in mice (for more on carcinogenicity effects in these studies, see Section 5.3.2) . In the study of Creutzenberg et al. (1990), decreased pulmonary clearance (overload) was clearly demonstrated by using two sizes of tracer particles after nano-TiO_2 exposure. During the 24-month exposure to nano-TiO_2 (see Table 5-6 for concentrations), rats inhaled (nose-only) two types of radioactive tracers at 3, 12, and 18 months after the beginning of the experiment. The half-times for pulmonary clearance of the smaller tracer particles (0.35-µm $^{59}Fe_2O_3$) were more than 3-times longer in rats exposed to nano-TiO_2 at all three tested time points, indicating overload. For the larger tracer particles (3.5-µm ^{85}Sr polystyrene), overload was seen at 3 and 12 months, and the clearance was back to control level at 18 months, which may be due to increased lung weight, altered lung structure, and altered breathing pattern, all of which could consequently change the deposition of ^{85}Sr polystyrene particles (Creutzenberg et al., 1990).

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

The effects of respiratory exposure to nano-TiO_2 are not limited to the respiratory system. In rats exposed to 5-mg nano-TiO_2/kg BW of rutile nano-TiO_2 rods through a single intra-tracheal instillation, observed effects included increases in the numbers of monocytes and granulocytes in the blood (signs of systemic inflammation); decreases in the number of platelets in the blood (platelet aggregation); and cardiac edema (Nemmar et al., 2008). In mice exposed to rutile and anatase nano-TiO_2 through intranasal instillation, pathological changes were observed in the kidney, and temporary liver injury was suggested by changes in serum biomarkers (Wang et al., 2008b).

Endothelium-dependent arteriolar dilation was impaired (decreased) by both fine TiO_2 and nano-TiO_2 inhaled by rats, more so by nano-TiO_2 than fine TiO_2 at similar lung load mass doses (Nurkiewicz et al., 2008). This microvascular dysfunction was due to fine TiO_2- and nano-TiO_2-induced increases in ROS in the microvascular wall, increases in nitrotyrosine expression in spinotrapezius microcirculation, and decreases in microvascular NO production (Nurkiewicz et al., 2009). In both fine TiO_2-and nano-TiO_2-treated groups, vascular smooth muscle sensitivity to NO was not altered, but the microvascular NO bioavailability was compromised (Nurkiewicz et al., 2009).

Effects in Brain

Since 1970, scientists have known that inhaled ultrafine air pollutants and engineered nanoparticles translocate into the brain (Oberdörster et al., 2004). Inflammatory responses, altered neurotransmitter levels, and pathological changes have been observed in rodent brains after inhalation of manganese oxide (Elder et al., 2006); instillation of nano carbon black (Tin Tin Win et al., 2008); and inhalation of ultrafine elemental ^{13}C particles (Oberdörster et al., 2004). A few recent studies showed that anatase and rutile nano-TiO_2 translocate into the brain following intranasal instillations (Wang et al., 2008a; Wang et al., 2008b, 2007b).

The only available studies of nano-TiO_2 effects on the central nervous system are from a research group that has administered nano-TiO_2 to mice using intranasal instillation (Wang et al., 2008a; Wang et al., 2008b; Wang et al., 2007b). These researchers have reported increased oxidative stress and inflammatory response, altered concentrations and metabolism of neurotransmitters, and pathological changes in the mouse brain. When mice were given 25-nm rutile, 80-nm rutile, or 155-nm anatase nano-TiO_2 though intranasal instillation (50 mg nano-TiO_2/kg BW every two days for 2, 10, 20, or 30 days), changes in neurotransmitter levels in the brain were observed only in mice exposed to 80-nm and 155-nm nano-TiO_2, whereas brain TiO_2 concentrations were similar for all three sizes of nano-TiO_2 (Wang et al., 2007b). After intranasal instillation of 80-nm rutile or 155-nm anatase nano-TiO_2 (500 μg per mouse every other day for up to 30 days), the highest titanium concentrations in the brain were in the hippocampus and olfactory bulb, the two regions where most pathological changes were also seen (Wang et al., 2008a; Wang et al., 2008b). The hippocampus and astrocytes seem to be the targets of nano-TiO_2 toxicity in the brain (Wang et al., 2008a; Wang et al., 2008b). At the ultra-structural level, mitochondria appear to be a target of nano-TiO_2 in nerve cells after both in vivo and in vitro exposures (Long et al., 2006; Wang et al., 2008b). For the whole brain, inflammatory responses and oxidative stress, including lipid peroxidation and protein oxidation, were detected as elevated levels of oxidative markers and cytokines in mice exposed to 80-nm rutile and 155-nm anatase nano-TiO_2 (Wang et al., 2008a; Wang et al., 2008b).

Levels of several neurotransmitters, including norepinephrine, 5-hydroxytryptamine, homovanillic acid, 5-hydroxyindole acetic acid, dopamine, and glutamic acid, were altered after intranasal instillation of nano-TiO_2 (Wang et al., 2008a; Wang et al., 2008b; Wang et al., 2007b). Nitric oxide, which serves as a neurotransmitter and an important player in inflammatory responses, was also increased in the brain of mice exposed to 80-nm and 155-nm nano-TiO_2 (Wang et al., 2008a). Additionally, the activity of cholinesterase, which inactivates the neurotransmitter acetylcholine, increased (Wang et al., 2008a). These changes showed that the concentrations and metabolism of neurotransmitters in the brain were affected by nano-TiO_2 given through intranasal instillations.

Table 5-6. Summary of health effects of nano-TiO_2 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]	Nano-TiO_2 (Nanostructured and Amorphous Materials), anatase, 5 nm, measured BET surface area 219±3 m^2/g, surface functionalization: O, O-H, H_2O. Aerosol size: 119±1.56 nm (inhalation high dose), 122.9±1.55 nm (inhalation low dose) Nano-TiO_2 (Degussa), anatase/rutile, 21 nm, BET surface area 41±1.1 m^2/g, surface functionalization: O, O-H, H_2O. Aerosol size: 138.8±1.44 m^2/g (inhalation high dose), 152.9±1.38 m^2/g (inhalation low dose)	Single inhalation exposure for 4 hours Particle concentration in chamber: 5 nm TiO_2: Low: 0.77 mg/m^3 (necropsy immediately after exposure) High: 7.22 mg/m^3 (necropsy immediately after exposure); 7.35 mg/m^3 (necropsy 20 hours after the end of exposure) 21 nm TiO_2: Low: 0.62 mg/m^3 (necropsy immediately after exposure) High: 7.16 mg/m^3 (necropsy immediately after exposure); 7.03 mg/m^3 (necropsy 20 hours after the end of exposure)	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). No changes in histology of the lung, total protein, LDH activity, or neutrophil number in BAL fluid. Nano-TiO_2 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. Calculated/estimated particle mass per mouse (µg) and particle surface area (cm^2): 5 nm TiO_2 Low: 1.3 µg/mouse and 3.2 cm^2 (immediately after exposure) 5 nm TiO_2 High: 12.5 µg/mouse and 30.3 cm^2 (immediately after exposure) 12.7 µg/mouse and 30.7 cm^2 (20 hours after exposure) 21 nm TiO_2 Low: 1.1 µg/mouse and 2.2 cm^2 (immediately after exposure) 21 nm TiO_2 High: 12.4 µg/mouse and 24.8 cm^2 (immediately after exposure) 12.2 µg/mouse and 24.4 cm^2 (20 hours after exposure)	Grassian et al. (2007a)
		Single intra-nasal instillation Particle concentration in instillation solutions: 5 nm TiO_2: Low: 0.1 mg/mL Medium: 0.4 mg/mL High: 0.6 mg/mL 21 nm TiO_2: Low: 0.5 mg/mL Medium: 2.0 mg/mL High: 3.0 mg/mL Necropsy 24 hours after instillation	21 nm TiO_2 induced more inflammation than 5 nm TiO_2: 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. No pathological changes in lung; no changes in TNF-α in BAL fluid. 21 nm anatase/rutile TiO_2 and 5 nm anatase TiO_2 do not share the same dose-response curve for neutrophil concentration in BAL fluid as a function to either particle mass or surface area. Calculated/estimated particle mass per mouse (µg) and particle surface area (cm^2): 5 nm TiO_2 Low: 5 µg/mouse and 12.1 cm^2 5 nm TiO_2 Medium: 20 µg/mouse and 48.4 cm^2 5 nm TiO_2 High: 30 µg/mouse and 72.6 cm^2 21 nm TiO_2 Low: 25 µg/mouse and 12.5 cm^2 21 nm TiO_2 Medium: 100 µg/mouse and 50 cm^2 21 nm TiO_2 High: 150 µg/mouse and 75 cm^2	

Table 5-6. Summary of health effects of nano-TiO_2 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-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [female CDF (F344)/CrlBR] Mouse [female B6C3F1/CrlBR] Hamster [female Syrian golden (Lak:LVG [SYR] BR)]	Fine TiO_2 (DuPont), rutile; aerosol 1.36 – 1.44 µm MMAD Nano-TiO_2 (P25), photocatalytic, average primary particle size 21 nm, 1.37 µm MMAD; aerosols: 1.29-1.44 µm MMAD	Subchronic inhalation Fine TiO_2: 0, 10, 50 or 250 mg/m^3 Nano-TiO_2: 0, 0.5, 2, or 10 mg/m^3 6 hr/day, 5 days/wk for 13 weeks 0 (immediately after exposure), 4, 13, 26, or 52 (up to 46 and 49 for hamsters exposed to fine TiO_2 and nano-TiO_2, respectively) weeks of recovery before sacrifice	Lung burden of fine TiO_2: Immediately after exposure: lung burden of fine TiO_2: mice > rats > hamsters at 50 and 250 mg/m^3: rats > mice > hamsters at 10 mg/m^3. The lung burden decreased with time after exposure. 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). Pulmonary clearance kinetics of fine TiO_2: 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/m^3. Lung burden of nano-TiO_2: Lung burden of nano-TiO_2: rats ≥ mice > hamster. Immediately after exposure, at 10 mg/m^3, rats and mice had same lung burdens for nano-TiO_2. At 2 or 0.5 mg/m^3, rats had more lung burden. Mice and rats, but not hamsters, have pulmonary particle overload at 10 mg/m^3. Pulmonary clearance kinetics of nano-TiO_2: At 10 mg/m^3, 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/m^3, rats, mice and hamsters had biphasic decreases in lung burn, and rats only had detectable nano-TiO_2 after the whole recovery period. Burden in the lymph nodes associated with lung: During the whole recovery time, burden increased with time in rats of 10 and 5 mg/m^3 groups, and in mice of 10 mg/m^3 group. No nano-TiO_2 was detected in hamster lymph nodes at any time point or treatment group. General health of rats, mice and hamsters: 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/m^3 fine TiO_2 had a consistent lower weight during the recovery period, but rats exposed to 250 mg/m^3 fine TiO_2 had a consistent heavier weight. Hamster exposed to fine TiO_2 had decreases in weight gain after exposure, and recovery 6 weeks post exposure. Hamsters exposed to nano-TiO_2 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.	Fine TiO_2: Bermudez et al. (2002) Nano-TiO_2: Bermudez et al. (2004) Comparison of fine and nano-TiO_2 data reported in Bermudez et al. (2002) and Bermudez et al. (2004): Hext et al. (2002, 2005)

