United States Environmental Protection Agency

EPA/600/R-09/057F

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

November 2010

National Center for Environmental Assessment–RTP Division
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Disclaimer

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table of Contents

List of Figures

List of Tables

Authors, Contributors, and Reviewers

Principal Authors

J. Michael Davis (Project Leader)—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Thomas C. Long—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Jo Anne Shatkin—The Cadmus Group (currently with CLF Ventures)

Amy Wang—Oak Ridge Institute for Science and Education, Postdoctoral Fellow to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Co-Authors

Judith A. Graham—Private Consultant

Maureen Gwinn—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Brent Ranalli—The Cadmus Group

Contributors

Christian Andersen—National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Walter Cybulski—Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Genya Dana—Oak Ridge Institute for Science and Education, Postdoctoral Fellow to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

J. Allen Davis—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Steve Diamond—National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Kevin Dreher—National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Patricia Gillespie—Oak Ridge Institute for Science and Education, Postdoctoral Fellow to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Connie Meacham—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Emily Monosson—Private Consultant to the Cadmus Group

Jeffery Morris—Immediate Office of the Assistant Administrator, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Christine Ogilvie-Hendren—Oak Ridge Institute for Science and Education, Postdoctoral Fellow to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Stephanie Rosch—The Cadmus Group

Chon Shoaf—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

John Vandenberg—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Debra Walsh—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Sally White—Student Services Contractor to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

William Wilson—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

External Reviewers[1]

Pratim Biswas—Washington University

Bernard Goldstein—University of Pittsburgh

Fred Klaessig—Degussa

Rebecca Klaper—University of Wisconsin

Terry Medley—DuPont (with David Warheit, Gary Whiting, Scott Frerichs, and Brian Coleman)

Srikanth Nadadur—National Institute of Environmental Health Sciences

Gunter Oberdörster—University of Rochester

John A. Small—National Institute of Standards and Technology (with Richard Holbrook)

Jeffrey Steevens—U.S. Army Corps of Engineers

Mark Wiesner—Duke University

[1] Reviewers for the November 2007 draft of case study on nano-TiO_2 in drinking water treatment.

Peer Reviewers[1]

Steffen Foss Hansen—Technical University of Denmark

Kiril D. Hristovski—Arizona State University

Stephen J. Klaine—Clemson University

Bernd Nowack—Swiss Federal Laboratories for Materials Testing and Research (EMPA)

Annette B. Santamaria—ENVIRON International Corporation

Kathleen E. Sellers—ARCADIS

Workshop Participants[2]

David Andrews–Environmental Working Group

Jeff Baker–TSI Incorporated

Brenda Barry–American Chemistry Council

Catherine Barton–DuPont

Eula Bingham–University of Cincinnati

Pratim Biswas–Washington University in St. Louis

Jean-Claude Bonzongo–University of Florida

Steven Brown–Intel Corporation

Mark Bunger–Lux Research, Incorporated

Carolyn Nunley Cairns–Consumers Union

Richard Canady–McKenna, Long & Aldridge LLP

Janet Carter–U.S. Occupational Safety and Health Administration

Elizabeth Casman –Carnegie Mellon University

Sylvia Chan Remillard–HydroQual

Shaun Clancy–Evonik Industries AG

Ramond David–BASF Corporation

Joan Denton–California Environmental Protection Agency

Gary Ginsberg–Connecticut Department of Public Health

Pertti (Bert) Hakkinen–National Institutes of Health, National Library of Medicine

Jaydee Hanson–International Center for Technology Assessment

Patricia Holden–University of California, Santa Barbara, CA

Paul Howard–U.S. Food and Drug Administration

Sheila Kaplan–University of California, Graduate School of Journalism, Berkeley, CA

---

[1] Reviewers of the April 2010 draft of *Nanomaterial case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen*.

[2] EPA's "Nanomaterial Case Studies Workshop: Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Titanium Dioxide," Research Triangle Park, NC, September 29-30, 2009

Fred Klaessig–Pennsylvania Bio Nano Systems, LLC

Rebecca Klaper–University of Wisconsin, Great Lakes Water Institute

Todd Kuiken–Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies

John LaFemina–Battelle

Thomas Lee–Minneapolis Star Tribune

Shannon Lloyd–Concurrent Technologies Corporation

Christopher Long–Gradient Corporation

Margaret MacDonell–Argonne National Laboratory

Fred J. Miller–Independent Consultant

Nancy Monteiro-Riviere–North Carolina State University

Paul Mushak–PB Associates

Srikanth Nadadur–National Institutes of Health, National Institute of Environmental Health Sciences

Michele Ostraat–Research Triangle Institute

Anil Patri–Science Applications International Corporation, contractor with National Cancer Institute, Nanotechnology Characterization Laboratory

Maria Victoria Peeler–Washington State Department of Ecology

Richard Pleus–Intertox, Incorporated

John Small–National Institute for Standards and Technology

Jeff Steevens–U.S. Army Corps of Engineers Research and Development Center

Geoffrey Sunahara–National Research Council, Canada, Biotechnology Research Institute

Treye Thomas–U.S. Consumer Product Safety Commission

John Veranth–University of Utah

Donald Versteeg–The Procter & Gamble Company

Nigel Walker–National Toxicology Program, National Institutes of Health, National Institute for Environmental Health

William Warren-Hicks–EcoStat, Incorporated

Paul Westerhoff–Arizona State University

Mark Wiesner–Duke University

EPA Workgroup[1]

Christian Andersen[#]
Rochelle Araujo
Fred Arnold
Ayaad Assaad
Norman Birchfield
Deborah Burgin
Jim Caldwell
David Cleverly
Michele Conlon
Mary Ann Curran
Walter Cybulski[#]
J. Michael Davis*[+]
Jane Denne
Steve Diamond[#]
Jaimee Dong
Kevin Dreher[#]
Jeremiah Duncan
Brian Englert
Patricia Erickson
Cathy Fehrenbacher
Gina Ferreira
Kathryn Gallagher
Michael Gill
Michael Gonzalez
Maureen Gwinn[+]
Kathy Hart
Tala Henry
Ross Highsmith
Lee Hofmann
Marion Hoyer
Joe Jarvis
Bernine Khan
David Lai
Wen-Hsiung Lee
Laurence Libelo
Diana Locke
Jacqueline McQueen*
David Meyer
Gregory Miller
J. Vincent Nabholz
Nhan Nguyen
Carlos Nunez
David Olszyk
Martha Otto
Scott Prothero
Kim Rogers
Nora Savage
Phil Sayre
Rita Schoeny
Walter Schoepf
Najm Shamim
Deborah Smegal
Jose Solar
Neil Stiber
Timothy Taylor
Susan Thorneloe
Dennis Utterback
Amy Wang[+]
Eric Weber
Randy Wentsel
Doug Wolf

U.S. EPA Editorial Support

J. Sawyer Lucy—Student Services Contractor to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Deborah Wales—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Barbara Wright—Senior Environmental Employee to National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Contractor Editorial Support

ICF International

[1] *Co-Chair; [+]Co-author; [#]Contributor

Abbreviations

α-HBDH	Alpha-hydroxybutyrate dehydrogenase
ACGIH	American Conference of Governmental Industrial Hygienists
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	Degree(s) Celsius
C_{60}	Spherical fullerene composed of 60 carbon atoms; commonly "Bucky ball"
Ca^{2+}	Calcium cation
CCOHS	Canadian Centre for Occupational Health and Safety
CE	Capillary electrophoresis
CEA	Comprehensive environmental assessment
CK	Creatinine kinase
cm^2	Square centimeter(s), Centimeter(s) squared
cm^3	Cubic centimeter(s), 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
EC_{50}	Half-maximal effective concentration, Effective concentration 50; the concentration at which 50% of subjects show a response
EDS	Energy-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
ºF	Degree(s) Fahrenheit
F344	Fischer 344 (Rat strain)
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
γH2AX	Phosphorylated form of histone H2AX (phosphorylation of H2AX at serine 139)
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}	Concentration that results in a 20% (or 25%) change (inhibition) from the control response, 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}	Concentration of a chemical that kills 50% of a sample population, 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
4-MBC	4-methylbenzylidene camphor
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	Micron(s), Micrometer(s)
$\mu m^2/cm^3$	Square microns per cubic centimeter, Micrometer(s) squared per centimeter cubed
m^2	Square meter(s), Meter(s) squared
m^2/g	Square meter(s) per gram, Meter(s) squared per gram
m^3	Cubic meter(s), Meter(s) cubed
MARA	Microbial array for risk assessment (assay)
MCL	Maximum contaminant level
mg	Milligram(s)
mg/cm^2	Milligram(s) per square centimeter, Milligram(s) per centimeter squared
mg/kg	Milligram(s) per kilogram
mg/L	Milligram(s) per liter
mg/m^3	Milligrams per cubic meter, 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
MMPD	Multiple Path Particle Dosimetry (name of a computer model)

MPPS	Maximum penetrating particle size
mSv	Milliseviert
MT	Metric ton(s)
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 Environmental Assessment
Nano-TiO_2	Nanoscale titanium dioxide
ng/mL	Nanogram(s) per milliliter
NHEERL	(U.S. EPA) National Health and Environmental Effects 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
NOM	Natural organic matter
NOSH	Nanoparticle Occupational Safety and Health (Consortium)
O_2^-	Superoxide radical anion
OC	Octocrylene
OECD	Organization for Economic Co-operation and Development
OH	Hydroxyl
· OH	Hydroxyl radical(s)
OM	Octyl methoxycinnamate
· OOH	Hydroperoxyl radical(s)
OPC	Optical particle counter
OPPT	(U.S. EPA) Office of Pollution Prevention and Toxics
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
π	Pi, approximately equal to 3.14159
p	Pink-eyed dilution (gene)
P25	Degussa Aeroxide® P25 (uncoated nano-TiO_2)
P805	Degussa Aeroxide® P805 (hydrophobic nano-TiO_2)
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
PGF	Placenta growth factor
PMN	Polymorphonuclear neutrophil
PNEC	Predicted no-effect concentration

ppb	Part(s) per billion
PPE	Personal protective equipment
ppm	Part(s) per million
PTFE	Polytetrafluoroethylene
Pt	Platinum
PTM	Particle tracking model
p^{un}	Pink-eyed unstable (locus)
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
σ_g	Geometric standard deviation
s.c.	Subcutaneous
SAXS/WAXS	Small- and wide- angle X-ray scattering
SCCNFP	(European Commission) Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers
SCCP	(European Commission) 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)
T805	Degussa Aeroxide® T805 (hydrophobic nano-TiO_2)
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
USP	U.S. Pharmacopeia
UV	Ultraviolet (light/radiation), wavelengths in the range of 10 to 400 nm

UV-A	Ultraviolet A, wavelengths in the range of 320 to 400 nm
UV-B	Ultraviolet B, wavelengths in the range of 290 to 320 nm
VEDIC	Video-enhanced differential interference contrast
WHMIS	(Canadian) 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

Foreword

Nanoscale materials (nanomaterials) have been described as having at least one dimension on the order of approximately 1 to 100 nanometers (nm) (National Nanotechnology Initiative, 2006, 091186). Such materials often have 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: (1) as an agent for removing arsenic from drinking water; and (2) 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. Chapter 6 highlights the information that is currently available in each of these areas, and it describes information gaps and research questions identified in the case studies. It also discusses the role of the case studies in informing research planning and future assessment efforts. Appendices A through C provide supplementary information on the use of nano-TiO_2 in topical sunscreens, manufacturing processes for nano-TiO_2 and sunscreen formulations, and examples of laboratory and workplace exposure control practices, respectively.

The intent of these case studies is to characterize the current state of knowledge on the environmental impacts of nano-TiO_2 as used in these two specific applications, as well as areas where information is missing. Note that some information gaps are specific to nano-TiO_2, either as a drinking water treatment agent or as an ingredient in topical sunscreen. Other gaps may pertain more broadly to nano-TiO_2 irrespective of its application, and still other gaps may pertain even more widely to nanomaterials in general. In this way, the case studies may be used in developing research strategies that will support comprehensive environmental assessments of nanomaterials.

The case studies document has undergone a formal external peer review performed by scientists in accordance with Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2006, 194566). Six external peer reviewers reviewed the April 2010 draft of this document and provided responses to charge questions on the extent to which the case studies accurately and sufficiently characterize the state of understanding regarding the use of nano-TiO_2 in drinking water treatment and sunscreens. This final document incorporates revisions in response to the peer review comments.