Table 5-6. Summary of health effects of nano-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
(continuation from previous page)			Pulmonary inflammation after fine TiO_2 exposure: Rats, mice and hamsters had pulmonary inflammation, and only hamsters had full recovery. Rats generally had more severe inflammation, and hamsters had the least. Fine TiO_2 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. Fine TiO_2 exposure: LDH levels in BAL fluid transiently increased in mice and rats Pulmonary inflammation after nano-TiO_2 exposure: Rats and mice have pulmonary inflammation. Nano-TiO_2 exposure: Rats and mice, but not hamsters, in the 10 mg/m^3 groups have increased numbers of macrophage and neutrophil and concentrations of LDH and protein in BAL fluid. Pulmonary lesions are most severe in rats, and least in hamsters. Fine TiO_2 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. Only rats had a progressive fibroproliferative lesion and alveolar epithelial metaplasia (bronchiolization). Fine TiO_2 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. Nano-TiO_2 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/m^3 group. With increasing time post exposure, the lesions became more severe. Species and particle differences: Overload was seen in rats and mice (but not hamsters) exposed to 50 and 250 mg/m^3 fine TiO_2 or 10 mg/m^3 nano-TiO_2. Lung TiO_2 burdens and tissue responses in mice, rat and hamsters exposed for 13 weeks to 10 mg/m^3 nano-TiO_2 or to 50 mg/m^3 fine TiO_2 were similar for all three species.	

Table 5-6. Summary of health effects of nano-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [female Wistar] Mouse [female NMRI]	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 dye to increases in lung weight, altered lung structure and breathing pattern, which lead to more in the tracheo-bronchial region of the long and apparently higher clearance rates.	Creutzenberg et al. (1990)
			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-TiO_2 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_2O (manufacturer reported primary particle 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-TiO_2 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	Nano-TiO_2 caused more severe and prolonged (~1 year) pulmonary inflammatory response (i.e., increase in alveolar macrophages, polymorphonuclear neutrophils, and lavagable protein) than fine TiO_2. 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-TiO_2 and fine TiO_2 shared the same dose response curve. More severe and prolonged impairment of alveolar macrophage-mediated particle clearance in rats exposed to nano-TiO_2 than rats exposed to fine TiO_2. Seven months after TiO_2 exposure, fine TiO_2 exposed (but not nano-TiO_2 exposed) rats showed normal clearance rates. Pathological changes in the lung: Nano-TiO_2 caused greater epithelial effects (Type II cell proliferation, occlusion of pores of Kohn) and more interstitial fibrotic foci than fine TiO_2. Dosimetry: Nano-TiO_2 and fine TiO_2 had a similar mass deposition in the lower respiratory tract and same retention in the alveolar space up to 1 year after exposure. Nano-TiO_2 showed longer total pulmonary retention (retention halftime: ~500 days for nano-TiO_2, ~170 days for fine TiO_2), 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-TiO_2 depends mainly on mucocillary clearance, while fine-TiO_2 depends on clearance to the gastrointestinal tract) than fine TiO_2.	Oberdörster et al. (1994)

Table 5-6. Summary of health effects of nano-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [male Wistart (strain Crl: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 polymophonuclear 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 neutrophillic 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-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rats [female Sprague-Dawley [Hla:(SD)CVF]]	Fine TiO_2, primary particle <5 µm, 99% rutile (reported vendor), BET surface area 2.34 m^2/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 Nano-TiO_2 (P25), primary particle 21 nm, 80% anatase, 20% rutile (reported by vendor), BET surface area 48.08 m^2/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	Short-term inhalation Whole body chamber exposure Exposures selected for not alter BAL markers of pulmonary inflammation or lung damage Exposure to fine TiO_2: aerosol concentration x exposure time (actual deposition in lung) 15 mg/m^3 x 480 min (90 µg) 16mg/m^3 x 300 min (67 µg) 12 mg/m^3 x 240 min (36 µg) 6 mg/m^3 x 240 min (20 µg) 3 mg/m^3 x 240 min (8 µg) Exposure of nano-TiO_2: aerosol concentration x exposure time (calculated/actual deposition in lung) 10 mg/m^3 x 720 min that took place over 3 days (38 µg) 12 mg/m^3 x 240 min (19 µg) 6 mg/m^3 x 240 min (10 µg) 3 mg/m^3 x 480 min (10 µg) 12 mg/m^3 x 120 min (10 µg) 3 mg/m^3 x 240 min (6 µg) 1.5 mg/m^3 x 240 min (4 µg) Shame exposure (control): 0 mg/m^3 x 240 min 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	Histology of the lung: No significant inflammation. Particle accumulation in alveolar macrophage. Anuclear alveolar macrophages were seen in both nano-TiO_2 and fine TiO_2 exposed rats, but not in shame exposed rats. Anuclear alveolar macrophages are presumed to be an apoptotic change. Endothelium-dependent arteriolar dilation as measured after intraluminal infusion of the Ca^{2+} ionophore A23187 in exteriorized spintrapezius muscle: Both fine TiO_2 and nano-TiO_2 exposures impaired arteriolar dilation in a dose-dependent manner, and nano-TiO_2 exposure produced greater impairment than fine TiO_2 at similar pulmonary load doses. No-effect dose of fine TiO_2 was 8 µg (as in lung deposition), and for nano-TiO_2 was 4 µg. On a mass base, nano-TiO2 was about one order of magnitude more potent than fine TiO_2; on total particle surface area base calculated by BET surface area, fine TiO_2 would be more potent than nano-TiO_2 (the authors suspected overestimation of the total nano-TiO2 surface area delivered, since no agglomeration was considered). Additional nano-TiO_2 exposure conditions (12 mg/m^3 x 2 h; 4 mg/m^3 x 6 h; 8 mg/m^3 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.	Nurkiewicz et al. (2008)

Table 5-6. Summary of health effects of nano-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
		Same exposure conditions as above (Nurkiewicz et al., 2008) for endogenous microcascuarl NO production tests, but only three groups in all other tests: aerosol concentration x exposure time (actual deposition in lung) Shame exposure (control): 0 mg/m^3 x 240 min Fine TiO_2: 16mg/m^3 x 300 min (67 µg) Nano-TiO_2: 6 mg/m^3 x 240 min (10 µg) 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, microvascualr oxidative stress, and nitrotyrosin staining	Same impairment of arteriolar dilation at 67 µg fine TiO_2 and 10 µg nano-TiO_2: more than 50% decrease compared to shame treated controls after Ca^{2+} ionophore A23187 injection at 20 and 40 psi ejection pressures. No change in arteriolar dilation in response to sodium nitroprusside (NO donor) in either 67 µg fine TiO_2 or 10 µg nano-TiO_2 exposed rats, indicating no change in vascular smooth muscle sensitivity to NO. Increased ROS amount in the microvascular wall in both 67 µg fine TiO_2 and 10 µg nano-TiO_2 groups at the same level as measured by ethidium bromide fluorescence. Increased nitrotyrosine expression in 10 µg nano-TiO_2 treated rats (not measured in fine TiO_2 group) in lung (3 folds) and spinotrapezius microcirculation (4 folds), as compared to shame exposure, suggesting nitrosative injury in lung and systemic microcirculation. Decreased Ca^{2+} ionophore A23187-stimulated endogenous microvascular NO production in fine TiO_2 and nano-TiO_2 treated groups in a dose-dependent manner: Similar to shame control, the NO production was sensitive to nitric oxide synthase inhibition caused by N^G-monomethyl-Larginine. 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 Ca^{2+} ionophore A23187) in 67 µg fine TiO_2 and 10 µg nano-TiO_2 groups.	Nurkiewicz et al. (2009)
Instillations				
Mouse [male ICR]	Nano-TiO_2 (Degussa), rutile, highly dispersed and hydrophilic fumed nano-TiO_2, diameter 19–21 nm (average primary particle size 21 nm), surface area of 50±15 m^2/g, purity ≥99.5% To avoid aggregation, the nano-TiO_2 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.	Single intra-tracheal instillation 0, 0.1, or 0.5 mg/mouse 3 days (for hyper-acute response), 1 wk (acute) or 2 wk (chronic) of recovery before sacrifice	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. 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. 1 wk after instillation, 0.1 mg/mouse increased alveolar macrophage infiltration, type II pneumocyte proliferation, and apoptosis in macrophage and type II pneumocyte. Gene expression in lung 1 wk after instillation of 0, 0.1, and 0.5 mg/mouse: cDNA microarray showed up-regulation in pathways involved in cell cycle regulation, apoptosis, chemokines, and complementary cascades. RT-PCR showed up-regulation in *plgf*, chemokines (*cxcl1*, *cxcl5*, and *ccl3*), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and prostaglandin E receptor 4. 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.	Chen et al. (2006)

Table 5-6. Summary of health effects of nano-TiO_2 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 Wistart]	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-TiO_2 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-TiO_2 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-TiO_2 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 are 7.9 m^2/g Ultrafine carbon black, mean diameter 14.3 nm, BET surface 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 Crl: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-TiO_2 particles in mammalian animal models: respiratory route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat [Crl:CD®(SD)IGS BR]	Nano-TiO_2 (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 m^2/g (uf-1) Nano-TiO_2 (P25) (Evonik), photocatalytic, 80% anatase/20% rutile, not coated, average particle size 129.4 nm in water, average BET surface area 53.0 m^2/g Nano-TiO_2 (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 m^2/g (uf-2) Fine TiO_2 (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 m^2/g Quartz	Single intra-tracheal instillation (subchronic effects) 0, 1, or 5 mg/kg 90 days recovery period	No sustained adverse pulmonary effects for photostable nano-TiO_2 (both types of coated rutile). Pulmonary inflammation and cytotoxic effects at highest exposure of photocatalytic nano-TiO_2 increased bronchoalveolar lavage fluid LDH and BAL fluid microprotein concentrations. Increased tracheobronchial and lung parenchymal cell proliferation rates at highest exposure of photocatalytic nano-TiO_2. Lung inflammation/cytotoxicity/cell proliferation and histopathological responses: quartz > nano-TiO_2 P25 (anatase and rutile) > fine TiO_2 (rutile) = nano-TiO_2 uf-1 (rutile) = nano-TiO_2 uf-2 (rutile).	Warheit et al. (2007a) Warheit et al. (2007c)

Table 5-6. Summary of health effects of nano-TiO_2 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. Malondiadehyde 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-TiO_2 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-1β – 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-TiO_2 (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 m^2/g (Llames, 2008a).

5.3.1.2.4. Toxicity by Other Exposure Routes

Ocular exposure, intravenous injection, and subcutaneous injection have also been investigated in nano-TiO_2 toxicity studies (Table 5-7). Ocular exposure to sunscreen containing nano-TiO_2 could occur accidentally when sunscreen spray and sunscreen lotion are applied. At least one brand of sunscreen lotion that contains nano-TiO_2 is in a tear-free formula and marketed for children (Project on Emerging Nanotechnologies, 2007). A single ocular exposure to photocatalytic nano-TiO_2 caused conjunctival redness for 1 or 2 days in rabbits (Warheit et al., 2007a).

One journal article and two professional meeting abstracts are available on the effects of injected nano-TiO_2 in rats and mice. In the Fabian et al. (2008) study, an intravenous injection of 5 mg/kg nano-TiO_2 with unknown photoreactivity did not induce changes in blood tests diagnostic for inflammatory responses, kidney toxicity, or liver toxicity. Two meeting abstracts presented immunological effect studies in mice exposed to nano-TiO_2 through subcutaneous and intravenous injections (Miller et al., 2007b; Weaver et al., 2007). Preliminary results showed that photocatalytic nano-TiO_2 in suspension (Degussa W740X) appeared to have very limited inflammatory ability, and very high doses (560 mg/kg for intravenous injections and 5,600 mg/kg for subcutaneous injections) were needed to produce immunological effects (Weaver, 2008).

Table 5-7. Summary of health effects of nano-TiO_2 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 Crl: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-TiO_2 particles in mammalian animal models: other (injection, ocular) route (continued). [a]

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Occular 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

Some of the non-carcinogenic effects shared by conventional and nano-TiO_2 were similar in the nature or type of the effects, but differed in dose-response. For example, pulmonary inflammation in laboratory animals and overload in rats were observed after respiratory tract exposures to either conventional TiO_2 or nano-TiO_2, and nano-TiO_2 often caused more severe or more persistent responses than conventional TiO_2 at the same mass concentrations/doses. Systemic effects were also observed: increased inflammatory cell numbers and decreased platelet numbers in the blood, renal pathology, potential hepatic toxicity, and changes in the brain morphology and neurotransmitters. Except for the effects in the brain, the aforementioned effects outside the lung have been reported only once and have not been confirmed by other laboratories. While topically applied photostable nano-TiO_2 is not expected to cause adverse effects in healthy skin, data are lacking on the effects in healthy flexed human skin and damaged human skin.

5.3.2. Carcinogenic Effects

The carcinogenicity of TiO_2 to humans has been reviewed by various international health organizations and workplace regulatory agencies. Currently, TiO_2 (including nano-TiO_2, but not considered separately) is classified as "possibly carcinogenic to humans" (Group 2B) by the International Agency for Research on Cancer (IARC) (Baan, 2007; IARC, 2006) and as "carcinogenic" (Class D2A) by the Workplace Hazardous Materials Information System (WHMIS), a program administered by the Canadian Centre for Occupational Health and Safety (CCOHS) (2006).

In a 2005 draft evaluation, TiO_2 was not designated as a "potential occupational carcinogen," due to insufficient evidence (NIOSH, 2005). For nano-TiO_2, NIOSH expressed concern in the 2005 draft about the potential carcinogenicity of ultrafine TiO_2 (nano-TiO_2) if exposure levels were at the current mass-based occupational limits of 1.5 mg/m^3 for respirable dust or 15 mg/m^3 for total dust, and recommended controlling exposure to as low as feasible below the recommended exposure limit (NIOSH, 2005). Based on calculated lung cancer risks, NIOSH (2005) stated a draft recommendation for an exposure limit of 0.1 mg/m^3 for ultrafine TiO_2, which is more than 10-fold lower than the exposure limit of 1.5 mg/m^3 for fine TiO_2 (less than 2.5 μm), as time-weighted average concentrations for up to 10 hr/day during a 40-hour work week.

This section reviews studies in humans and in animals on carcinogenicity of nano-TiO_2 and briefly discusses the mode of action of conventional TiO_2 and nano-TiO_2 carcinogenicity. Conventional TiO_2 has been shown to induce lung cancer through inhalation in rats at 250 mg/m^3 (6 hr/day, 5 days/week for 24

months) (Lee et al., 1985a, 1985b), but not at 50 mg/m^3 or below (Lee et al., 1985a, 1985b; Muhle et al., 1991). No increases in tumors were observed in mice receiving a single intra-tracheal instillation of 0.5 mg of TiO_2, in mice and rats fed with TiO_2 in the diet at up to 5.0% daily for 103 weeks, or in hamsters given 3 mg of TiO_2 via intra-tracheal instillation weekly for 15 weeks (Baan, 2007). Similarly, epidemiological studies did not show increased lung cancer in people exposed to conventional TiO_2 (Boffetta et al., 2001; Boffetta et al., 2004; Chen and Fayerweather, 1988; Fryzek et al., 2003; Ramanakumar et al., 2008; Siemiatycki, 1991). The carcinogenicity studies of conventional TiO_2 are not discussed in detail in this case study, and readers are referred to studies cited here and in the IARC Monographs Working Group report (Baan, 2007).

5.3.2.1. Studies in Humans

Seven epidemiological studies of TiO_2 carcinogenicity have been reported: two population-based case-control studies (one for lung cancer (Boffetta et al., 2001) and the other for 20 types of cancer (Siemiatycki, 1991)); two retrospective cohort mortality studies (Boffetta et al., 2004; Fryzek et al., 2003); one mortality, morbidity, and case-control study (lung cancer and chronic respiratory diseases) (Chen and Fayerweather, 1988); and two case-control studies (lung cancer) (Ramanakumar et al., 2008). Based on these studies, IARC (2006), the Canadian Centre for Occupational Health and Safety (CCOHS) (2006), and NIOSH (2005) concluded that the evidence is insufficient to conclude that TiO_2 exposure increases the risk of lung cancer in human beings. Furthermore, none of these studies were designed for nano-TiO_2 exposure, and none of them provided information on TiO_2 particle sizes. The risks posed by nano-TiO_2 (ultrafine primary particles) and the relationship between particle size and lung cancer risk in humans cannot be discerned from these studies.

5.3.2.2. Animal Studies

Carcinogenicity of nano-TiO_2 was observed in three animal studies using photocatalytic nano-TiO_2 in rodents (Borm et al., 2000; Heinrich et al., 1995; Pott and Roller, 2005). Increased lung tumor incidences were observed in rats (Borm et al., 2000; Heinrich et al., 1995; Pott and Roller, 2005), but not in mice (Heinrich et al., 1995), exposed to P25 through inhalation or intra-tracheal instillation. Photocatalytic nano-TiO_2 given through intraperitoneal injections did not increase tumors in the abdominal cavity in rats (Pott et al., 1987). Intramuscular implantation of nano-TiO_2 with unknown photo-reactivity also did not increase tumors at the sites of implantation in rats (Hansen et al., 2006). Data specifically on photostable nano-TiO_2 carcinogenicity are inconclusive (2005).

5.3.2.2.1. Intratracheal Instillation

Female Wister CRP/WU rats received fine and nano-TiO_2 via intra-tracheal instillations, and the tumor incidence and pulmonary inflammation were measured 2.5 years after administration (Borm et al., 2000). Fine TiO_2 (250 nm) was given 6 times at 10 mg each, and the photocatalytic nano-TiO_2 (21 nm, 80% anatase, 20% rutile, uncoated, P25) was given 5 times at 6 mg each (Borm, pers. comm, 2008). At these total doses (60 mg for fine TiO_2 and 30 mg for nano-TiO_2), lung clearance might be expected to be severely compromised. The authors found evidence of alveolar and interstitial inflammation 2.5 years after instillation. The histologically confirmed tumor incidences were 27% for fine TiO_2 and 66% for nano-TiO_2, while the macroscopic tumor incidences were only 20.9% for fine TiO_2 and 50% for nano-TiO_2. In vehicle-treated controls, the microscopic tumor incidences were between 5 and 6%. Although particles that induce high tumor incidences generally also cause high inflammatory cell counts, nano-TiO_2 caused a high tumor incidence and low inflammatory cell counts. Borm et al. (2000) suggested that tumor formation was directly related to high interstitialization rather than overload and subsequent tissue response, similar to the premise that lung burden is correlated to surface area of the particles (Oberdörster et al., 1994).

Pott and Roller (2005) reported increases in pulmonary tumors in rats exposed to hydrophilic fine TiO_2 and hydrophilic nano-TiO_2, but were unable to draw conclusions about the carcinogenicity of hydrophobic nano-TiO_2. Female Wistar (HsdCpb:WU) rats received weekly intra-tracheal instillations of three types of TiO_2: hydrophilic nano-TiO_2 (P25), hydrophobic nano-TiO_2 (Aeroxide® P805/Degussa P805, see Footnote c in Table 5-8), and hydrophilic fine TiO_2 (232033 from Sigma). If the products used in the study are the same as those currently available, both the hydrophilic nano-TiO_2 and fine TiO_2 were photocatalytic and the hydrophobic nano-TiO_2 was photostable. The tested TiO_2 physicochemical properties, doses, and key results are listed in Table 5-8. The types of primary benign lung tumor were adenoma and epithelioma, and the primary malignant tumors were adenocarcinoma and squamous cell carcinoma. At the tested doses, 42–46 rats out of 48 rats/group survived in the hydrophilic nano-TiO_2 and hydrophilic fine TiO_2 groups, and statistically significant increases in benign or malignant lung tumors, or both, were observed in these two groups.

Table 5-8. Treatments and pulmonary tumor incidences in rats exposed to fine and nano-TiO_2 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-TiO_2 (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 m^2/g.

Hydrophobic nano-TiO_2 (Degussa P805) showed high acute mortality in the Pott and Roller (2005) study. Nano-TiO_2 P805 was given at a much lower amount in each instillation than nano-TiO_2 P25 and fine TiO_2, because instilled P805 showed acute lethality. A single intra-tracheal instillation of P805 at 0.5, 1.0, and 1.5 mg caused death in 25%, 58%, and 92% female Wistar rats, respectively, within 24 hours. Pott and Roller (2005) originally ordered Degussa T805 for their study, and were unable to confirm that the received P805 was the same as T805. The physicochemical properties of T805, but not P805, were used for calculation and reported in the study (Pott and Roller, 2005). In contrast to the high acute toxicity of hydrophobic nano-TiO_2 reported in the Pott and Roller (2005) study, very low toxicity of hydrophobic nano-TiO_2 was reported in an earlier study by Rehn et al. (2003). Rehn et al. (2003) reported that a single intra-tracheal instillation of P805 at 0.15, 0.3, 0.6, or 1.2 mg caused no death in

female Wistar rats. Furthermore, P805 induced only mild, reversible inflammatory responses in the lung, and was less biologically active than P25 (Rehn et al., 2003). The reasons for the great discrepancy in the toxicity of hydrophobic nano-TiO_2 (P805 vs. T805 manufactured by Degussa) remain unclear.