Chapter 1. Introduction

1.1. Background

Nanoscale materials (nanomaterials) have been described as having at least one dimension on the order of approximately 1-100 nm (National Nanotechnology Initiative, 2006, 091186). Engineered nanomaterials are intentionally made, as opposed to being an incidental by-product of combustion or a natural process such as erosion, and they often have unique or novel properties that arise from their small size. These materials are being used in an expanding array of consumer products (The Project on Emerging Nanotechnologies, 2009, 196052), and, like all technological developments, nanomaterials offer the potential for both benefits and risks. The assessment of such risks and benefits relies on 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.

This document focuses on two specific uses of nano-TiO_2: as a drinking water treatment agent, and as an active ingredient in 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.

Part of the rationale for focusing on specific applications of selected nanomaterials is that such materials and applications can have highly varied and complex properties that make considering them in the abstract or in generalities quite difficult. Different materials and different applications of a given material could raise unique questions or issues, as well as some issues that are common to various applications of a given nanomaterial or even to different nanomaterials. After several individual case studies have been examined, refining a strategy for nanomaterials research to support long-term assessment efforts should be possible.

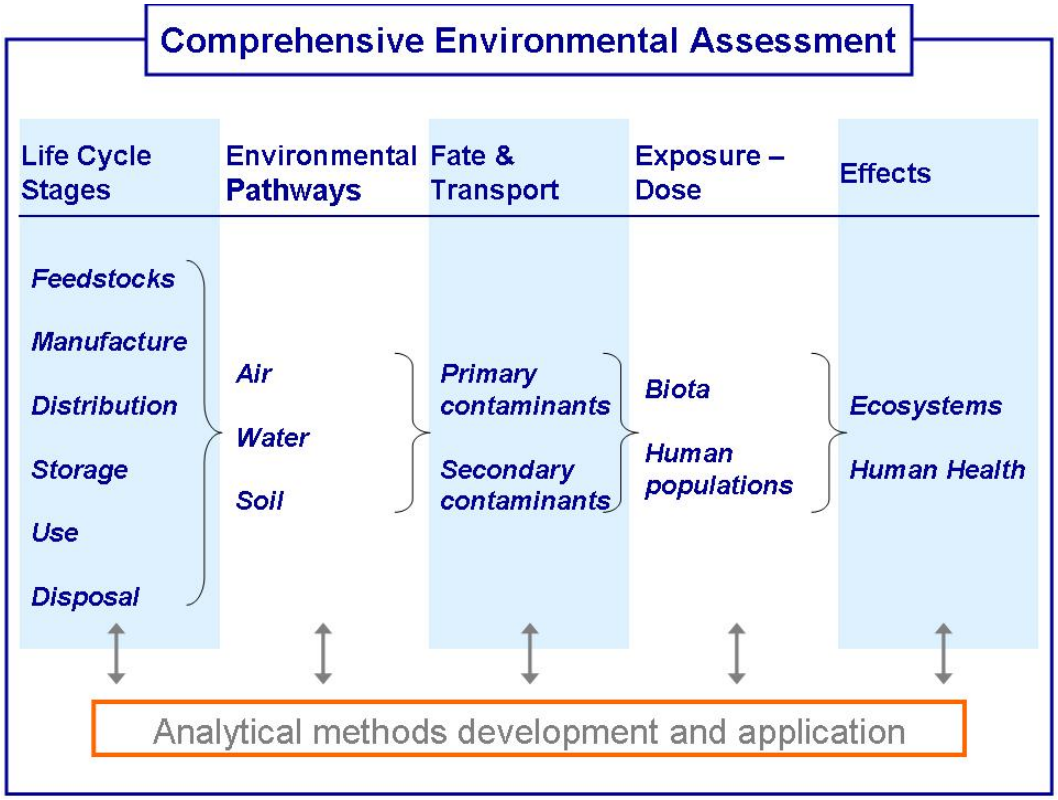
The process for selecting case studies of nano-TiO_2 in drinking water treatment and in topical sunscreen involved a workgroup representing several EPA program offices, regional offices, and Office of Research and Development (ORD) laboratories and centers. The EPA workgroup considered several candidate nanomaterials and identified their preferences based on, among other things, apparent relevance of the nanomaterial to EPA programmatic interests. The choice of specific applications was determined by a smaller team directly involved in the production of the case studies document. Among the factors guiding the selection process at each stage was the potential for exposure of ecological receptors and human populations to the nanomaterial as a function of a particular application. This is not to say, however, that the selection of these case studies signifies a determination that they present the greatest potential for exposure of all possible applications, or, for that matter, that any exposure actually occurs. Rather, the case studies simply provide a means to focus thinking about the types of information that would be instructive in assessing the potential ecological and health implications of selected nanomaterials.

The case studies follow the CEA approach, which combines a product life-cycle framework with the risk assessment paradigm (Davis, 2007, 089803; Davis and Thomas, 2006, 089638). In

---

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

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 (e.g., air, water, sediment, 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 (e.g., manufacturing by-products) and secondary (e.g., environmental transformation products) contaminants can be spatially distributed in the environment (Column 3, Figure 1-1).



Source: Adapted from Davis and Thomas (2006, 089638) and Davis (2007, 089803).

Figure 1-1. Basic structure of 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);

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

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.

The CEA framework is highly simplified in Figure 1-1. Among the many direct and indirect impacts that could conceivably be included in a CEA are effects on other materials (e.g., damage to surfaces of structures, statuary, vehicles), hedonic or aesthetic qualities (e.g., alterations in visibility, taste, odor), and other possible large scale impacts such as energy consumption, resource depletion, and global climate change. Although none of these effects are being excluded a priori from consideration here, their inclusion would depend on having a plausible premise for expecting a discernible impact. If such a premise can be articulated for additional types of effects, the case study can be expanded to encompass their consideration within the CEA framework.

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 either an assessment tool or as a framework for developing a research strategy, CEA is also a *process* that draws upon formal, structured methods to reach collective judgments by a diverse group of 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, 090565; Shatkin, 2008, 180065; Thomas and Sayre, 2005, 088085) or by using collective expert judgment methods (e.g., Kandlikar et al., 2007, 091626; Morgan, 2005, 088831), 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 CEA 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 nano-TiO_2 in the future. The goal is not to provide an actual CEA or to state conclusions regarding possible ecological or health risks related to nano-TiO_2, but to enable decisions on prioritizing research that would support future efforts to provide the input to policy and regulatory decision-making

Although the differences between the applications of nano-TiO_2 as a drinking 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 drinking 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 drinking water treatment is presented first, followed by discussion of sunscreen. In some sections, the discussions are not strictly parallel, reflecting differences in 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, drinking 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. The focus of a comparative CEA would be guided by risk management objectives. For example, the use of nano-TiO_2 for arsenic removal in drinking water might be compared to one or more current methods for arsenic removal; use of nano-TiO_2 for topical sunscreen might be compared to sunscreen containing conventional TiO_2 or to sunscreen with organic ultraviolet (UV) radiation blocking agents. Given that a number of different options could be of interest to risk managers, considering every potential option in the present case study is not feasible. Therefore, this document focuses solely on nano-TiO_2 in drinking water treatment and topical sunscreen, which is also consistent with the fact that the case studies are not intended to be assessments at this time, but rather are meant to assist in identifying and prioritizing research needs related to nano-TiO_2. 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, 196826) and the Environmental Working Group (EWG) (2009, 196367). 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.

This document is not intended to provide an exhaustive review of the literature, and focuses instead on findings most clearly relevant to assessment objectives. 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. Such information sources are used because of the limited amount of published materials on nano-TiO_2 and its applications in the peer-reviewed literature, coupled with the limited mechanisms for making manufacturer-specific data publicly available. This document is not an assessment but, rather, a means to identify information gaps and research questions, thus a range of information sources was used.

1.3. Terminology

A number of terms used in the field of nanotechnology have specialized meanings, and definitions of certain terms could have important legal, regulatory, and policy implications. Not surprisingly, perhaps, defining such words, including the term nanomaterial itself, has often been a matter of considerable interest and debate. For the purposes of this document, however, it is not deemed necessary to have a connotative definition that states the necessary and sufficient conditions that define a nanomaterial. Instead, a denotative approach is used; that is, the term “nanomaterial” in the case study denotes something that most persons would agree is (or at least appears to be) an

example of a nanomaterial or a product that incorporates a nanomaterial, regardless of whether a consensus exists regarding what properties or characteristics qualify it as such.

Although this case study focuses on "nano-TiO_2," readers should note that this term encompasses a variety of materials that might possess a range of physicochemical properties. As a result, not all materials referred to as nano-TiO_2 will necessarily behave in the same manner and exert the same biological effects. Thus, caution in extrapolating from one nano-TiO_2 formulation to another when assessing hazards is appropriate. Conversely, until more information is available to discern more precisely how various formulations differ in behavior and effects, pooling information from multiple sources can be useful for the purposes of this document, namely to identify potential research directions to pursue.

Some other terms used throughout this document are discussed below, primarily to explain how the terms are used here rather than to attempt to provide a formal definition of them.

Nano-TiO_2

This document focuses primarily on engineered nano-TiO_2, which usually is in the form of particles in the 1 to 100 nm size range. The term "nano-TiO_2," as it is used in this document, refers to a variety of formulations containing titanium dioxide particles that meet this size-based definition. When reading this document, it is important to understand that the general use of this term encompasses specific formulations that can display a range of characteristics and behaviors depending on the properties of the particle, the experimental or environmental conditions, and other factors.[1] Where information is not specific to nano-TiO_2, the term "titanium dioxide" (TiO_2) is used without the "nano-" prefix.

Conventional TiO_2

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 in this document. Even so, materials described as conventional often contain a range of particle sizes, including some with nanoscale dimensions. In the scientific and technical literature, the terms "bulk" and "pigmentary" also are often used to distinguish conventional from nano-TiO_2. Additionally, terms such as "ultrafine," "PM-0.1" (which means particulate matter up to 0.1 micrometer [µm] diameter), and "micronized grade" have been used to denote nanoscale particles, but typically in a particular context or field of specialization such as aerosols and air pollution.

Aggregate and Agglomerate

As discussed in Chapter 3, in many circumstances primary nanoscale particles can aggregate or agglomerate into secondary particles with dimensions greater than 100 nm (a cluster that is sometimes referred to as a colloid, as described below). Specifically, the terms "aggregate" and "agglomerate" are used in the literature on nanomaterials and other fields to indicate the clustering of particles into a single entity of such particles. These two terms can have specific meanings. For example, the British Standards Institution (BSI, 2007, 202162) defines aggregate as a "particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components" and notes that "the forces holding an aggregate together are strong forces, for example, covalent bonds, or those resulting from sintering or complex physical entanglement." The BSI defines agglomerate as a "collection of loosely bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components" and

[1] Where sources have provided documentation on size, surface coating, extent of aggregation or agglomeration, and other salient properties or characteristics, this information is included in the case study with sources referenced appropriately.

notes that "the forces holding an agglomerate together are weak forces, for example van der Waals forces, as well as simple physical entanglement." However, the meanings of aggregate and agglomerate have sometimes been interchanged, as noted by Nichols et al. (2002, 202114). This lack of consistency in terminology usage across, and sometimes within, the various fields that contribute to nanomaterials research exemplifies the challenges posed by the multidisciplinary nature of the nanotechnology field. The nanotechnology community is an amalgam of investigators who all study nanoscale materials but whose scientific roots are in various other mature fields spanning toxicology, ecology, colloid science, materials science, and many other disciplines. The customary terminology for aggregates and agglomerates may be well established within one field, but use of these terms may elicit different interpretations within another; as a result, the definitions for these terms are not specific, nor are they consistent. Given this inconsistency in usage and, more importantly, the frequent lack of adequate information to determine which term might be more appropriately applied in a particular study or report, the term "cluster" is used in this document to subsume both aggregates and agglomerations of nanoparticles. This term has precedent within multiple disciplines and avoids confusion between potentially inconsistent connotations of the other terms. Note that, in addition to being used as a noun (as explained above), the word "aggregate" is used as an adjective (primarily in Chapter 5) to refer to exposure to a given material from multiple sources, pathways, and routes.