5.3.2.2.2. Inhalation

Heinrich et al. (1995) reported increased lung cancer in rats (but not in mice) that inhaled photocatalytic nano-TiO_2. Animals were exposed to P25 aerosols (18 hours/day, 5 days/week) in whole-body exposure chambers. Generated by a dry dispersion technique, the nano-TiO_2 aerosol had a mass median aerodynamic diameter of 0.80 μm, with a geometric standard deviation of 1.80.

For female NMRI (Naval Medical Research Institute) mice, the nano-TiO_2 exposure was stopped after 13.5 months and followed by clean air exposure for 9.5 months. The 13.5-month nano-TiO_2 aerosol exposure was 4 months at 7.2 mg/m^3, 4 months at 14.8 mg/m^3, and 5.5 months at 9.4 mg/m^3. Although nano-TiO_2 exposures decreased lifespan in mice (50% mortality at 17 months after birth, compared to 20 months in controls), the exposures did not increase lung tumor incidence at the end of the study (13.8% in nano-TiO_2 exposed, compared to 30% in controls). Even though the reported spontaneous lung tumor rate seemed to be higher than historical data (20.7% lung cancer in the natural lifespan of female NMRI mice (Lohrke et al., 1984); 12% bronchiole-alveolar lung adenoma and 10% bronchiolo-alveolar lung carcinoma in female Han:NMRI mice up to 104 weeks old (Rittinghausen et al., 1997), 13.8% would not be considered as an increase compared to historical controls.

For female Wistar rats, the nano-TiO_2 exposure was stopped after 24 months, and followed by clean air exposure for 6 months. The 24-month nano-TiO_2 aerosol exposure consisted of 4 months at 7.2 mg/m^3, 4 months at 14.8 mg/m^3, and 16 months at 9.4 mg/m^3. At the end of the 30-month study, 32 of 100 nano-TiO_2-exposed rats had benign or malignant lung tumors (20 benign squamous cell tumors, 13 adenocarcinoma, 4 adenoma, and 2 squamous cell carcinoma), while only 1 of 217 control rats had lung adenocarcinoma (Heinrich et al., 1995). The lung particle loading was 23.2 mg/lung after 6 months, and 39.2 mg/lung after 24 months (Gallagher et al., 1994). The exposure to nano-TiO_2 did not increase the levels of DNA adducts in the lung (Gallagher et al., 1994). This study showed that inhaled photocatalytic nano-TiO_2 is a lung carcinogen in female rats, but no dose-response relationship can be calculated due to the dosing design. In a parallel study, decreased pulmonary clearance (overload) was clearly demonstrated (Creutzenberg et al., 1990).

The aerosol concentrations used in this study, ranging from 7.2 mg/m^3 to 14.8 mg/m^3, are occupationally relevant, for example, the OSHA PEL (Occupational Safety and Health Administration permissible exposure limit) is 15 mg/m^3 and the ACGIH TLV (American Conference of Governmental Industrial Hygienists threshold limit value) is 10 mg/m^3.

5.3.2.2.3. Intraperitoneal Injection

Pott et al. (1987) intraperitoneally injected Wistar and Sprague-Dawley rats with photocatalytic nano-TiO_2 (P25)[11] and examined abdominal cavities for tumors. The treatment doses ranged from a single intraperitoneal injection of 5 mg nano-TiO_2 to 5 injections of 20 mg nano-TiO_2 (for a total of 100-mg nano-TiO_2) over 5 weeks (Table 5-9). Tumor incidences were based on rats with sarcoma, mesothelioma, or carcinoma in the abdominal cavity. Rats with uterine tumors were excluded from the rats-with-tumor count, because 5–10% of the controls had malignant tumors of the uterus and some with metastases. Tumor incidences in the abdominal cavity in nano-TiO_2-treated rats ranged from 0% to 10% in the 5 experiments using nano-TiO_2 (Table 5-9). Although controls were not available in all experiments, Pott et al. (1987) concluded there were no increases in tumor incidence (in the abdominal cavity) in nano-TiO_2 treated rats.

Table 5-9. Incidence of tumor in the abdominal cavity of rats intraperitoneally injected with photocatalytic nano-TiO_2.

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).

5.3.2.2.4. Intramuscular Implantation

No tumors were observed in rats receiving implantations of either conventional TiO_2 or nano-TiO_2 for up to 12 months (Hansen et al., 2006). Each of the 10 male Sprague-Dawley rats was surgically implanted with conventional TiO_2 (a 9-mm x 2-mm disk containing 100% rutile) subcutaneously on the left side, and with nano-TiO_2 (20–160 nm, mean size 70 nm, 90% anatase and 10% rutile) intramuscularly on the right side of paravertebral muscle. The implanted doses were one disk of conventional TiO_2 and 0.1 mL nano-TiO_2. Four rats were sacrificed after 6 months, and the remaining six were sacrificed after 12 months. Inflammation (but not granuloma) was observed at the site of conventional TiO_2 implantation, and granuloma (localized nodular inflammation; non-cancerous inflammation) was observed at the site of nano-TiO_2 implantation at both 6 and 12 months. No tumors were observed at either time.

5.3.2.3. Modes of Action for Carcinogenicity

The mode of action of lung cancer induced by poorly soluble particles with no specific toxicity is believed to be particle deposition in respiratory epithelium, decreased lung clearance (to the degree of overload), persistent inflammation, cellular injury and persistent cell proliferation, fibrosis, and secondary genotoxicity (mutation) in the lung cells (Baan et al., 2006; Muhle and Mangelsdorf, 2003). TiO_2 is traditionally considered chemically inert and falls into the category of poorly soluble particles with no specific toxicity. When dose-response is expressed as surface area (dose) to tumor proportion (response), TiO_2, nano-TiO_2, and other poorly soluble particles with no specific toxicity appear to share the same dose-response curve[12] (Dankovic et al., 2007).

With the exception of mutation, all the events described in the previous paragraph (Baan et al., 2006; Muhle and Mangelsdorf, 2003) have been reported in rats exposed to both fine TiO_2 and photocatalytic nano-TiO_2 through inhalation or instillation (Borm et al., 2000; Heinrich et al., 1995; Hext et al., 2002; Pott and Roller, 2005). Figure 5-1 illustrates that, at low or medium exposure levels, lungs with normal clearance show inflammation that diminishes over time after exposure ceases. When the exposure level is high enough to decrease clearance, rats show persistent pulmonary inflammatory responses (even after exposure ends), cell proliferation and fibrosis, and eventually tumors. In mice, when the exposure is high enough to cause decreases in clearance, pulmonary inflammatory responses gradually decrease after the exposure ceases and no persistent pathological changes or tumors are

[12] Because the nano-TiO_2 data used in this dose-response curve were from studies using the same photocatalytic nano-TiO_2 product, this dose-response curve might not be applicable to nano-TiO_2 with a different crystalline type/ratio, purity, shape, surface treatment, or some other characteristic. Although such factors are known to affect nano-TiO_2 toxicity, their role in carcinogenicity remains unknown.

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

Increased mutation frequency in hypoxanthine-guanine phosphoribosyl transferase (hprt) was seen in type II alveolar cells isolated from rats exposed to 100 mg/kg fine TiO_2 through intra-tracheal instillation (Driscoll et al., 1997). No studies that investigated mutations in lungs of rats exposed to nano-TiO_2 are available. In vitro studies also support the mode of action stated above. Both macrophage- and neutrophil-enriched BAL cell populations from rats exposed to high concentrations of fine TiO_2 showed increased mutations in cultured cells (rat alveolar type II epithelial cell line; RLE-TN) in vitro (Driscoll et al., 1997). Because catalase, an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen, decreased BAL-cell-induced mutation in RLE-TN cells, ROS released from inflammatory cells could contribute to secondary genotoxicity and eventually to the carcinogenicity of TiO_2 (Driscoll et al., 1997). This sequence of events, however, does not appear to occur in mice. At an inhalation dose that causes overload, nano-TiO_2 does not appear to increase lung tumors in mice. More specifically, overload occurs in mice at an inhalation concentration of 10 mg/m^3 nano-TiO_2 (P25), based on the increase of clearance half-life of nano-TiO_2 from 40 days at 2 mg/m^3 to 395 days at 10 mg/m^3, after 13 weeks (6 hr/day, 5 days/week) of exposure (Hext et al., 2002). After 13.5 months of inhalation exposure to the same type of nano-TiO_2 (P25) at approximately 10 mg/m^3 (including 4 months of exposure at 14.8 mg/m^3), mice showed no increased lung tumors over the 2-year study period (Heinrich et al., 1995).

Although the evidence available to date for nano-TiO_2 carcinogenesis is consistent with the mode of action of other poorly soluble particles and suggests that particle overload is a sufficient condition for nano-TiO_2 to induce lung cancer, this does not definitively establish that particle overload is a necessary condition for nano-TiO_2-induced lung cancer. For example, it has been suggested that nano-TiO_2-induced lung tumors are directly related to high interstitialization rather than overload (Borm et al., 2000). Given the paucity of nano-TiO_2 cancer studies and the lack of consensus on exposure-dose metrics, the question arises whether there may be other effects or modes of action unique to nano-TiO_2 or nanomaterials in general that are yet to be found.

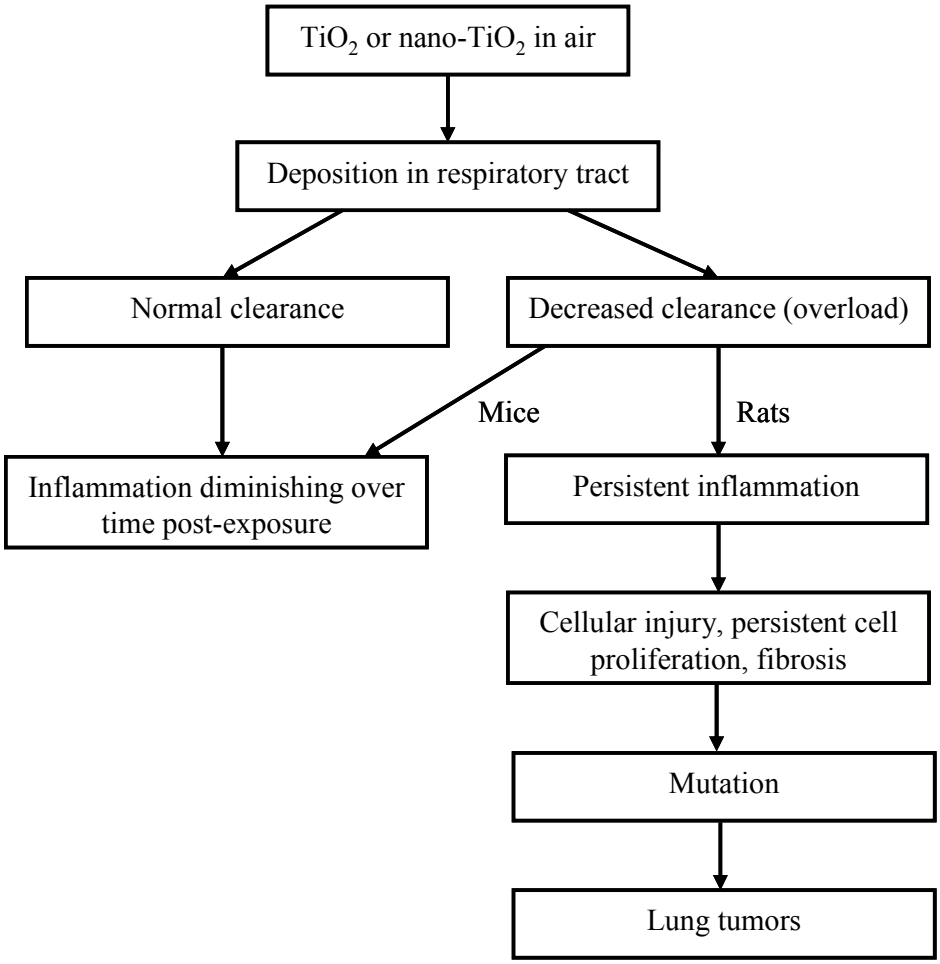


Figure 5-1. The pulmonary effects of fine TiO_2 and nano-TiO_2 exposure through inhalation or instillation.

Although the carcinogenicity of TiO_2 and nano-TiO_2 in rats at high doses has been shown repeatedly in inhalation and instillation studies, the relevance of this rat-specific response to human health is under debate. Rats have been suspected to be more sensitive to poorly soluble particle-induced lung cancer because they are more prone to pulmonary inflammation (Muhle and Mangelsdorf, 2003). Furthermore, lung tumors induced by poorly soluble low-toxicity particles are limited to rats with severely compromised particle clearance in lung (overload) (Hext et al., 2005). In human exposures, people working in dusty environments, such as coal miners, could encounter high concentrations of particles and have impaired lung clearance (Baan et al., 2006). Coal miners, however, are likely to be exposed to a mixture of particles (i.e., not limited to poorly soluble low-toxicity particles). Evidence of *persistent* or *chronic* inflammation in humans exposed to TiO_2 is suggested only by case studies of

workers exposed to TiO_2 and other minerals (Keller et al., 1995; Moran et al., 1991; Yamadori et al., 1986).

5.3.2.4. Summary of Carcinogenic Effects

The results of nano-TiO_2 carcinogenicity studies in animals are summarized in Table 5-10. No data are available for nano-TiO_2 carcinogenicity in humans or for photostable nano-TiO_2 in animals. TiO_2 (not specific to nano-TiO_2) was classified as "possibly carcinogenic to humans" (Group 2B) by an IARC Monographs Work Group in 2006 (Baan, 2007), and "carcinogenic" (Class D2A) by WHMIS (CCOHS, 2006). NIOSH (2005) proposed not designating TiO_2 as a "potential occupational carcinogen" because of insufficient evidence, but expressed concern about the potential carcinogenicity of ultrafine TiO_2 (nano-TiO_2) at the current exposure limits. Based on calculated lung cancer risks, the draft NIOSH recommendation was an exposure limit of 0.1 mg/m^3 for ultrafine TiO_2 and 1.5 mg/m^3 for fine TiO_2 (less than 2.5 μm), as time-weighted average concentrations. The relevance of rat-specific nano-TiO_2 carcinogenicity to human health remains to be elucidated.

Table 5-10. Results of nano-TiO_2 carcinogenicity studies in animals.

Exposure route	Species	Result	Lowest effective dose (highest ineffective dose)	References
Photocatalytic nano-TiO_2				
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-TiO_2 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/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months and then 9.4 mg/m^3 for 16 months

[b] 7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months and then 9.4 mg/m^3 for 5.5 months

[c] Nano-TiO_2 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-TiO_2?

Dermal toxicity

5.3-2. Is the current information on nano-TiO_2 skin penetration sufficient for risk assessment?

5.3-3. Would nano-TiO_2 penetrate into living cells in flexed, "soaked," or damaged skin (such as sunburned, scratched, eczematous skin)?

5.3-4. How important is testing nano-TiO_2 skin penetration on different races and at different ages?

5.3-5. Do certain formulations of nano-TiO_2 sunscreens generate hydroxyl radicals when applied to skin?

5.3-6. Given that nano-TiO_2 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-TiO_2 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-TiO_2 occupational exposure and health effects in humans?

Reproductive toxicity

5.3-9. What is the potential for reproductive and developmental effects of nano-TiO_2?

Carcinogenicity

5.3-10. Is ingested nano-TiO_2 carcinogenic?

5.3-11. Is inhaled nano-TiO_2 carcinogenic at exposure levels below those that induce particle overload?

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Appendix A. Nano-TiO_2 in Sunscreen: Background Information

Nanoscale titanium dioxide (nano-TiO_2) has been used in topical sunscreen products since around 1990 (Environmental Working Group, 2008). Between 1995 and 2002, the market for inorganic sunscreen ingredients (both nanoscale and non-nanoscale) increased from a value of roughly $30 million to a value of about $38 million, and has maintained about a 20% share of the sunscreen ingredient market as a whole (Dransfield, 2005). Dransfield (2005) projected that the inorganic active sunscreen ingredient market would grow to approximately $75 million by 2010, and that inorganic active ingredients would account for one-third of the total active sunscreen ingredient market. Dransfield (2005) suggested that the projected increase in the popularity of inorganics can be attributed to improved transparency in the products, which would imply particularly rapid growth in the market for nanoscale inorganics. In 2006, the Australian Therapeutic Goods Administration (TGA) estimated that 70% of titanium sunscreens and 30% of zinc sunscreens in Australia were formulated with nanoparticles (TGA, 2006).

The U.S. topical sunscreen market in 2000 was approximately $553 million (65%) of the $853 million "sun-care" market (a category that includes self-tanning products, after-sun products, etc.) (Packaged Facts, 2001). The size of the U.S. sunscreen market had apparently not changed substantially since 1993, when retail sales were reportedly in the range of $550–575 million (Davis, 1994). The total U.S. sun-care market reached $1.1 billion in 2005, and is projected to reach $1.2 billion by 2010 (Jeffries, 2007). If sunscreens continue to account for 65% of the U.S. sun-care market, that would translate to $715 million in sunscreen sales in 2005, and a projected $780 million in sunscreen sales in 2010. Globally, sales of sun protection products that presumably include topical sunscreens and cosmeceuticals were expected to exceed $820 million in 2006 (Newman, 2006). As a "mature" market in the United States, sun protection products are expected to have a growth rate of only about 2% per year (Jeffries, 2007). Between 2005 and 2010, however, growth in the sun-care market was expected to be much faster abroad than in the United States (Jeffries, 2007). If the growth in cosmeceuticals has dampened demand for conventional sunscreen, this growth has led to even greater demand for active sunscreen ingredients, including micronized TiO_2 (Davis, 1994).

A.1. Sunscreen Chemistry, and the Role and Properties of Nano-TiO_2

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

The traditional sunburn protection factor (SPF) rating system measures protection against UV-B radiation only. The Food and Drug Administration (FDA) proposed an official rating system that also takes UV-A radiation into account, awarding sunscreens between one and four stars based on their UV-A protection. This system was expected to go into effect in November 2008 or later (72 FR 49070). Various other UV-A protection ratings systems are in use or have been proposed in Australia, New Zealand, Europe, Japan, China, and Korea (Moyal, 2008).

A.1.1. Size of Nano-TiO_2 Particles (Mean and Distribution)

The composition of nano-TiO_2–based sunscreens is determined or constrained by several factors, including peculiar properties of nano-TiO_2, general principles of sunscreen chemistry, and aesthetic and other concerns. The size of nano-TiO_2 particles (both the primary particle size and the effective particle size of aggregates and agglomerates) affects protection against UV-A and UV-B radiation, the opacity of the sunscreen, and the stability of the dispersions. In most cases, a range of nano-TiO_2 sizes is present due to various primary particle sizes and aggregation.

The size of nano-TiO_2 particles affects how much UV-A and UV-B the particles transmit and scatter, and therefore, the degree of protection the particles provide against UV-A and UV-B radiation. Shao and Schlossman (1999) found that a nano-TiO_2 dispersion with a primary particle size of about 15 nm transmitted less UV-B and more UV-A and visible light than did dispersions with primary particle sizes of 35, 100, and 200 nm. (The particles were present in aggregates of mean sizes 125.3, 154.1, 251.1, and 263.4 nm, respectively.) The results of this study indicate that smaller nano-TiO_2 particles are better for UV-B protection, and larger nano-TiO_2 particles are better for UV-A protection. Dransfield (2005) presented data indicating that TiO_2 particles (not specifying whether they were primary or secondary particles) in the range of 40–100 nm provide the best UV-A protection, and particles in the

range of 60–220 nm provided the best UV-B protection. According to Hewitt (2002), theoretical calculations suggest that the optimal mean TiO_2 primary particle size for good UV-B and UV-A protection is about 50 nm. Chaudhuri and Majewski (1998) noted that nano-TiO_2 with a primary crystal size of 10–20 nm and an effective particle size of about 100 nm is expected to have a "very high UV scattering effect."

Particle size also determines the opacity of nano-TiO_2 formulations. Larger primary particles transmit less visible light (Shao and Schlossman, 1999). Aggregation will also make a formulation more opaque (Chaudhuri and Majewski, 1998). TiO_2 particles larger than 200 nm in sunscreen or cosmetics leave a white hue on the skin and are considered aesthetically unacceptable in many applications. Nano-TiO_2 particles smaller than 100 nm are generally not visible, and the sunscreen appears transparent when applied. A presentation by Schlossman et al. (2006) included pictures demonstrating the opacity of formulations with different particle sizes when applied to skin. Formulations with an effective agglomerated particle size of 100–120 nm (primary particle size of 10 nm) or 120–150 nm (primary particle size of 15 nm) were transparent or nearly transparent. Schlossman et al. (2006) noted that, in addition to particle size, two other factors affected the opacity/transparency of formulations: the difference between the refractive index of the particle and that of the media, and the uniformity of particle dispersion.