Colloid

The term "colloid" is used in the literature to refer to a particle or cluster of particles suspended within a given medium and that are smaller than microscale (i.e., $<10^{-6}$ m). Luoma (2008, 157525) describes a colloidal particle as containing multiple atoms of a substance measuring between 1 nm and 1,000 nm, and thus a colloid might or might not be a nanoparticle in that context. In this case study, although the term "colloid" is used at times to refer to a sub-microscale particle (especially if a cited publication uses this terminology), either the more specific term "nanoscale" or a specific size range is used when the particle size is salient to the discussion.

The extent to which the properties of a cluster of primary nano-TiO_2 particles that exceeds 100 nm are similar to the properties of conventional TiO_2 is unclear. For example, inhalation of nano-TiO_2 (20-nm diameter) induced more pulmonary inflammation in the rat than inhalation of fine TiO_2 (approximately 250-nm diameter) at a similar mass concentration, even though particles in both groups had similarly sized agglomerates (0.71 μm mass median aerodynamic diameter [MMAD] nano; 0.78 μm MMAD fine) (Oberdörster, 2000, 036303; Oberdörster et al., 1994, 046203). Additional analysis revealed that effects were similar when expressed on the basis of surface area. Also unclear is the extent to which changes in conditions might initiate the formation, decomposition, or dissolution of a cluster; and there is uncertainty as well regarding what specific factors drive important changes in conditions. As discussed in Chapter 3 (Fate and Transport), disaggregation can occur under some conditions. Given these considerations, this document does not use 100 nm as the essential and exclusive criterion for considering what might be relevant to an evaluation of nano-TiO_2. This view is consistent with a statement by the European Commission (2008, 196378) that extends the term "nanomaterial" to encompass "nanostructured materials," defined by the International Organization for Standardization (ISO) (2004, 190006) 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."

Naturally Occurring, Incidental, and Engineered Nanoparticles

In addition to distinctions based on size of particles, The Project on Emerging Nanotechnologies (2009, 196052) divides nanoscale materials into three classes based on the origin of the particles. The first class, naturally occurring nanosized particles, includes, for example, particles that originate from volcanic explosions, ocean spray, and soil and sediment weathering and

biomineralization processes (which can result in crystals of aluminum and iron oxides with nanometer-scale dimensions). The second class is incidental nanosized particles, which are generated as by-products of processes such as combustion, cooking, or welding. The focus of this report is on the third class of nanoscale materials, *engineered* nanomaterials. This class comprises materials purposely generated for a specific function, such as the carbon nanotubes used in tennis rackets to make them lighter and stronger. In this case study, unless otherwise specified, references to nano-TiO_2 indicate engineered nanoscale materials. Nonengineered types of nanosized TiO_2 (from the first or second class) are referred to as "nanoscale TiO_2."

Degussa Aeroxide® P25 (hereafter referred to as P25) is a commercial-grade, uncoated nano-TiO_2 product (Evonik, 2008, 157578) 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 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. Elemental Ti 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, 091188). 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 U.S., surface-mining operations in Virginia and Florida produce concentrated Ti-containing minerals (ilmenite and rutile) suitable as feedstock for TiO_2 production (USGS, 2009, 157454). Other countries that produce significant amounts of Ti ores include Australia, Canada, China, India, Norway, and South Africa (USGS, 2009, 157454).

With exposure to ultraviolet (UV) radiation (wavelengths less than ~400 nm), pure TiO_2 is photocatalytic.[1] Studies suggest anatase and rutile have different photocatalytic properties, with anatase being the more reactive (Sayes et al., 2006, 090569; Uchino et al., 2002, 090568). 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 wastewater, drinking water, and indoor air. For more information on conventional TiO_2, see the article by Diebold (2003, 193342) and the Current Intelligence Bulletin published by the National Institute for Occupational Safety and Health (NIOSH) (2005, 196072).

[1] Photocatalysis is the phenomenon by which a relatively small amount of material, called a photocatalyst, increases the rate of chemical reaction without itself being consumed. (*adj.* photocatalytic).

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 nm^3 ($4/3\ \pi\ r^3$) whereas a 500-nm particle would have a volume of 65,000,000 nm^3. 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 nm^2 ($4\ \pi\ r^2$), whereas the surface area of a 500-nm particle equals approximately 800,000 nm^2. 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 nm^2, 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, 093301).

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, drinking 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.

Surface areas of nano-TiO_2 primary particles, aggregates, and agglomerates can be expressed as total (inner and outer) surface area and external surface area. The total surface area includes the inner surface area of porous or aggregated or agglomerated nanoparticles (Scientific Committee, 2007, 157639), and can be measured by the Brunauer, Emmett, Teller method (BET) and other methods. The external surface area, which is insensitive to particle porosity, can be measured indirectly by microscopy, diffusion chargers, scanning mobility particle sizers, and other methods (LeBlanc, 2009, 625209). Whether the total surface area or external surface area is more relevant for nanoparticle effects has not been determined. In one instance, humic acid caused nano-TiO_2 micropore blockage and consequently decreased the total surface area, but not the external surface area (Yang et al., 2009, 190513). Humic acid-coated nano-TiO_2 had lower zeta potential (i.e., increased electrostatic repulsion), which leads it to be more easily dispersed and suspended than uncoated nano-TiO_2 (Yang et al., 2009, 190513). External surface area alone, however, does not always predict the nature and magnitude of effects. When possible, the method of measuring surface area is provided when discussing studies in this document.

Several types of nano-TiO_2 are available with differing physicochemical properties. Commercially available brands of nano-TiO_2 can vary in particle size[1], surface area, purity (e.g., due to doping, coating, or quality control), surface characteristics, crystalline form, chemical reactivity, and other properties (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. 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, 089849; Uchino et al., 2002, 090568), but less effective in others (Nagaveni et al., 2004, 090578). 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 P25 nano-TiO_2 that has been treated with trialkoxyoctyl silane on the surface to increase hydrophobicity, has lower surface reactivity than P25 as indicated

[1] The sizes of nanoparticles may be reported either as crystallite size (sometimes called primary crystallite size, such as size determined by X-ray Diffraction analysis) or as primary particle size, which is typically larger than crystallite size. (Barton, personal communication, 2009, 625563).

by a vitamin C oxidation assay (Degussa, 2003, 193916). 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. This document presents information on both coated and uncoated nano-TiO_2, recognizing that differences in properties and environmental behavior may limit the applicability of information from one particle type to another.

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 U.K. Department for Environment, Food, and Rural Affairs (2007, 195461); Used with permission from Oxford University Press, Powers et al. (2006, 088783); Used with permission from Informa Healthcare, Powers et al. (2007, 090679); Used with permission from Informa Healthcare, Warheit et al. (2007, 091305); and data from Organisation for Economic Co-operation and Development (OECD) (2008, 157512).

External factors can also influence photoreactivity. Krishna and co-authors (2006, 193482), for example, found that the presence of fullerenes, which scavenge photogenerated electrons, enhances the photocatalytic efficacy of nano-TiO_2. Likewise, Komaguchi and colleagues (Komaguchi et al., 2006, 193479) saw significant increases in photocatalytic efficiency of P25 after exposure to an oxidizing environment.

Photocatalytic nano-TiO_2 is preferred for drinking 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 nano-TiO_2. For example, although doped rutile nano-TiO_2 can be extremely photostable, rutile nano-TiO_2 produced by a certain specific powder-preparation method can be highly photocatalytic (Kim et al., 2003, 157861). Similarly, not all coatings decrease nano-TiO_2 photoreactivity.

A report by Barker and Branch (2008, 180141) has noted that nano-TiO_2 in some sunscreens might not be photostable. 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 with manganese (Barker and Branch, 2008, 180141).

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 values for a limited set of 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 (T805).

Another characteristic of significance is the aggregation or agglomeration of nano-TiO_2 particles. 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, 193919). 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, 090584; Kormann et al., 1988, 090582; Lecoanet et al., 2004, 089258). The mean aggregated particle diameter of the product P25 is claimed to be approximately 3.6 µm, with the smallest 4% of aggregated particles reported to have an average diameter of 160 nm (Klaessig, personal communication, 2006, 196041). After being subjected to bath 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, 193917; Wahi et al., 2006, 090580). Ridley et al. (2006, 090599) 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 1- to 30-µm diameter range, and that these size ranges persisted even under sonication and other conditions that would favor disaggregation. Reported particle size values for aggregates and agglomerates are influenced by factors such as initial concentration, sonication power input, pH, and measurement technique. Such variables are germane to interpreting the results of reported size distributions.

Despite the presence, and sometimes the predominance, of larger aggregates and agglomerates, several researchers investigating laboratory-synthesized anatase and commercial nano-TiO_2 products such as P25 have also found free particles or aggregates with diameters <100 nm in varying amounts. The quantity of such particles has been found to depend on synthesis method, temperature, solution pH, and the presence of buffers (Jiang et al., 2009, 193450). Moreover, some preparations are specifically designed to generate dispersed particles (e.g., Seok et al., 2006, 091198) which would be important in using nano-TiO_2 as a catalyst.

The pH_{pzc} 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 pH_{pzc} of the particles, the aggregates tended to be smaller (Dunphy Guzman et al., 2006, 090584; Dunphy Guzman, personal communication, 2007, 091184). Sampled aggregates ranged up to 150 nm in size, and contained an estimated 8-4,000 nanoparticles (Dunphy Guzman et al., 2006, 090584). The pH_{pzc} also depends at least in part on the crystallinity of the nano-TiO_2 particles; Finnegan et al. (2007, 193379) reported pH_{pzc} values of approximately 5.9 for rutile and approximately 6.3 for anatase.

Coatings and surface treatments also affect particle aggregation/agglomeration behavior. A preliminary report by Wiench and colleagues (2007, 090635) indicated that coated nano-TiO_2 particles (rutile, size 50 × 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 (20-30 nm, anatase/rutile 80/20, surface area 48.6 m^2/g).

A recent study showed that a type of coated nano-TiO_2 used in sunscreens quickly lost its outermost coating and became easily dispersed soon after contact with water (Auffan et al., 2010, 625063). The tested nano-TiO_2, T-Lite™ SF (manufactured by BASF company, Germany) had a mainly rutile TiO_2 core, coated with $Al(OH)_3$ and an outermost hydrophobic coating with polydimethylsiloxane (PDMS). The structural changes were seen within 30 minutes of aging (stirring) when the initially hydrophobic nano-TiO_2 started to disperse into the aqueous phase of the suspension. The PDMS coating was dissolved, and the dissolution rate was accelerated by UV exposure. Aluminum was not detected, suggesting that the $Al(OH)_3$ outer layer was more stable.

The complexity of nano-TiO_2 characterization is illustrated in Table 1-2, from Warheit et al. (2007, 091075). 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 as measured by dynamic light scattering. 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. Even with detailed information such as provided here, additional details may be necessary to fully characterize the complex nature of nano-TiO_2 nanomaterials. In particular, the presence of a surface coating changes the nature of the interface between the nano-TiO_2 particle and the environment or an organism, and it is not clear whether the surface coating or the core material dominates particle-environment and particle-organism interactions.

Table 1-2. Characterization of three nano-TiO_2 particle types

Particle Type	BET Surface Area (m^2/g)	Chemical Composition	Chemical Reactivity[a]	Median Particle Size and Size Range[b]		pH in Deionized Water
				in Water[c]	in PBS	
Uf-A	18.2	98% TiO_2 (100% rutile), 2% alumina	10.1	136 nm ± 35%	1,990 nm ± 25%	5.64
Uf-B	35.7	88% TiO_2 (100% rutile), 5% alumina, 7% silica	1.2	149.4 nm ± 50%	2,669 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

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

PBS – Phosphate buffered saline

[a]Chemical reactivity was tested using a Vitamin C (antioxidant) yellowing assay.

[b]After sonication for 15 min at 60 Hertz (Hz).

[c]0.1% tetrasodium pyrophosphate solution.

Source: Modified with permission from Elsevier, Warheit et al. (2007, 091075).

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, 193478) 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 approximately 10 nm to approximately 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 physicochemical 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. As discussed in Section 1.6.1, ideal characterization to support toxicological testing would include analysis of the raw material (as received from the manufacturer or supplier), analysis of nanomaterials in the testing

media for the duration of the experiment; and analysis of nanomaterials (and possibly degraded products or biotransformed products) in biological samples. 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 nonrepresentative 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, 090679). Further, different particle characterization techniques can produce different estimates of particle size, 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), and the type and extent of characterization is an important consideration in interpreting the results of nano-TiO_2 research.

1.5.1. Drinking Water Treatment

This document assumes that one use of nano-TiO_2 would be specifically for arsenic removal in a drinking water treatment facility. In addition to arsenic removal (Li et al., 2009, 193506), however, nano-TiO_2 could be used for disinfection of pathogens (Alrousan et al., 2009, 157461; Coleman et al., 2005, 089849; Li et al., 2008, 157538; Rincon and Pulgarin, 2003, 157856) or for remediation of ground water or wastewater contaminated with various organic and inorganic pollutants (Adams et al., 2004, 193250; Chen and Ray, 2001, 193310; Han et al., 2009, 193407; Kim et al., 2003, 193472; Lee et al., 2008, 098739; Lin and Valsaraj, 2003, 193511; Ryu and Choi, 2008, 157501). 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 drinking water treatment facilities.

Most of the relevant literature 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, 157845; Ferguson et al., 2005, 090572; Pena et al., 2006, 090573). 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 drinking water treatment plants (Dionysiou, personal communication, 2009, 193921; Pichat, 2003, 196037; Purifics, 2008, 196040; Richardson et al., 1996, 193612).

For arsenic removal from water, both conventional and nanoscale TiO_2 have been developed to photocatalytically oxidize arsenic and adsorb arsenic. Studies have shown that TiO_2 can oxidize As(III) to As(V) and adsorb inorganic arsenic (Dutta et al., 2004, 157845; Fostier et al., 2008, 193381; Hristovski et al., 2007, 193436). 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, 193641). Recently, nano-TiO_2 was shown to mineralize methylated arsenic and to adsorb methylated arsenic (Xu et al., 2007, 193725; Xu et al., 2008, 193727). 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, 193725; Xu et al., 2008, 193727).

The mechanism of arsenic adsorption onto TiO_2 surfaces has been demonstrated to be 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., 2005, 193452; Jing et al., 2005, 193454; Pena et al., 2006, 090573). In ground water containing As(III), As(V), MMA(V), and

DMA(V), nano-TiO_2 adsorbed As(III) and As(V) most, followed by MMA(V), but almost no DMA(V) (Jing et al., 2009, 193453). The difference in competitive adsorption could be due to the increased thermodynamic stability of the bidentate ligands formed by other arsenic species with TiO_2 compared with the monodentate surface structure formed between TiO_2 and DMA(V). However, in the presence of high concentrations of competing ions, the other arsenic species may be forced to form monodentate rather than bidentate ligands.

Photocatalytic oxidation is also the mechanism for TiO_2 degradation of organic pollutants in wastewater. Photocatalytic degradation is based on the formation of radicals (hydroxyl radicals [·OH], superoxide radical anions [$\cdot O_2^-$], and hydroperoxyl radicals [·OOH]), which serve as oxidizing species in the photocatalytic oxidation process (Lu et al., 2009, 193528). 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, 193278).

One generally accepted mechanism of nano-TiO_2 antimicrobial action is the generation of reactive oxygen species (ROS), which can cause cell wall or cell membrane damage (Kuhn et al., 2003, 090597; Neal, 2008, 196069), such as lipid peroxidation (Maness et al., 1999, 193538). 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, 157782; Coleman et al., 2005, 089849). Because TiO_2 generates ROS (mainly highly reactive hydroxyl radicals) in the presence of UV light and oxygen (Reeves et al., 2008, 157506), 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, 180383).

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, 093309) 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 to 320 nm, and are primarily absorbed by nano-TiO_2; UV-A wavelengths are in the range of 320 to 400 nm, and are primarily scattered by nano-TiO_2 (Shao and Schlossman, 1999, 093301). 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, 196248) 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. FDA, 2006, 157728). 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 (by weight) of TiO_2 in sunscreen that the U.S. Food and Drug Administration (FDA) allows is 25% (U.S. FDA, 1999, 196374), 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% (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 (Comparative study on cosmetics legislation in the EU and other principal markets with special attention to so-called borderline products, 2004, 157826; Oxonica, 2005, 157793; Steinberg, 2007, 193656).

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 approximately 1 micron (or μm, 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, 093301), as it was formerly considered distinct from nano (e.g., EWG, 2008, 196343) but such a distinction is no longer made by the European Working Group (EWG, 2009, 196367). 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 (with at least one dimension less than 100 nm (La Farge, personal communication, 2007, 196047), they found nanoparticles in all seven products (La Farge, personal communication, 2007, 196047; Sunscreens: Some are short on protection, 2007, 155618). 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, personal communication, 2007, 196047). FDA does not specify the use of nano or other terms to describe TiO_2 (64 FR 27671), and some nano-TiO_2 sunscreens are therefore simply 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. The standardization of characterization method and sample preparation protocols will also greatly facilitate the physicochemical characterization of the nanomaterials.

Section 1.5 addressed some general aspects of characterization of nanomaterials, particularly nano-TiO_2. This section provides a brief overview of analytical methods that could be suitable for nano-TiO_2, with a focus on currently available methods. Different methods for measuring the same parameter may yield different results for the same material (Hewitt, 2009, 625212), and therefore stating the testing method is important. 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 methods used for identifying their molecular components and characterizing certain surface properties), but these methods also are not discussed in Section 1.6. Some of the chemical analysis methods suitable for nanomaterials are discussed in Powers et al. (2006, 088783) and U.S. EPA (2008, 157480). Methods for analyzing nanoparticles in the environment are summarized by Hoyt (2009, 633900). For detailed

comparison of various methods, see review articles by Maynard and Aitken (2007, 090674), Powers et al. (2006, 088783; 2007, 090679), and Domingos et al. (2009, 193346).

1.6.1. Methods for Laboratory Research

The physicochemical properties of nano-TiO_2 can change over time (Kolár et al., 2006, 193478) 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 collected and tested, such as in urine, organs, and cells.

The equipment and methods for measuring nanomaterials in the laboratory are numerous and are evolving. Table 1-3 summarizes methods that can be used for characterizing nanomaterials in aerosols and liquids (including biological fluids) (Maynard and Aitken, 2007, 090674; Nanosafe, 2008, 594868; Powers et al., 2007, 090679). Methods for properties relating to chemical reactivity or charge, such as surface charge and pH_{pzc}, are not included in the basic characterization methods summarized in Table 1-3. Specialized methods are also available that are specific for radio-labeled or fluorescent nanomaterials. The following methods have been used to measure various characteristics of nanomaterials in biological samples: dark field microscopy, transmission electron microscopy (TEM), energy-dispersive X-ray analysis (EDS), and inductively coupled plasma mass spectroscopy (ICP-MS) for presence and location, with additional information on size, shape, and aggregation/agglomeration state available from analysis of TEM images; 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, 193539); and scanning probe microscopy (SPM) for size and three-dimensional images. ICP, X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) can be used to determine chemical composition. The combination of flow FFF and ICP-AES has been used to detect nano-TiO_2 in tested commercial sunscreen, with information on mass-size distribution and Ti content of extracted nano-TiO_2 from sunscreen (Contado and Pagnoni, 2008, 157585). Although combinations of these methods can be used to infer the presence of nanomaterials in tissue (e.g., by metal content), no broad-spectrum techniques are currently available to measure the total amount of nanomaterials in tissues.

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

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

[a]For some particle shapes, electron microscopy can be used to estimate surface area (LeBlanc, 2009, 625209). SMPS, ELPI, diffusion charger, and electron microscopy measure external surface area and may underestimate total surface area for porous particles. BET, on the other hand, will measure total surface area, which includes the inner surface area of porous or aggregated or agglomerated particles (LeBlanc, 2009, 625209).

Source: Modified with permission from Informa Healthcare, Maynard and Aitken (2007, 090674); Used with permission from Oxford University Press, Powers et al. (2006, 088783); Used with permission from Informa Healthcare, Powers et al. (2007, 090679); and data from Nanosafe (2008, 594868)

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., 2009, 193346; Englert, 2007, 193367; Simonet and Valcárcel, 2009, 193648). Also, many analytical methods require sample treatment and extraction (Englert, 2007, 193367),

which may include solvent evaporation, and consequently could cause nanoparticle aggregation and salt precipitation (Simonet and Valcárcel, 2009, 193648). 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 differing 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, 193648).

Analytical methods that are currently available for nanomaterials in soil, sediment and ground water were summarized in a recent EPA State of Science Review (U.S. EPA, 2008, 157480) (Table 1-4). Methods can be coupled to enable detection of more than one parameter at a time. For example, flow FFF can be coupled with ICP-MS for both size and chemical analysis. Methods for properties relating to chemical reactivity or charge, such as surface charge and pH_{pzc}, are not included in the basic characterization methods summarized in Table 1-4.

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 FFF), Domingos et al. (2009, 193346) 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 that can interfere with interpretation of results. Using multiple analytical techniques or multiple preparation techniques, or both, has been recommended (Domingos et al., 2009, 193346; Englert, 2007, 193367).

The measurement and detection of engineered nanoparticles across a variety of environmental media is an active and growing area of research, though an extensive review of analytical methods falls outside the scope of this case study. A growing body of research focuses on developing methods to detect and characterize nanomaterials and their behavior within environmentally relevant matrices (Boxall et al., 2007, 157712; Hassellöv et al., 2008, 157559; Stone et al., 2010, 633898; Tiede et al., 2008, 196278; Tiede et al., 2009, 633895; Tiede et al., 2009, 193680).

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 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 crystalline structure	Electron diffraction	
	X-ray diffraction (XRD)	
	X-ray absorption spectroscopy (XAS)	
	Raman spectroscopy	

Source: Data from U.S. EPA (2008, 157480).

1.6.3. Methods and Instrumentation to Assess Workplace Exposure

Workplace exposure monitoring thus far has focused on measuring nanoparticles in the air. Instruments that can be used for aerosol sampling are available, but most are designed for laboratory use (Nanosafe, 2008, 594868) and lack one or more of the following desired attributes: portability, ease of use, capacity to distinguish nanoparticles from non-nanoparticles, different size bins in the 1-100 nm range, or ability to sample personal breathing zones (Ostraat, 2009, 196077).

Several governmental and nongovernmental organizations have begun addressing the need for instrumentation 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, 196073), in which sampling and monitoring methods and equipment are discussed. The Nanoparticle Occupational Safety and Health (NOSH) Consortium, an industry led consortium of participants from academia, governmental, and nongovernmental organizations, is helping to define best practices for working safely with engineered nanoparticles (Ostraat et al., 2006, 667690; Ostraat et al., 2008, 193591). 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, 090674) summarized available devices and approaches for evaluating particle number, surface area, and mass concentration of nanoparticles for use in 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 that use various types of on-line and off-line monitoring instruments (Nanosafe, 2008, 594868). The report provided examples of new nano-aerosol 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, 193820; TRS Environmental, 2009, 196057; van den Brink, 2008, 196075).

1.6.4. Summary of Analytic Methods

Many techniques can be used to measure and characterize nanomaterials in the laboratory and manufacturing workplace, and some are available for detecting nanomaterials in the environment. However, no single instrument can characterize all of the physicochemical properties of interest. Technical difficulties still exist in certain aspects, such as measuring and characterizing nanomaterials in organisms, and distinguishing naturally-occurring nanomaterials from engineered nanomaterials in the environment.

Chapter 2. Life Cycle Stages

This chapter discusses the life cycle of nano-TiO_2 as either a drinking 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, 155625). 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, 155622) and supplies approximately 90% of Ti minerals worldwide. For the 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 used in approximately 60% of current manufacturing (Hext et al., 2005, 090567). Creating synthetic rutile from ilmenite is often more economical than eliminating impurities from natural rutile.

World ilmenite production in 2007 was approximately 5.6 million metric tons (MT), and world rutile production was approximately 0.5 million MT. The nations that produce the greatest quantities of ilmenite are Australia, South Africa, Canada, China, Norway, India, the U.S., and Ukraine. Significant producers of rutile include Australia, Ukraine, South Africa, India, and the U.S. (Gambogi, 2008, 155622). 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, 2009, 195905).

In the U.S., 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 MT. Mine and mill employment at these sites was estimated at 229 persons in 2007, down from 344 in 2003 (Gambogi, 2008, 155622).

Low levels of radioactive materials are present in ilmenite and natural rutile (Collier et al., 2001, 155617; Haridasan et al., 2008, 155625). 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 approximately 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, 155625).