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

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

A.1.2. Active Ingredient Purity

The U.S. Pharmacopeia (USP) sets reference standards for TiO_2 and other active ingredients in over-the-counter and prescription drugs. The 2006 edition of the USP national formulary monographs, USP-NF 30 (U.S. Pharmacopeia, 2006), declares that TiO_2 "contains not less than 99.0% and not more than 100.5 percent of TiO_2." For "attenuation grade" TiO_2, that determination is made on an ignited basis.

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

A.1.3. Photostability and Surface Coating/Doping

Nano-TiO_2 is a natural semiconductor with photocatalytic properties. Its electrons can easily become excited by energy absorbed from UV radiation. When the electrons return to ground state, longer wavelength radiation is emitted. Alternatively, if the energized electrons escape from the particle, they can catalyze chemical reactions (oxidation/reduction processes) in nearby molecules. These reactions can create free radicals, which can damage skin cells or degrade other sunscreen ingredients. The choice of nano-TiO_2 crystal affects photostability. In particular, rutile is much more photostable than anatase (Chaudhuri and Majewski, 1998; Maynard, 2008). Although anatase is less photostable, it appears to be in common use. Barker and Branch (Barker and Branch, 2008) studied five TiO_2 sunscreens purchased over the counter and found that one was pure rutile and the other four were anatase/rutile mixes in which anatase predominated.

To increase TiO_2 and nano-TiO_2 photostability (i.e., to reduce the likelihood that excited electrons will escape), the crystals are commonly given a surface coating. Coating TiO_2 with silicon dioxide and alumina (3.5% by weight) can reduce photocatalytic activity by 99% (SCCNFP, 2000). Other TiO_2 or nano-TiO_2 surface coatings mentioned in the literature include inorganic oxides (Bird, 2002), simethicone (Chaudhuri and Majewski, 1998), methicone, lecithin (Schlossman et al., 2006), stearic acid, glycerol, silica, aluminum stearate, dimethicone (SCCNFP, 2000), metal soap, isopropyl titanium triisostearate (ITT), triethoxy caprylylsilane, and C9-15 fluoroalcohol phosphate (Shao and Schlossman, 1999). Alumina is often used in combination with other coating materials. The amount of surface coating applied varies substantially from product to product. For examples of common coating concentrations and combinations, see Appendix B, Table B-2.

Another technique for increasing photostability is "doping" the TiO_2 or nano-TiO_2 particles by embedding within them minute amounts of metals such as manganese, vanadium, chromium, and iron (Park et al., 2006). Doping rutile nano-TiO_2 with manganese is reported to increase UV-A absorption, reduce free radical generation, and increase free radical scavenging behavior (Reisch, 2005; Wakefield et al., 2004). Doped TiO_2 is colored instead of white, which can have desirable cosmetic effects in products such as skin lighteners (Park et al., 2006).

Recent research by Barker and Branch (2008) has found that the surface coatings on nano-TiO_2 in many sunscreens might not be stable or effective. The investigators studied the weathering of paint in contact with sunscreen. Out of five nano-TiO_2 sunscreens tested, four released photocatalytically generated hydroxyl radicals that accelerated the weathering of the paint. All four of those sunscreens used an anatase/rutile mix. The one nano-TiO_2 sunscreen that showed no appreciable effect on paint weathering was Oxonica's Optisol, which is 100-percent rutile, and is doped with manganese rather than surface-coated. It is not know whether nano-TiO_2 sunscreens generate hydroxyl radicals when applied to skin or whether such hydroxyl radicals would penetrate the skin and pose a threat to the health of the sunscreen user (Brausch and Smith, 2009; Maynard, 2008).

A.1.4. Dispersion and pH Considerations

Nano-TiO_2 can exist as a dry powder, but most sunscreen applications require the particles to be suspended in a fluid medium. This liquid is called a "dispersion" because special care must be taken to ensure that nano-TiO_2 will be distributed evenly and to minimize further aggregation and agglomeration (which could negatively impact UV scattering performance, transparency, etc., by increasing the effective particle size). Sunscreen manufacturers can purchase nano-TiO_2 powder and formulate their own dispersion, or they can purchase ready-made "predispersions."

In an effective dispersion, suspended particles are attracted to the dispersion medium and repel each other. Surface coatings influence the interaction of nano-TiO_2 with the dispersion medium, which can be water-based (aqueous), oil-based, or silicone-based. Early TiO_2 dispersions were generally oil-based (Bird, 2002). Surface coatings that make TiO_2 dispersible in non-aqueous media can be lipophilic (e.g., metal soap, ITT, lecithin); hydrophobic (e.g., methicone, dimethicone, triethoxy caprylylsilane); or both (e.g., C9-15 fluoroalcohol phosphate) (Shao and Schlossman, 1999). For methicone and C9-15 fluoroalcohol phosphate, silicone might be the preferred medium (Shao and Schlossman, 1999). Bird (2002) states that coatings have been developed to enable TiO_2 to be dispersed effectively in aqueous media as well, but provides no examples. Chaudhuri and Majewski (1998) describe one product, an "amphiphilic" powder (Eusolex® T-2000) containing about 80-percent USP-grade rutile coated with alumina and simethicone, that is easily dispersible in both water and oil.

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

At most pH values, nano-TiO_2 particles suspended in a dispersion have a positive electrical charge or a negative electrical charge and repel each other. At the pH_{pzc}/IEP, however, there is no electrostatic repulsion, and particles tend to agglomerate (Hewitt, 1995). To maintain electrostatic repulsion and prevent agglomeration, the dispersed product must be maintained at a pH other than the IEP (usually at a lower pH) at every stage of production and storage.

Surface coating can affect a particle's pH_{pzc}/IEP and can potentially extend the pH range at which the dispersion can be handled. For example, uncoated nano-TiO_2 has an IEP of pH 6, and nano-TiO_2 coated with alumina and simethicone has an IEP of pH 9 (Chaudhuri and Majewski, 1998). Bird (2002) cites lecithin as another coating that is advantageous for electrostatic reasons.

Experimental tests show additional pH considerations. Nano-TiO_2 performance can be adversely affected by strongly acidic formulations (effects include more agglomeration, lower SPF, and greater opacity), unless special formulating techniques are used (Hewitt, 1995).

Additional compounds can be added to the dispersion as "dispersants." "[The] proper dispersant can help particles to disperse into [the] vehicle so as to shorten the dispersion time and increase the degree of dispersion. It can reduce the viscosity and yet stabilize the dispersion by either electrostatic or steric repellency" (Shao and Schlossman, 1999). Different dispersants are used in water- and oil- (or silicone-) based formulations. PEG-10 dimethicone is used as a dispersant for nano-TiO_2 in a cyclopentasiloxane carrier in the predispersion CM3K25VM Kobo Products, Inc. manufactures. Polyhydroxystearic acid is used as a dispersant in a C12-15 alkyl benzoate carrier in Kobo's TNP40TPPS predispersion (Shao and Schlossman, 2004). Mitchnick and O'Lenick (1996) mention lecithin and phosphate esters as potential "dispersing aids" for TiO_2 dispersions, but they also use language suggesting that they might actually mean surface coatings.

A.1.5. Distribution of Active Ingredient in Emulsion

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

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

(w/Si, Si/w) (Hewitt, 2000). As noted above, nano-TiO_2 is most easily dispersed in oil, but emulsions can be formulated with nano-TiO_2 in a water phase, an oil phase, or a silicone phase. The nano-TiO_2 can be present in the dispersed phase or the continuous phase of a sunscreen emulsion (Dransfield, 2005).

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

A.1.6. Other Ingredients, Active and Inactive

Nano-TiO_2 can be combined with other physical UV blockers, such as zinc oxide (ZnO) (which can also be micronized), or with chemical UV filters to improve the UV protection the sunscreen provides. The sunscreen formula can also include a diverse array of inactive compounds for a variety of purposes.

TiO_2 and ZnO can form agglomerates. This attribute presents an obstacle to using TiO_2 and ZnO in the same sunscreen. A solution is to put one active ingredient in the oil phase of the emulsion and the other in the water phase (Hewitt, 1995).

Combining nano-TiO_2 with chemical UV filters often provides better UV-B protection than expected, based on the SPF of each ingredient. The improved protection is probably due to the scattering the physical UV blocker provides, which increases the optical path length of the radiation and creates more opportunities for absorption by the chemical filter (Bird, 2002; Chaudhuri and Majewski, 1998).

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

Increasingly, nano-TiO_2 is found in "cosmeceuticals," products that combine a variety of active ingredients to perform multiple health and beauty functions. These products include moisturizers and color cosmetics (see below for more on cosmeceuticals). The manganese added to some nano-TiO_2 formulations to prevent formation of free radicals during UV exposure can also help scavenge free radicals generated by other means, thus providing extra skin-protection benefits.

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

A.2. Some Sunscreens with Nano-TiO_2 or Micronized TiO_2 as Active Ingredient

Table A-1 was compiled in 2007 from information contained in the Environmental Working Group's cosmetic database "Skin Deep" (Environmental Working Group, 2008) and from on-line shopping sources. Products labeled as containing TiO_2 of unspecified particle size were excluded. The list of products provided in Table A-1 is likely not exhaustive. Also, product formulations and labels could change over time.

Table A-1. Titanium dioxide (TiO_2) 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).

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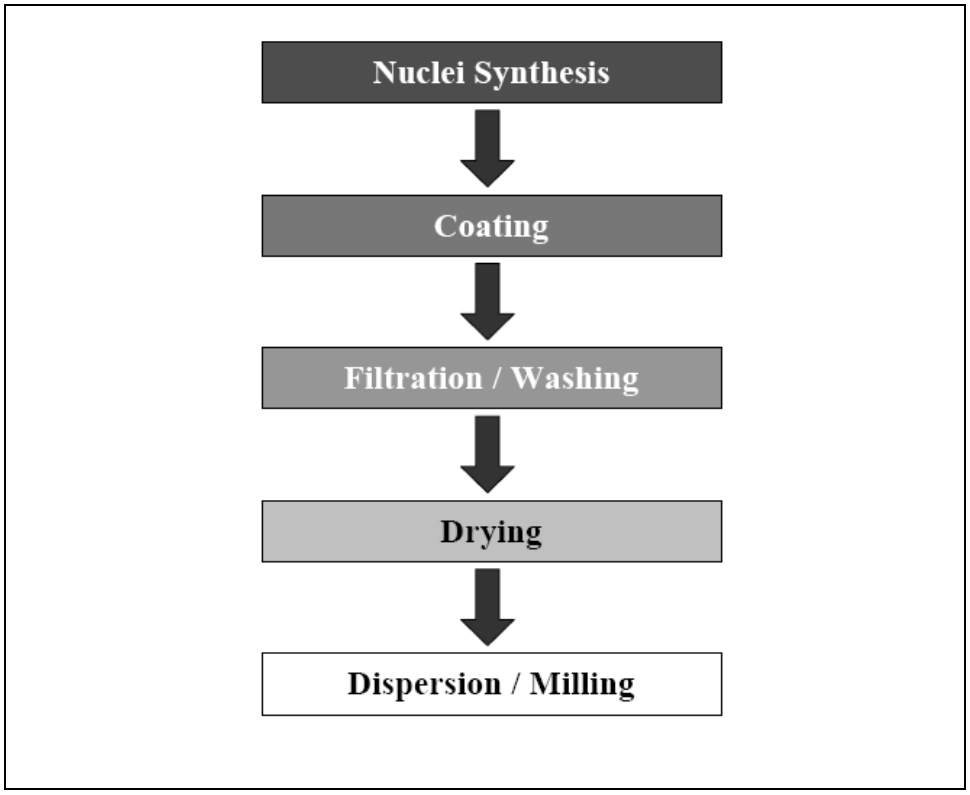
Appendix B. Nano-TiO_2 in Sunscreen: Manufacturing Processes

B.1. Overview of Nano-TiO_2 Manufacturing Process

A generic manufacturing process for nano-TiO_2 for sunscreen applications is outlined in Figure B-1.

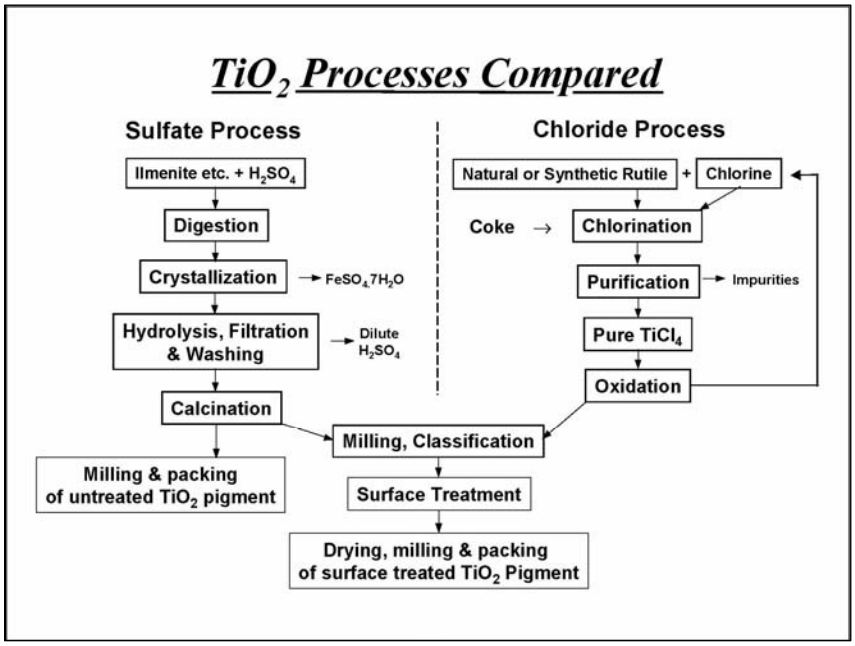


Source: Reprinted with permission from Dransfield (2005).

Figure B-1. Generic manufacturing process for nano-TiO_2 for sunscreens.

B.1.1. Titanium Dioxide Nuclei Synthesis

Commercial-scale TiO_2 synthesis is mostly by sulfate or chloride processes. In this section, a sulfate process, chloride process, and patented Altair process are described. These three processes can be used to synthesize both conventional (or pigmentary) and nanoscale TiO_2. There are many new processes being developed in the laboratory, but it is outside the scope of this Appendix to cover them (see review of nano-TiO_2 synthesis by (Chen and Mao, 2007). The sulfate process and the chloride process, illustrated in Figure B-2, are two common methods used to produce TiO_2 in a variety of grades for many different applications.



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

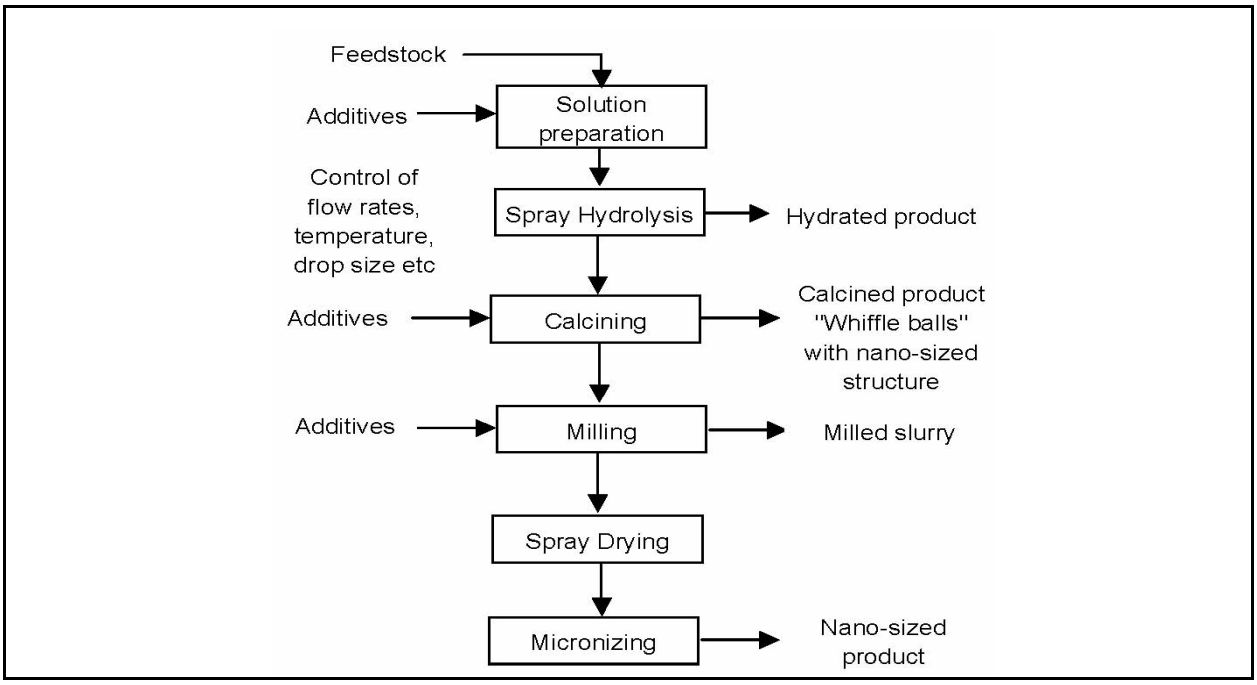
Figure B-2. Sulfate and chloride processes for TiO_2 manufacture.

The sulfate process, a wet process for creating pigmentary TiO_2, dates from around 1930, and it was the dominant method used to produce TiO_2 until the chloride process was developed in the 1950s (Hext et al., 2005). The chloride process now accounts for about 60% of worldwide TiO_2 pigment production (Hext et al., 2005). The chloride process, a gas-phase process, is more energy efficient than the wet-phase sulfate process; it can produce finer particles and particles with specific morphologies

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

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

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

Details of the sulfate process, chloride process, and the Altair Process (derived from spray hydrolysis) are provided in the following paragraphs. The steps unique to each process are presented first, followed by steps shared in these processes. Additionally, processes specific to manufacturing nano-TiO_2 include an additional gas-phase process ($TiCl_4 + 2H_2O \rightarrow TiO_2 + 4HCl$) and three additional wet processes ($TiOCl_2 + 2NaOH \rightarrow TiO_2 + 2NaCl + H_2O$; $Na_2TiO_3 + 2HCl \rightarrow TiO_2 + 2NaCl + H_2O$; and $Ti(OR)_4 + 2H_2O \rightarrow TiO_2 + 4ROH$) (Dransfield, 2005). The gas-phase process is similar to the chloride method except that the titanium tetrachloride is hydrolyzed rather than oxidized. It is also similar in some aspects to the Altair method. These three wet processes rely on feedstocks that are not found in nature, and thus require some additional, unspecified preparatory steps. Waste products from the various processes include hydrochloric acid, salt, water, and compounds formed from impurities.

Specific Steps in the Sulfate Process. The sulfate process begins with ilmenite ore ($FeTiO_3$), which is dried, ground, and treated with concentrated sulfuric acid (H_2SO_4) in an exothermic digestion reaction, producing a cake of titanyl sulfate ($TiOSO_4$) and other metal sulfates. This cake is then dissolved in water or a weak acid. After chemical flocculation, a clear solution and an insoluble mud are produced. The clear solution is cooled to crystallize ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, known as "copperas"). The ferrous sulfate heptahydrate is separated and sold as a by-product (Millennium Inorganic Chemicals, 2007).

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

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

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

$$FeTiO_3 + 2H_2SO_4 \rightarrow TiOSO_4 + FeSO_4 + 2H_2O$$

$$TiOSO_4 + H_2O \rightarrow TiO_2 + H_2SO_4$$

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

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

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

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

$$TiO_2 \text{ (impure)} + 2Cl_2 + C \rightarrow TiCl_4 + CO_2$$

$$TiCl_4 + O_2 \rightarrow TiO_2 + 2Cl_2$$

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

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

Milling and Micronizing for Sulfate, Chloride, and Altair Processes. Milling breaks apart the 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.

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

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

B.1.2. Surface Treatments and Doping

Some, but not all, nano-TiO_2 particles used for sunscreen undergo surface treatment to prevent the creation of free radicals, which could degrade the sunscreen or damage the skin (DuPont, 2007; Schlossman et al., 2006; Wakefield et al., 2004). Surface coatings for nano-TiO_2 in sunscreen can include combinations of inorganic oxides, simethicone, methicone, lecithin, stearic acid, glycerol, silica, aluminum stearate, dimethicone, metal soap, isopropyl titanium triisostearate (ITT), triethoxy caprylylsilane, and C9-15 fluoroalcohol phosphate.

In a patent they hold, Mitchnik and O'Lenick (1996) describe a sample protocol for applying a silicone surface treatment to TiO_2 for sunscreen. The patent does not specify the size of the TiO_2 particles. A quantity of silicone compound (generally between 0.1% and 25% by weight of the total formulation) is combined with TiO_2 powder. The mixture is heated to 40-100 °C for 2-10 hours, or long enough to remove 97% of the alcohol produced in the reaction. The patent holders claim that the resultant coated particles provide superior performance because the coating "preserves the structure of the TiO_2 crystals, eliminates the reactivity in water, and makes them hydrophobic."