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

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

2.2. Manufacturing

Around 2005, annual global production of nano-TiO_2 was estimated at 2,000 MT, with an overall market value of $70 million (Dransfield, 2005, 157809; Osterwalder et al., 2006, 157743). Approximately 65% of production at that time 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 5,000 MT/year, and more than 10,000 MT/year predicted for years 2011-2014 (UNEP, 2007, 196074). In spite of some advantages of nano-TiO_2 over conventional TiO_2, nano-TiO_2 cannot replace all conventional uses of TiO_2. For instance, as a whitening agent, conventional TiO_2, and not nano-TiO_2, is needed to scatter visible light. From an economic point of view, the cheaper conventional TiO_2 is likely to be used in applications that can use either TiO_2 or nano-TiO_2. Nonetheless, nano-TiO_2 production based on a predicted trend of graduate and a theoretical upper bound of total replacement of conventional TiO_2 was recently presented (Robichaud et al., 2009, 193617). The current and future worldwide production levels of nano-TiO_2 was estimated to have an upper estimate of approximately 2.5 million MT by 2025 (Robichaud et al., 2009, 193617). Thus far, nano-TiO_2 production has represented a small fraction of overall TiO_2 production, which commanded a market of 4.5 million MT and $9 billion (Dransfield, 2005, 157809; Osterwalder et al., 2006, 157743).

Manufacturers and researchers report nano-TiO_2 synthesis by various techniques, including chemical vapor deposition (CVD) and flame hydrolysis (Wahi et al., 2006, 090580). Further information on manufacturing of nano-TiO_2 is provided in Appendix B. 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, 090581; Nagaveni et al., 2004, 090578). 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, 090566).

According to one industrial manufacturer of nanoscale titania, flame hydrolysis can generate high-purity nano-TiO_2 using $TiCl_4$ as a feedstock (Mulenweg, 2004, 090592). 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 (HCl) and a mixture of sizes of the metal oxide TiO_2 (Tok et al., 2009, 196054). Flame hydrolysis is used for manufacturing P25 and yields agglomerated particles with a mean diameter of approximately 3.6 µm, with the smallest 4% of particles having an average diameter of 160 nm (Klaessig, personal communication, 2006, 196041).

Anticipated by-products of this flame hydrolysis method of TiO_2 production include those resulting from chlorine contamination of the TiO_2 (from the $TiCl_4$ precursor). Warheit et al. (2007, 090594) have suggested that solutions of P25 in water are acidic (pH = 3.28) because of chloride ion artifacts on the particle surface. Manufacturer information, however, indicates that a steam washing step during the manufacturing process removes HCl adsorbed on the surface of P25 (Vormberg, 2004, 157822).

Another production method used to manufacture pigmentary-grade TiO_2 is the sulfate process, although it can also be used to manufacture nano-TiO_2 in certain commercial settings (Medley, personal communication, 2008, 196038). Details on this and other processes used in producing nano-TiO_2 can be found in Appendix B.

When photocatalytic or other applications require smaller particles, additional post-manufacturing processes of sufficient energy can be utilized to break apart the aggregates/agglomerates. Surfactants or solvents can be used to help keep the smaller particles from reaggregating after separation (Hewitt, 1996, 157936; Porter et al., 2008, 157508). Also, nanoscale particles might be sonicated to increase dispersion (Bihari et al., 2008, 157593).

2.2.1. Drinking Water Treatment

No information was found on processes used in preparing or formulating nano-TiO_2 specifically 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 wastewater treatment (NSF International, 2009, 196092; Pichat, 2003, 196037; Purifics, 2008, 196040). For this treatment system, P25 is neither specially prepared nor coated (Powell, personal communication, 2009, 196056).

2.2.2. Sunscreen

Unlike drinking 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 readily available. The choice of a specific nano-TiO_2 crystalline form is a key issue in manufacturing sunscreens because various forms differ in photostability. In particular, rutile is much more photostable than anatase (Chaudhuri and Majewski, 1998, 093308; Maynard, 2008, 157522). Although less photostable, anatase appears to be in common use. Barker and Branch (2008, 180141) studied five sunscreens containing nano-TiO_2, purchased over the counter, and found that one was pure rutile, while the other four were anatase/rutile mixtures in which anatase predominated.

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 by "doping" nano-TiO_2 particles by embedding minute amounts of metals within them, such as manganese, vanadium, chromium, and iron (Park et al., 2006, 193593).

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. Aggregation and agglomeration can negatively impact 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.

2.3. Distribution and Storage

Limited information is available regarding nano-TiO_2 distribution and storage. P25 is shipped as a powder in 10-kg "multilayer ventilated paper bags, equipped with an additional polyethylene lining when required" (Degussa, 2007, 090576). 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, 090576). Releases could occur if bags were damaged during shipping or storage, although such releases should be minimized by proper implementation of standard good management practices.

Another brand of photocatalytic nano-TiO_2 (KRONOS vlp 7000, 7001, and 7500) is also shipped in 10-kg paper bags (KRONOS International, 2006, 196046). Nano-TiO_2 powders from

Sigma-Aldrich Corporation (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.

As a dispersion formulation, nano-TiO_2 is shipped in pails, drums, or totes (Klaessig, personal communication, 2008, 196042). 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 in cold climates. 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 wastewater treatment systems during storage.

2.3.1. Drinking Water Treatment

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

2.3.2. Sunscreen

Topical sunscreen products are generally packaged in retail-sized bottles at the production facility and shipped in large 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, shed light on the distribution chain of sunscreens. Sales in supermarkets, drugstores, and mass merchandise outlets accounted for approximately two-thirds of the total U.S. sun-care retail sales in 1992-1993, according to Davis (1993, 157949). 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, 157949).

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.

2.4. Use

2.4.1. Drinking 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, is limited to nano-TiO_2 that would be used to remove arsenic at drinking water treatment facilities.

Roughly 54,000 community water systems in the U.S. serve more than 95% of the population (U.S. EPA, 2006, 091194). 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 wells (U.S. EPA, 2002, 091192). Public water systems are required to keep arsenic concentrations in delivered water at or below a maximum contaminant level (MCL) of 0.01 mg/L (U.S. EPA, 2006, 091193). Approximately 5% of community water systems in the U.S. (i.e., approximately 3,000 systems serving 11 million people) have taken some action to be in compliance with the arsenic MCL (U.S. EPA, 2007, 091224). Likewise, approximately 5% of 20,000 nontransient noncommunity water systems that serve at least 25 of the same people for more than 6 months of the year, such as schools, churches, nursing homes, and factories (i.e.,

approximately 1,100 systems serving 2 million people) have also taken some action to comply with the arsenic MCL (U.S. EPA, 2007, 091224). Altogether, approximately 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 drinking water treatment system, nano-TiO_2 might be used as powder (e.g., in a slurry) or fixed on a supporting material as a component of adsorptive media. 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 out and/or recycle all of the photocatalyst is required (Dionysiou, personal communication, 2009, 193921; Pichat, 2003, 196037). 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, personal communication, 2009, 193921).

Zhang et al. (2008, 193735) investigated the removal of nano-TiO_2 in a simulated conventional drinking 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-liter 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, personal communication, 2008, 157573; Kline, personal communication, 2008, 157545), nor is it available in most whole-house filtration systems (Johnson, 2005, 157799).

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 system 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 UV lamps illuminate the outside (Dionysiou, personal communication, 2009, 193921; Pichat, 2003, 196037). 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, 196037). A ceramic membrane filters out nano-TiO_2 (Purifics, 2008, 196040). 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, 2008, 196040). 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, 196037; Purifics, 2008, 196040). 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, 157845; Ferguson et al., 2005, 090572; Lee and Choi, 2002, 193498; Li et al., 2003, 090581; Meridian, 2006, 090595). Higher arsenic oxidation rates occurred using a slurry that was continuously stirred (compared to immobilized nano-TiO_2) (Li et al., 2003, 090581). 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, 193622). Additionally, numerous chemicals can be added for drinking water treatment (NSF International, 2009, 196092), 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, 193465; Keeney et al., 2009, 193466). 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, 193321; Geller et al., 2002, 193390; Santmyire et al., 2001, 193629; Weinstock et al., 2000, 193716). In three studies, 31-45% of survey respondents said they routinely or often use sunscreen (Cokkinides et al., 2001, 193321; Geller et al., 2002, 193390; Weinstock et al., 2000, 193716). In another study, 30% of respondents said they were very likely to use sunscreen when they were outdoors (Santmyire et al., 2001, 193629). More recently, data from the 2005 Health Information National Trends Survey in the U.S. 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, 193252). In the 2007 iVillage survey, the Skin Cancer Foundation (2008, 594955) 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 the U.S. FDA (2009, 196372) 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 2 hours, the Skin Cancer Foundation's recommended rate of reapplication (Skin Cancer Foundation, 2008, 594955).

While the use of sunscreen may be lower in young adults and adolescents than adults (Kasparian et al., 2009, 193465) sunscreen use is likely to be higher in young children. Robinson et al. (2000, 193618) 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, 191128) 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 U.S. is unknown. Furthermore, the available survey data do 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 Ti and 30% of sunscreens containing zinc in Australia were formulated with nanoparticles (TGA, 2006, 089202). As noted in Section 2.2, annual global production of nano-TiO_2 was estimated at 2,000 MT around 2005, with approximately 65%, or 1,300 MT, used in "personal care" products such as topical sunscreens and cosmetics (Dransfield, 2005, 157809; Osterwalder et al., 2006, 157743).

A poster presentation by Johnson et al. (2009, 644432) at SETAC Europe's 19th Annual Meeting suggested that possible concentrations of nano-TiO2 in water, as a result of sunscreen use, are between 2,000 and 8,000 ng/L. This range is based on modeling assumed rates of sunscreen use over the course of a day, how much is expected to wash off, and how much will be removed by sewage treatment plants during various summer-time scenarios in the River Thames region of the UK.

2.5. Disposal

2.5.1. Drinking 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, 091181). This process creates wastewater, which might be recycled in the treatment train or discharged (e.g., to a municipal sewer). For processes in which nano-TiO_2 would be introduced prior to or during the sand filtration process, the eventual disposal of the filter sand or other filter materials could result in nano-TiO_2 entering landfills along with the filter.

After nano-TiO_2 is used in drinking 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; this is the case with approximately 30% of sludge generated from drinking water treatment (U.S. EPA, 2010, 635678). Whether TiO_2 could diffuse (and thus be released) from a solid matrix such as sludge is unknown. Some newly developed landfills are designed to collect and treat leachate, but leaks are still possible, and the ultimate fate of nano-TiO_2 in the treatment process is unknown. In addition, some older landfills without leachate collection measures may still be in use. 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 (U.S. EPA, 2010, 635678). This is the case with approximately 20% of sludge generated from drinking water treatment, which applied to land to improve soil conditions or to fertilize the soil. The sludge is plowed directly into the soil to limit water runoff and for sanitary reasons (U.S. EPA, 2010, 635678). Nano-TiO_2 and other contaminants such as residual arsenic would then be present in these agricultural soils.

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 wastewater 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. Additional exposure pathways other than the specific handling of sunscreen containers are acknowledged as potentially important, and will be addressed as part of the fate and transport discussion in Chapter 3.

Chapter 3. Fate and Transport

Chapter 3 explores what might happen to 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 also 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.

The preceding chapter discussed life cycle stages of nano-TiO_2 with some considerations specific to its use in drinking water treatment for arsenic removal and in sunscreens. As this chapter focuses on the various pathways by which nano-TiO_2 can potentially enter and propagate through environmental compartments, information related to wastewater treatment pathways and by-products will also be pertinent. Throughout this document, it is important to note the distinction between the two types of water treatment being discussed. The case study application of nano-TiO_2 used in drinking water treatment for arsenic removal is distinct from the potential downstream appearance of nano-TiO_2 in municipal wastewater treatment plants. The former scenario deals with the use of nano-TiO_2, while the latter deals with its impacts after release to the environment. Because the processes for drinking water treatment and municipal wastewater treatment are different, they will lead to different scenarios for the fate and impacts of nano-TiO_2.

Although most studies cited in this chapter consider nano-TiO_2 in aggregate or agglomerate form (as discussed in Chapter 1), it is unclear whether all constituent primary particles remain in clusters if conditions change. Disagglomeration, 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 et al., 2006, 090584; Dunphy Guzman, personal communication, 2007, 091184). Sampled aggregates ranged up to 150 nm in size, and contained an estimated 8-4,000 nanoparticles (Dunphy Guzman et al., 2006, 090584). The pH_{pzc} depends in part on the crystal form of the nano-TiO_2 particles. Finnegan et al. (2007, 193379) reported pH_{pzc} values of approximately 5.9 for rutile and 6.3 for anatase. The degree of aggregation generally increases with increases in ionic strength (Domingos et al., 2009, 193347; French et al., 2009, 193384). The interaction between natural organic matter (NOM) and the aggregation state of nano-TiO_2 is complex, and whether aggregation is enhanced or inhibited by the presence of these organic species can depend on factors such as concentration of NOM, concentration of nano-TiO_2, pH, and the presence of divalent cations such as calcium (Kim et al., 2009, 635778).

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, 090582; Li et al., 2003, 090581; Nagaveni et al., 2004, 090578; Pena et al., 2006, 090573; Ryu and Choi, 2006, 090579; Sun et al., 2007, 193662; Wahi et al., 2006, 090580). Moreover, some preparations are specifically

---

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

designed to generate dispersed particles (e.g., Seok et al., 2006, 091198) to increase the efficacy of nano-TiO_2 as a catalyst, increasing the potential for the presence of disagglomerated or even disaggregated nano-TiO_2 to occur in the environment. However, a limited number of studies of nano-TiO_2 agglomeration/disagglomeration behavior under "real-world" ambient environmental conditions, irrespective of medium, have been conducted (Kiser et al., (2009, 225305)Battin et al., 2009, 201604).

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 indicated 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, 157415).

Several physicochemical properties of nano-TiO_2 can contribute directly to its environmental fate and transport in water. Long et al. (2006, 089584) 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, 090599) 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 showed that environmental pH can affect the surface charge properties.

Schmidt and Vogelsberger (2006, 193634) 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. However, since the equilibrium solubilities of the four types of nano-TiO_2 ranged from micro-to nano moles per liter, while the saturated suspensions were in the range of milligrams per liter, dissolved Ti concentrations were negligible compared with the initial TiO_2 input.

Although many studies have demonstrated the potential to use the photocatalytic properties of nano-TiO_2 in biocidal applications, including wastewater treatment (Chen and Ray, 2001, 193310; Han et al., 2009, 193407; Khataee et al., 2009, 193468; Rincon and Pulgarin, 2003, 157856; Wang et al., 2008, 193705; Watlington, 2005, 196080; Xu et al., 2009, 193726), data on the fate of nano-TiO_2 in actual wastewater treatment facilities are scarce. The Water Environment Federation released a report including the behaviors and effects of nanomaterials in wastewater treatment, although very few studies were on nano-TiO_2 (Effects of nanoparticles on the wastewater treatment industry (Report No, 2008, 195800). Kiser et al.(2009, 225305), however, have reported the occurrence of nano-TiO_2 at full-scale wastewater treatment plants (in both raw and finished waters). The authors measured total Ti concentrations, which included some nano-scale particles, on the order of 10 µg/L in tertiary effluent from wastewater treatment. Another investigator studied the effects of nano-TiO_2

on aquatic microbial communities under environmental conditions, which has implications on both natural waters and on wastewater treatment environments (Battin et al., 2009, 201604). Several recent studies have used mass balance modeling to predict the accumulation of nanomaterials within various environmental compartments, including nano-TiO_2 accumulation in wastewater treatment plants (Gottschalk et al., 2009, 633897; Mueller and Nowack, 2008, 157519).

Other types of nanoparticles also have been studied in wastewater treatment plants. Limbach et al. (2008, 155628) studied the fate of cerium oxide nanoparticles (20- to 50-nm diameter) in a model wastewater 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 wastewater 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 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.

Kiser et al. (2010, 634458) investigated biosorption rates of eight nanoparticles, including TiO_2, to wastewater treatment sludge. Investigators found that different nanoparticles biosorbed at different rates when placed in solutions with varying concentrations and types of biomass, which were designed to represent wastewater treatment sludge. For example, 23% of nanoscale TiO_2 was removed via biosorption in biomass solution of 400 mg/L total suspended solids, compared to 88% of aqueous fullerenes in the same solution, 39% of functionalized Ag nanoparticles, and 13% of fullerol nanoparticles (Kiser et al., 2010, 634458). The authors noted that further research is needed to understand the specific mechanisms responsible for sorption.

A limited number of studies are available on nano-TiO_2 and its interactions with microorganisms and other NOM under "real-world" environmental conditions (Battin et al., 2009, 201604; Kiser et al., 2009, 225305; Kiser et al., 2010, 634458). Battin et al. (2009, 201604) investigated damage to microorganisms from aggregated, agglomerated, and polydisperse nano-TiO_2 under natural conditions in river microcosms. Their toxicity results correlated poorly with lab experiment results on monodisperse nano-TiO_2 with monocultures, and contribute to the small but growing body of literature of nanoparticle toxicity in natural aquatic systems. It has long been recognized that anatase TiO_2 can photogenerate fairly long-lived ROS such as hydrogen peroxide via photoinduced redox reactions or modification of the TiO_2 surface in aqueous laboratory environments (Harbour et al., 1985, 090632). It is not clear how relevant results from such experiments would be for anticipating nano-TiO_2's behavior in wastewater or drinking water treatment plants.

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, 190513) found that humic acid, a common type of natural organic matter, is easily adsorbed onto nano-TiO_2 in aqueous media. Because humic acid adsorption decreased the zeta 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, 190513).

Sediment, the solid fragments of inorganic or organic material that are carried by and settle to the bottom of natural waters, is another environmental matrix that could be affected by the release of nanomaterials. One study was identified on the transport and deposition of nano-TiO_2 in natural streams and streambeds (Boncagni et al., 2009, 634454). Partitioning of nanomaterials, including

nano-TiO_2, to sediments and other environmental compartments was modeled by Gottschalk et al. (2009, 633897).

3.1.1. Drinking Water Treatment

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. The removal efficiencies of commercial nano-TiO_2 in conventional water treatment processes (coagulation, flocculation, sedimentation, and filtration) have been reported in one study using jar testing (Zhang et al., 2008, 157462), although the condition was set for nano-TiO_2 in source water and not as an agent in drinking water treatment. The study showed that more than 20% of initial 10 mg/L nano-TiO_2 remained in the water after up to 24 hours of flocculation and 1 hour of sedimentation (in buffered nanopure water with $MgCl_2$); more than 30% initial 10 mg/L nano-TiO_2 remained in water after alum coagulation, flocculation, and sedimentation (in nanopure water); and filtration using a 0.45 µm membrane as a final process was able to leave only 1% to 8 % of the total TiO_2 mass (in flocs smaller than 500 nm) in the water (Zhang et al., 2008, 157462). It is expected that the actual removal efficiencies in drinking water treatment facilities would be different from these tested conditions due to the differences in process time, source water, and other factors. For instance, under the tested conditions (Zhang et al., 2008, 157462), the most efficient nano-TiO_2 removal was seen after 8- or 24-hours flocculation and 1-hour sedimentation. Flocculation is typically less than 1 hour in drinking water treatment plants, which may result in less removal than observed in the Zhang et al. study, while sedimentation is commonly several hours, which may result in more removal (AWWA Staff, 2003, 193818). In addition, the removal efficiencies of nanoparticles, not limited to nano-TiO_2, were lower in tap water containing natural organic matter compared to nanopure water (Zhang et al., 2008, 157462). Since the removal of nano-TiO_2 initially received as a suspension (200-nm aggregates) was less efficient than the removal of nano-TiO_2 initially received as dry powders (500-nm aggregates), the authors speculated that the removal efficiencies would be lower for small aggregates than large aggregates at the same alum (coagulation agent) concentration.

Several different waste streams that could contain nano-TiO_2 could be generated from drinking water treatment facilities. For nano-TiO_2 that settles with floc in the sedimentation step, nano-TiO_2 presumably could become part of the sludge (AWWA Staff, 2003, 193818). The discarded sludge could be transported off-site for disposal or reuse, such as being buried in municipal solid waste landfills or directly applied to agricultural or recreational land.

Theoretically, nano-TiO_2 might become part of the filter matrix during the filtration step of water treatment. U.S. EPA's Filter Backwash Recycling Rule (U.S. EPA, 2002, 644800) requires that, when the filter is backwashed, the water used must be recycled back into the coagulation process. This could reintroduce nano-TiO_2 into the treatment process, but the implications for concentrations of nano-TiO_2 in finished water are not clear.

Various fate pathways could apply to nano-TiO_2 used as a drinking water treatment agent. For example, if nano-TiO_2 is not completely filtered out or otherwise removed from the final effluent, nano-TiO_2 might remain in the water as aggregates/agglomerates and enter municipal 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 in the subsurface environment. If nano-TiO_2 were to enter ground water aquifers, nano-TiO_2 would presumably persist, given that other inorganic compounds are not readily broken down in that environment and nano-TiO_2 is poorly soluble; however, particle/agglomerate size and other characteristics could change. Conceivably, nano-TiO_2 could contribute to the release of (or modify the bioavailability of) other water contaminants of concern.

If nano-TiO_2 were present in the final drinking water product that reaches the tap, it eventually might enter the ambient environment or be captured by a wastewater stream, after which it could reach a wastewater treatment facility. If the particular wastewater treatment method employed there

did not completely remove nano-TiO_2, some level of nano-TiO_2 would likely enter downstream water sources.

3.1.2. Sunscreen

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 (Appendix B). Nano-TiO_2 in emulsion, dispersion, and possibly powdered form could be present in wastewater (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 could also be released to wastewater or to natural bodies of water through showering/bathing or through laundry water drainage following sunscreen use. Swimming after sunscreen use could result in an accumulation of sunscreen material in the swimming pool water and potentially be a point of release into the environment as untreated wastewater. If nano-TiO_2 remained 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.

Auffan et al. (2010, 625063) investigated how nano-TiO_2 particles formulated for use in sunscreens transform, or age, when placed in media that mimicked environmental conditions and conditions of product use. Their results showed that 90% wt of one coating constituent desorbed from the particle surfaces, that another constituent remained on the surface but was oxidized, and that the third constituent was chemically affected but remained sorbed at the surface. Though the remaining Al-based layer was still effective in protecting against the production of superoxide ions from the photoactive nano-TiO_2 particle core under their experimental conditions, these changes in coating characteristics illustrate that transformations may occur once nano-TiO_2 is released to the environment.

The potential for release is suggested by recent studies that have detected topical sunscreen constituents in untreated wastewater, treated wastewater, surface water (lakes and rivers), fish from lakes and rivers, and biosolids (Balmer et al., 2005, 157817; Fent et al., 2008, 157574; Rodil and Moeder, 2008, 157503). The organic compounds detected in these studies were UV filter compounds such as 4-MBC (4-methylbenzylidene camphor) and octocrylene (OC), which generally biodegrade slowly and can bioaccumulate. Some evidence also indicates that nano-TiO_2 can bioaccumulate (Zhang et al., 2006, 157722). 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, 157722) 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 wastewater or natural bodies of water.

3.2. Soil

Three studies addressed the fate and transport of nano-TiO_2 in soil. Dunphy Guzman et al. (2006, 090584) 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 range where electrostatic forces between nano-TiO_2 aggregates and the experimental Pyrex surface should have been strong (pH 2.5-5.9), 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 transport of nanoparticles and aggregated nanoparticles. These 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, 089258) 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. Approximately 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 were specific to the controlled experimental conditions, they suggest that particle size affects mobility of nanoparticles and that anatase might be mobile in ground water (Lecoanet et al., 2004, 089258).

A recent study using soil samples from 11 different sites found that nano-TiO_2 could remain suspended in soil suspensions for 10 days (Fang et al., 2009, 193371). 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 deeper 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, liquid waste would be separated 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 and reach 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 (Ti is not regulated under U.S. EPA's Biosolids Rule, Part 503) (see U.S. EPA, 1994, 090659). The treatment might include high temperature or strong alkaline pH processing, or both (U.S. EPA, 1994, 090659). 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, 090659). Roughly 50% of treated sewage sludge is applied to land, and treated sewage sludge is applied to less than 1% of all U.S. agricultural land (U.S. EPA, 2006, 090658).