Nano-TiO_2 particles can also be doped with various metals such as manganese, vanadium, chromium, and iron. Park et al. (2006) listed examples of doping methods, including: (1) combining particles of a host TiO_2 lattice with a second component in solution or suspension, and then baking at no lower than 300 °C. The second component is typically a salt, such as a chloride, or an oxygen-containing anion, such as a perchlorate or a nitrate; (2) mixing solutions of the dopant salt and of a titanium alkoxide, and then heating the solution to convert the alkoxide to the oxide and precipitate out the doped material; and (3) flame pyrolysis [3] 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-TiO_2 Particles and Products Used in Sunscreens

Several commercially-available nano-TiO_2 particles intended for sunscreen application and some of their characteristics are summarized in Table B-2 (SCCNFP, 2000). Although these nano-TiO_2 particles were selected for their applicability to the European market, they are likely to be fairly representative of nano-TiO_2 active ingredients used in the United States.

Table B-1. Selected list of nano-TiO_2 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).

Three manufacturers of United States Pharmacopeia (USP)-grade nano-TiO_2 for sunscreen applications provided information on their products and processes: Kobo Products Inc., which specializes

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

Kobo manufactures a line of 26 attenuation grade TiO_2 dispersions containing nano-TiO_2. The primary particle sizes are mostly 10-35 nm in 25 of 26 dispersions; one dispersion contains 90 nm primary TiO_2 particles. The nano-TiO_2 aggregate sizes in dispersions (measured by dynamic light scattering [DLS]) are mostly 103-165 nm in 25 of 26 dispersions, including the dispersion with 90 nm primary particles; one dispersion contains 230 nm aggregates (Kobo Products Inc., 2009). One of the Kobo TiO_2 dispersions called TNP40VTTS contains nano-TiO_2 particles coated with alumina and an isopropyl titanium tri-isostearate/triethyl caprylysilane crosspolymer (Kobo Products Inc., 2009; Shao and Schlossman, 2004). Polyhydroxystearic acid is used to disperse the product in the solvent/carrier, C12-15 alkyl benzoate, which is an ester (Kobo Products Inc., 2009; Shao and Schlossman, 2004). The particles in another dispersion, CM3K40T4, are surface-treated with alumina and methicone and are dispersed in the cyclopentasiloxane carrier with the help of PEG-10 dimethicone (Kobo Products Inc., 2009; Shao and Schlossman, 2004).

Optisol™ UV Absorber, a nano-TiO_2 product, is the first commercial product from Oxonica Materials (a branch of Oxonica), and the first commercial health product from Oxonica. Optisol™ is a powder composed of uncoated rutile nano-TiO_2 (size not specified) with approximately 0.67% manganese in the crystal lattice (Kobo Products Inc., 2009; Shao and Schlossman, 2004). Doping with manganese gives the sunscreen the advantages of increased ultraviolet-A (UV-A) absorption, reduced free radical generation, and increased free radical scavenging behavior (Reisch, 2005; Umicore, 2008).

Uniqema/Croda[5] manufactures several TiO_2 sunscreens, including a line of Solaveil™ Clarus using nano-TiO_2 (Chandler, 2006). Solaveil CT-100 and Solaveil CT-200, two of the products in the Solaveil Clarus line, are discussed here as examples. Solaveil CT-100 has more than 50% C12-C15 alkyl benzoate, 25-50% nano-TiO_2, and 1-5% each of aluminum stearate, polyhydroxysteric acid, and alumina (Croda, 2007). Solaveil CT-200 has 15-40% nano-TiO_2, 10-30% isohexadecane, 10-30% glycerol tri(2-ethylhexanoate), 3-7% aluminum stearate, and 1-5% each of polyhydroxysteric acid and aluminum oxide (Croda, 2008). The TiO_2 particle size distribution is very narrow, with the vast majority of particles falling in the nano range (Croda, 2008). Uniqema (no date) recommends using CT-200 at a concentration 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.

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

B.3. Formulations for Sunscreen Containing Nano-TiO_2

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

Generally, compatible ingredients are combined into a number of fluid phases. These phases are then energetically mixed in a particular sequence (sometimes at specified temperatures) to form an emulsion. Formulators have to take care not to allow the pH of the mixture to reach the isoelectric point (IEP) of the nano-TiO_2 or any other dispersed ingredient.

Table B-3 shows a sample formulation using Croda Solaveil CT-100W and Solaveil CT-200 (Croda, 2009). Table B-4 lists a sample formulation that uses nano-TiO_2 from Kobo for SPF 35 sunscreen 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 : *Talc (and) Methicone*	1.00
Velvesil 125 – Momentive/Kobo Products : *Cyclopentasiloxane (and) C30-45 Alkyl Cetearyl Dimethicone Crosspolymer*	3.00
Net-WO – Barnet : *Cyclopentasiloxane (and) PEG-10 Dimethicone (and) Disteardimonium Hectorite*	0.20
CM3K40T4 – Kobo Products : *Cyclopentasiloxane (and) Titanium Dioxide (and) PEG-10 Dimethicone (and) Alumina (and) Methicone*	35.00
Uvinul MC80 – BASF : *Ethylhexyl Methoxycinnamate*	7.00
Salacos 99 – Nisshin Oil : *Isononyl Isonanoate*	5.00
Lexol EHP – Inolex Chemical : *Ethylhexyl Palmitate*	4.00
Squalane – Fitoderm : *Squalane*	0.20
Tocopherol – Cognis : *Tocopherol*	0.20
SF96-350 – Momentive/Kobo Products : *Dimethicone*	1.00
SF96-100 – Momentive/Kobo Products : *Dimethicone*	1.00
SF1202 – Momentive/Kobo Products : *Cyclopentasiloxane*	27.10
Propyl Paraben NF – International Sourcing : *Propylparaben*	0.10
Part 2	
Sodium Citrate – Roche : *Sodium Citrate (and) Water*	2.00
Net-DG – Barnet : *Dipotassium Glycyrrhizinate*	0.10
Sodium Hyaluronate – Centerchem : *Sodium Hyaluronate (and) Water*	1.00
Keltrol CG-T – CP Kelco : *Xanthan Gum (and) Water*	2.00
Butylene Glycol – Ruger : *Butylene Glycol*	4.00
Methyl Paraben NF – International Sourcing : *Methylparaben*	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-TiO_2 Exposure Control in the Workplace and Laboratory

C.1. Workplace Exposure Controls

This section summarizes strategies that are currently in place or recommended to decrease exposures to nanomaterials in the workplace (Nanosafe, 2008b; NIOSH, 2009) and to ensure the effectiveness of personal protective equipment (PPE) against nano-TiO_2 (Golanski et al., 2008; Guizard and Tenegal, 2008; Nanosafe, 2008b). While this section focuses on workplace practice of nanomaterial manufacturers, some of the principles and use of PPE are also applicable to laboratories and other settings.

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

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

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

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

programs could also encourage the general public to reduce environmental releases. Some companies that manufacture nanoscale titanium dioxide (nano-TiO_2) have engineering safeguards and additional programs in place to reduce or eliminate occupational and environmental exposures (e.g., BASF, 2008; DuPont, 2007). Various production methods to decrease worker exposure are also being investigated [for nano-TiO_2, see Guizard and Tenegal (2008)].

With a goal to manage nanotechnology safely and effectively within industry, the Nanoparticle Occupational Safety and Health (NOSH) Consortium has investigated methods for monitoring workplace exposure and testing protective technologies. The NOSH Consortium has measured the effectiveness of standard respiratory filters with silicon dioxide (SiO_2) aerosol nanoparticles. With the exception of prolonged exposure (400 minutes or longer), the filter efficiencies for both charged and re-neutralized SiO_2 aerosol nanoparticles met the specifications of the filter type (Ostraat, 2009). The longest exposure time within which the N100 filter performed at or exceeded the efficiency specified by the filter ranking (>99.97-percent filtration efficiency) was 210 minutes (Ostraat, 2009). No PPE specific for nanomaterials exists or is under development (Klaessig, 2008). (For filter efficiency against nano-TiO_2 aerosol penetration tested by NanoSafe, see below.)

In the following section, two types of PPE are briefly discussed in terms of their protection against nano-TiO_2 aerosols: 1) filters for inhalation protection and 2) protective clothing and gloves for skin protection. Eye-protective gear is available as a third type of PPE commonly used for protection against nano-TiO_2 aerosols, but no information was found on this subject.

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

Electrostatic filters are charged polypropylene fibers, classified as FPP3—minimum filtration efficiency 99%—based on European Norm (EN) certification. When an electrostatic filter was tested with nano-TiO_2 aerosols, for which size ranged from 16 nm to greater than 76 nm, the MPPS was approximately 35 nm, which was very similar to graphite MPPS (Golanski et al., 2008). At the MPPS, however, nano-TiO_2 penetration was nearly five times higher than that for graphite. Near the MPPS, the differences between nano-TiO_2 and graphite particle penetration increase by an order of magnitude.

HEPA filters have a minimum filtration efficiency of 99.97%, are composed of glass fibers, and are classified as H12 for particles <1 micrometer (μm). Like electrostatic filters, HEPA filters showed one order of magnitude higher penetration of nano-TiO_2 (10–19 nm) than that of graphite (10–19 nm), with the highest penetration at approximately 0.2% for 19-nm TiO_2 (Golanski et al., 2008). The penetration of platinum (Pt) through HEPA filters was only slightly lower than that of nano-TiO_2. Golanski et al. showed that particle size alone might not be a sufficient indicator of HEPA filter performance and suggested that nano-TiO_2 might penetrate fibrous filters more than other nanomaterials, namely graphite and Pt (2008). The exposure duration of the Golanski et al. (2008) study was not reported, and therefore, it could be possible that the filtration efficiency of HEPA filters for nano-TiO_2 might decrease with prolonged exposure, as was found for the N100 filter for more than 400 minutes of exposure to SiO_2 aerosol nanoparticles (Ostraat, 2009).

The efficiency of protective clothing in preventing nano-TiO_2 penetration by diffusion was higher for non-woven fabric than woven cotton and polyester fabric (Golanski et al., 2008). Air-tight, non-woven, polyethylene Tyvek (115 μm thick) was more efficient against nanoparticle penetration than woven cotton (650 μm thick) and woven polyester (160 μm thick) for 10-nm nano-TiO_2 (Golanski et al., 2008), 10-nm nano-Pt (Golanski et al., 2008), and 40- and 80-nm graphite (Nanosafe, 2008a).

Nitrile, latex, and Neoprene gloves were reported to be efficient against nano-TiO_2 aerosol penetration via diffusion for a short exposure time (minutes). No penetration through gloves was detected when the gloves were exposed to aerosols of approximately 10-nm nano-TiO_2 and 10-nm Pt (Golanski et al., 2008) or 20- to 100-nm graphite (Nanosafe, 2008a). As these authors pointed out, aerosol penetration test results that examine diffusion do not indicate penetration against dispersion. In addition, continuous flex of gloves could lead to cracks and holes in the gloves (Schwerin et al., 2002), so changing gloves throughout the day is recommended (Harford et al., 2007).

C.2. Manufacturer and Laboratory Practices

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

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

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

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

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