Nano-TiO_2 in sewage 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, eventually 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 vegetation. 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 might differ from conventional TiO_2 due to nano-TiO_2 particles' greater surface area-to-volume ratio; the specifics of potential reactivity differences are largely unknown at this time.

3.2.1. Drinking Water Treatment

One scenario by which nano-TiO_2 could enter soils would be through direct land application of sludge after specifically being used as an agent in drinking water treatment. In addition to the sewage sludge produced in wastewater 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 sludge as well. Similarly, as described above, if nano-TiO_2 were present in finished drinking water, it would eventually enter sewage treatment facilities where any remaining residual nano-TiO_2 could also enter the sludge. The discarded sludge would be transported off-site and could be used as daily cover in a municipal solid waste landfill or used for direct 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 vegetation. These scenarios could have implications for soil microbes and could also be noteworthy in relation to nutrient uptake by edible vegetation.

3.2.2. Sunscreen

As described above, nano-TiO_2 in topical sunscreens could end up in the sludge produced at a wastewater 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. (2007, 157594) at a European nano-TiO_2 manufacturing facility that supplies the nanomaterials for sunscreens and cosmetics found that "outside the plant," the airborne TiO_2 particle concentration was approximately 13,000 particles/cm^3, with nearly 94% of particles 100 nm or less in size, and approximately 52% at 40-60 nm (Berges, 2007, 157594; Berges, 2008, 193274). The authors did not specify the duration or environmental conditions of the measurements.

Some potential could exist for environmental or occupational atmospheric emissions and releases of nano-TiO_2 if the transport or storage containers were to be compromised (e.g., due to a forklift error, train derailment, or truck accident). Direct land application of sludge, from either drinking water or wastewater treatment, might contribute nano-TiO_2 to the atmosphere if dried material were to be re-entrained from wind turbulence. Nano-TiO_2 is not expected to enter air via sunscreen application or from drinking water treatment processes.

The large surface area of nano-TiO_2 particles presents an opportunity for other co-occurring contaminants to adsorb onto their 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 gases (U.S. EPA, 2004, 056905). When nano-TiO_2 was dispersed for 0.5 hours in the air immediately next to thermal precipitators 1.5 m above the ground in various outdoor locations in the city of El Paso, Texas, USA, the collected nano-TiO_2 particles were not only in agglomerate/aggregate form, but were also associated with other airborne nanoparticles, in particular, nanosilicate particulates (Murr et al., 2004, 196310). Environmental conditions at the study sites were not described, other than the investigators avoided collections in high-humidity environments.

Chapter 4. Exposure–Dose Characterization

This chapter examines the potential for biota and humans to be exposed to 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[2] 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 ambient 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 manufactured product's 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 leach into bodies of water when the soil is saturated with water.

During the life cycle stages of 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 presence in wastewater after users bathe or shower to remove residual sunscreen on the skin and launder clothes containing traces of sunscreen. Discharges of nano-TiO_2 from wastewater treatment plants are not currently regulated, and are thus not designed or operated to remove nano-TiO_2, although early research suggests that some removal can occur (Kiser et al., 2009, 225305). Therefore, nano-TiO_2 might be present in the effluent and could lead to

---

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

[2] The distinction between *exposure* and *dose* in this document is consistent with risk assessment usage (U.S. EPA, 1992, 041875). 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.

aquatic species exposure. In the life cycle disposal stage, wastes from factories and research facilities containing nanomaterials are often incinerated, possibly releasing nano-TiO_2 into the air. Household waste containing consumer products made 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). For instance, an accident or a mechanical failure might occur in spite of good safety practices. Such occupational exposures could differ from exposure to the general public in various ways. For example, workers could be exposed to free nano-TiO_2, whereas the public might more commonly encounter nano-TiO_2 embedded in a product. Exposure durations and concentration levels are likely to be higher in occupational settings. Likewise, target tissue dose levels could differ between workers and the general population or even between workers in different occupations at the same facility, depending on factors such as respiration rates in relation to sedentary or strenuous activity in the presence of airborne nano-TiO_2.

4.1. 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. Some of these scenarios will be further explored in this section, specifically examining various TiO_2 exposure conditions and how they could affect aquatic and terrestrial organisms. The potential for bioaccumulation and entry of nano-TiO_2 into the food web is discussed in Section 4.6.

4.1.1. Aquatic Species

Data on concentrations of nano-TiO_2 in sediment, 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., 2008, 157562). 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 natural bodies of water. 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., 2008, 157562). 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 (Handy et al., 2008, 157562). Surface-acting metal toxicity of nano-TiO_2 has been suggested as a cause of gill damage in rainbow trout where the Ti concentration in gill tissue was not increased (Federici et al., 2007, 091222).

Because water flow is also slower near the interface with air, higher concentrations of nanoparticles are also expected at the air-water interface (Handy et al., 2008, 157562). 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.

4.1.2. Terrestrial Species

Terrestrial organisms could be exposed to nano-TiO_2 under various scenarios. For example, spillage during the life-cycle stages of 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 treated or wastewater 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.

4.2. 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 a function of 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 nonoccupational 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 use in drinking water treatment and in topical sunscreens.

4.2.1. General Population

4.2.1.1. Drinking Water Treatment

Although the actual use of nano-TiO_2 in water treatment facilities appears to be limited at present to pilot testing (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 U.S. 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 mL/kg-day for these infants versus 17 mL/kg-day for the older age group (see Table 3-9 in U.S. EPA, 2008, 196062). 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, 2008, 196062).

4.2.1.2. Sunscreen

As discussed in Section 2.4.2, iVillage survey data from 2007 suggest that sunscreen might be used on a daily basis by 33 million people in the U.S. and on an occasional basis by another 177 million. Moreover, sunscreen use appears to be increasing. According to the Skin Cancer Foundation (2008, 594955), 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, 157682). Nano-TiO_2 sunscreen powders are also available, according to the Project on Emerging Nanotechnologies at the Woodrow Wilson Center's nanotechnology-based consumer product inventory (Woodrow Wilson International Center for Scholars, 2006, 196083). Another sun protection option available to consumers is "cosmeceuticals," cosmetics that incorporate active sunscreen ingredients (Davis, 1994, 157946). In the mid-1990s, up to 30% of lipsticks and 20% of makeup were estimated to have SPF ratings, sunscreen claims, or both (Davis, 1994, 157946). 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, 157946).

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 mg/cm^2 of skin (roughly 1.5 ounces or 3 tablespoons for the entire body of an average adult), most consumers use 0.5-1.5 mg/cm^2 skin (Srinivas et al., 2006, 157734). 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/application, and a 3-year old would use an estimated 3-15 g/application (Table 4-1). 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/person/application for an adult, and 0.17 to 0.76 g/person/application for a 3-year old (Table 4-1). These exposure estimates are in line with estimates made by Hansen et al. (2008, 157560). 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 4-1, the ranges of applied nano-TiO_2 would be 12 to 55 mg/kg of body weight/application for a 3-year old and 8.0-37 mg/kg of body weight/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, 193435), 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, 193537). 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, 191128).

Table 4-1. Estimated dermal exposure to nano-TiO_2 from sunscreen containing 5% nano-TiO_2 for adults and 3-year-old children

Subject	Surface area[a] of skin (cm^2)	Applied sunscreen surface density (mg/cm^2)	Applied sunscreen amount (mg/person/ application)	Applied[b] nano-TiO_2 (mg/person/ application)	Applied nano-TiO_2 (mg/kg BW/ application)
3-yr-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-yr-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

[a]Body surface area values are based on Tables 6-6 and 6-16 of U.S. EPA (1997, 594981).

[b]Actual concentrations of nano-TiO_2 in commercial sunscreen on the market vary, with the high at nearly 15%. (Table A-1 in Appendix A.)

BW – Body weight. The body weights used in the calculation were 14 kg, the median for 36-month old females (2000, 157982), and 62 kg, the median for adults 18-74 years old; Table 7.5 of U.S. EPA (1997, 594981).

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 treated water containing nano-TiO_2, the characteristics of the resulting aerosol have not been documented in the published literature. Section 4.2.2 discusses inhalation exposure from 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) has been estimated between 2.5 and 5.4 mg/individual/day (Lomer, 2000, 635672; Lomer et al., 2004, 157382). 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/individual/day), and no particle size information was provided.

4.2.2. 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 drinking water treatment agents or topical sunscreens.

As a frame of reference, NIOSH (2005, 196072) proposed a draft occupational exposure limit of 1.5 mg/m^3 for fine TiO_2 (primary particle <10 μm, see below for details) and 0.1 mg/m^3 for ultrafine TiO_2 (primary particle <0.1 μm, see below for details), as time-weighted average concentrations for up to 10 hours/day during a 40-hour work week. The "fine" particles in this NIOSH draft were defined as all particle sizes that are collected by respirable particle sampling (i.e., 50% collection efficiency for particles of 4 μm, with some collection of particles up to 10 μm). "Ultrafine" particles were defined as the fraction of respirable particles with primary particle diameter <0.1 μm(2005, 196072). The NIOSH draft exposure limit was based on primary particle size, not the measured aggregate or agglomerate sizes. Agglomerates of ultrafine TiO_2, which may by larger than 0.1 um, often exhibit biologically similar behavior to ultrafine TiO_2 due to the surface area of the constituent particles, and therefore the recommended exposure limits for ultrafine TiO_2 should apply (NIOSH, 2005, 196072). The draft recommended exposure limits were extrapolated from rat-based critical dose estimated to humans, using specific surface area measured by the BET method (6.68 m^2/g for fine TiO_2 and 48 m^2/g for ultrafine TiO_2) to convert particle mass to surface area dose (NIOSH, 2005, 196072). Because the sizes and surface areas of fine and particularly ultrafine (nano) TiO_2 vary, the risk estimates will vary for other particle sizes and surface areas (NIOSH, 2005, 196072), as well as other crystal forms of TiO_2 and other conditions (Section 5.1).

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 TiO_2 manufacturing facilities located in the U.S. 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) (Fryzek et al., 2003, 157864). However, it is possible that the levels of exposure can vary depending on the facility.

The manufacturer of P25 has stated on its website that workplace inhalation exposures to TiO_2 are typically less than 0.5 mg/m^3 (Degussa, 2007, 090576). If such exposures are sustained for less than 2 hours/day, they would not exceed the NIOSH proposed occupational exposure limit of 0.1 mg/m^3, which is expressed as a 10-hour time-weighted average. 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 an enclosed process. This manufacturer is said to require the use of personal protective equipment during any repair work that could lead to dust exposure (Maier, personal communication, 2007, 091185). Such information suggests only 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 particle manufacturing, packaging, and distribution 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, 157699). No worker exposure data were available for materials incorporation, packing, or product fabrication for nano-TiO_2-containing polymer products. Although the potential for worker exposure was stated to be low (DuPont, 2007, 157699), the detection limit (0.3 mg/m^3) is above the draft NIOSH recommended limit for ultrafine or nano-TiO_2 of 0.1 mg/m^3 (NIOSH, 2005, 196072).

Preliminary estimates of workplace exposure in a factory that produces rutile nano-TiO_2 for sunscreen and cosmetics were reported by Berges (2007, 157594; 2008, 193274). Measurements were made in 2006, and then in 2007, when improvements to local exhaust systems were in operation (Berges, 2008, 193274). 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 approximately 100 µm]; *respirable* refers to particles that penetrate to the alveolar [pulmonary] region with a mass median aerosol diameter [MMAD] of approximately 4 µm[1] (CEN, 1993, 078032)) 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. It is not clear how applicable the results from this manufacturing facility are to occupational exposure in facilities where, for example, sunscreen products are formulated from the raw materials.

Liao et al. (2009, 157456) further reported and analyzed the Berges (2007, 157594; 2008, 193274) 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, 157594; 2008, 193274), 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 to 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 µm^2/cm^3 for "alveolar deposited" particles and 50 µm^2/cm^3 for "tracheobronchial deposited" particles. Under normal operating conditions, the particle surface area concentrations were 50 µm^2/cm^3 for the alveolar deposited particles and 13 µm^2/cm^3 for the tracheobronchial deposited particles. Outside the facility, the airborne TiO_2 particle concentration was approximately 13,000 particles/cm^3. 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). Employees classified as surface treatment workers (involved in drying, packing, and blending operations) had a higher TiO_2 burden in their 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, 157594; 2008, 193274) reports as well as conventional TiO_2 studies (Boffetta et al., 2004, 157849; Fryzek et al., 2003, 157864), Liao et al. (2009, 157456) 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, 157456) 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"

[1] The size for respirable particles cited here is the standard used by Berges (2007, 157594; 2008, 193274), and the size used in other studies may vary by standards set by different agencies or even laboratories. Some argue that approximate 50% of 5 µm particles are deposited in the alveolar region of humans who are engaged in activities requiring moderate ventilation levels, particularly associated with oronasal breathing. Thus, the respirable particulate size for the alveolar region in humans is closer to 7-8 µm (F.J. Miller, personal communication (2009, 625211)).

(Liao et al., 2009, 157456). Though these conclusions were not based on actual worker exposure, the combination of field data on relevant TiO_2 size, laboratory lung cell studies and computer modeling generates data on the potential nano-TiO_2 burden that could be faced by people handling these materials.

In a presentation at a professional conference, Li et al. (2008, 196055) displayed photographs of a factory in Shanghai, China 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 was uncertain as the factory also handled conventional "pigmentary grade" and "food grade" TiO_2 (Ichihara, personal communication, 2009, 196034). Li et al. (2008, 196055) 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. The authors noted that this factory exhibited particularly poor conditions compared to others with similar processes, but even so their investigation can be used to illustrate 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, personal communication, 2008, 196042) 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 were available.

The above information suggests that inhalation and dermal exposure could occur during manufacturing, packaging, shipping, and storage of nano-TiO_2. To fully characterize potential risk, toxicity data at conditions comparable to be the reported exposure conditions would be useful, although extrapolation from higher concentrations can be used as well.

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

Nano-TiO_2 is used in various manufactured products, raising the possibility that biota and humans could be exposed to nano-TiO_2 from more than one source. Such sources might include drinking water treatment agents, topical sunscreens, cosmeceuticals (traditional cosmetics such as moisturizers and color cosmetics that incorporate active sunscreen ingredients containing nano-TiO_2), sun-protective clothing, cleaning agents, air purifiers, coatings, and food packaging, among many others (The Project on Emerging Nanotechnologies, 2009, 196052). It should, however, be recognized that nano-TiO_2 particles from these different sources may have different properties such as size distribution, crystalline phase, and surface treatment. Kaegi et al. (2008, 193457), 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, 193437) 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 treated water and sunscreen could involve a person's ingesting the water (oral route), bathing (dermal) or showering (dermal and inhalation), 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 wastewater treatment facility and could also ingest prey whose contamination originated from ambient water, sediment, or other prey or plants that already contained sunscreen constituents. The seemingly widespread occurrence of nanoparticles of various types in aquatic media reported by Wigginton et al. (2007, 157415) lends plausibility to these scenarios.

4.4. Cumulative Exposure to Nano-TiO_2 and Other Contaminants

Nano-TiO_2 is not the only substance relevant 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 were 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, 090578; Pena et al., 2006, 090573). With a tendency to adsorb other pollutants and an ability to absorb into the body and cells (Sections 4.6.1, 4.6.3, and 4.6.4), the possibility cannot be ruled out that nano-TiO_2 could carry toxic pollutants to target sites where the pollutants would not normally go (Moore, 2006, 089839). Such activity could result in increased uptake of other pollutants or interactive effects that would otherwise not occur if these substances were only present individually.

4.5. Models to Estimate Exposure

The EPA uses various models to estimate exposures for chemical assessments, some of which are described on the websites for the Council for Regulatory Environmental Modeling (U.S. EPA, 2009, 196065) and the Center for Exposure Assessment Modeling (U.S. EPA, 2009, 196064). 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, 2007, 196060). Other fate and transport models also might be relevant, for example, the Particle Tracking Model (PTM) that the Army Corps of Engineers developed (Demirbilek et al., 2005, 193887); the Eulerian model that treats particles as a liquid (Kollias, 2009, 624994); Publicly Owned Treatment Works (POTW) models for evaluating fate in wastewater treatment plants (to sludge, effluent, and air); receiving water models for evaluating environmental transport and fate, and bioaccumulation models (Minerva, 2009, 625210); and over 20 models of aquatic fate and transport (for comparison, see a review by Paquin et al., 2003, 196867). 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) (Mueller and Nowack, 2008, 157519). PEC values were calculated for "realistic exposure scenarios" (based on nano-TiO_2 use, estimated as 25 MT per year in Switzerland) and for "high exposure scenarios" (based on 500 MT 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, Hund-Rinke and Simon, 2006, 090607), was divided by an assessment factor of 1,000, in accordance with the Technical Guidance Document on Risk Assessment published by the European Chemicals Bureau, because, as the authors noted, the "accuracy of the data was low" (European Chemicals Bureau, 2003, 196375; Mueller and Nowack, 2008, 157519). 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, 157519) stated that, given that the PEC was 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 (Umweltbundesamt, 2009, 196071). Gottschalk et al. (2009, 633897) followed up on this work using similar modeling procedures to predict environmental concentrations of several nanomaterials across a variety of compartments and regions. The team also investigated the overall applicability of probabilistic modeling to predict environmental exposure to nanomaterials (Gottschalk et al., 2010, 635674).

Based on available information about the applied concentration of nanoparticles in cosmetics, personal care products and paints, Boxall et al. (2007, 196111) used a series of algorithms to estimate the PECs of nanoparticles in soil and water. Although anticipating that 10% market penetration probably provides a conservative estimate (with the exception of sunscreens), the researchers calculated the PEC for three scenarios assuming that 10%, 50% and 100% of the products on the market contained nanoparticles. The total predicted concentrations in water were found to be 24.5-245 µg/L.

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. It is important to note that upon entering an organism, a substance may still be transported and undergo changes as it moves throughout the organism. For this reason, understanding dose includes understanding additional fate and transport considerations specific to the media encountered by a substance once it is taken up; several investigations have been identified in this area. Sager et al. (2007, 090633) 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 in determining the behavior of nano-TiO_2 within a given system.

Exposure to nano-TiO_2 in aquatic organisms has been studied mostly by measuring tissue concentrations in fish exposed to it in water. However, information related to exposure of substances other than nano-TiO_2 is often also mentioned, appropriately reflecting the multiple substances to which aquatic organisms may be exposed in the natural environment. For terrestrial organisms, including laboratory animals used for toxicological studies and used 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, dermal, or ingestion. While differences in animal models, such as the differences in human and rodent nasal pathways leading to the olfactory bulb, are known to underlie some differences in toxicological results between species, studies across a biological continuum are drawn upon herein to collect a spectrum of potentially informative data.

Additionally, this section discusses special biological barriers (blood brain barrier [BBB] and placenta), and issues related to dose-metrics for nano-TiO_2. Again, because internal transport of the materials will influence the ultimate dose to the organism, it should be noted that multiple routes of exposure will be considered, even though all routes may not be equally significant. Some routes (e.g., i.v., i.p., and i.m. injections) could have relevance when internal transport is considered.

4.6.1. Uptake in Aquatic Species

4.6.1.1. Bioaccumulation

Zhang et al. (2006, 157722) found that nano-TiO_2 can accumulate internally in carp (Table 4-2). The authors exposed carp to photocatalytic nano-TiO_2 (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 2,100 at 3 mg/L, and approximately 1,400 at 10 mg/L.

In contrast to the finding of bioaccumulation of nano-TiO_2 in carp that Zhang et al. (2006, 157722) reported, Federici et al. (2007, 091222) detected no accumulation in rainbow 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, 091222) study used photocatalytic nano-TiO_2 (also P25), but 80% of the water in the fish tank was changed every 12 hours. Similar to Zhang et al. (2006, 157722), Federici et al. (2007, 091222) 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 14ºC for rainbow trout and at 23ºC for carp, could influence the behavior or effects of nano-TiO_2 and contribute to the difference between these two studies. Furthermore, carp feeding behavior mainly consists of grubbing in sediments, and therefore carp could have a higher exposure to settled nano-TiO_2 aggregates than rainbow trout. Studies on kinetics of uptake and clearance, which could be valuable in understanding nano-TiO_2 bioaccumulation and relevant factors, were not available.

Although nano-TiO_2 may 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. (2008, 157563) 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., 2008, 157563). Handy et al. (2008, 157563) 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 cellular response to

nano-TiO_2 exposure (Xu et al., 2009, 157452). The concentration of nano-TiO_2 or Ti in cells was not measured (Xu et al., 2009, 157452).

4.6.1.2. 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 of chemical energy needed to fuel ecosystems. Many invertebrates, which are primary consumers of chemical energy in aquatic ecosystems, 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, 157477), they could be exposed to quite high numbers of nanoparticles in water (Griffitt et al., 2008, 157565). Even if nano-TiO_2 were not absorbed into tissues, nano-TiO_2 in the digestive tract of daphnids could still contribute to bioaccumulation in the food web. Although nano-TiO_2 has not been tested for trophic transfer in the food web, one study found evidence of transfer of carboxylated and biotinylated CdSe-based quantum dots to higher trophic organisms (rotifers) through eating ciliated protozoans exposed to quantum dots (Holbrook et al., 2008, 192383). Since quantum dot uptake in this study was inferred from quantitation of Cd^{2+} and assumed no dissolution of the nanoparticles, it is unclear to what extent these results are applicable to poorly soluble nano-TiO_2. Biomagnification was not observed at the top predator level (rotifers), because biomagnifications factors (BMF) values for the quantum dots ranged from between 0.29 and 0.62 (Holbrook et al., 2008, 192383).

4.6.1.3. Cumulative Dose of Nano-TiO_2 and Other Pollutants

Increased uptake of other pollutants in the presence of nano-TiO_2 has been reported by Sun et al. (2007, 193662) and Zhang et al. (2006, 157722; 2007, 090114) (Table 4-2). Sun et al. (2007, 193662) demonstrated that arsenic as arsenate [As(V)] strongly binds to Aeroxide® P25 (P25) in water and that fish (carp) exposed to water containing 10 mg/L of this photocatalytic nano-TiO_2 and 200 µg/L arsenate accumulated more arsenic than fish exposed to either nano-TiO_2 or arsenic alone. The bioconcentration factor of arsenic[1] was more than twice as high when nano-TiO_2 was present than when it was not (Sun et al., 2007, 193662). 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, 087896; Feng et al., 2001, 193374; Moore et al., 1997, 193553; Tian et al., 2001, 193679). Although data on nano-TiO_2 concentrations in the environment[2] are lacking, the tested nano-TiO_2 concentration (10 mg/L) may be higher than the likely environmental concentrations, with the exceptions of spills or accidents. 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, 193662) concluded that adsorption to nano-TiO_2 facilitated arsenic transport and uptake.

[1] The bioconcentration factor of arsenic = 1,000 × arsenic concentration in fish (µg/g dry weight)/arsenic concentration in water (µg/L).

[2] Limited information is available on nano-TiO_2 in the ambient air within or surrounding nano-TiO_2 production facilities, or from the runoff from structures painted with paints containing TiO_2. In the various compartments of the environment, however, nano-TiO_2 concentrations are unknown, and current technologies have not been able to distinguish man-made nano-TiO_2 from naturally-occurring nano-TiO_2.

Table 4-2. 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 hr)	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, 157722)
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, 193662)
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, 090114)
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 control fish on day 0, 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, 091222)

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

P25 – Aeroxide® P25

Zhang et al. (2007, 090114) showed that fish (carp) exposed to cadmium in water (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 4-2). 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. 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, 090114). Unlike nano-TiO_2, natural sediment particles (19 µm) (at equivalent concentrations) did not affect cadmium bioaccumulation. 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 tissue concentrations of cadmium and nano-TiO_2 (measured as Ti) 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). 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 drinking water treatment with conventional TiO_2 (Richardson et al., 1996, 193612) suggests the possibility that nano-TiO_2 could have the same effect. Richardson et al. (1996, 193612) compared

the organic disinfection by-products detected after using: (1) chlorine as the sole disinfectant; and (2) TiO_2/UV light treatment followed by chlorination. The authors reported detecting an additional by-product (tentatively identified as dihydro-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 nanomaterial; e.g., nano-TiO_2 and nano-silver (nano-Ag) have been used together in multiple products (The Project on Emerging Nanotechnologies, 2009, 196052).

4.6.2. Respiratory (Inhalation and Instillation)

Instillation can be performed in various ways, but essentially involves the direct administration of a substance to the respiratory tract. 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, 193534; Oberdörster et al., 1992, 045110; Oberdörster et al., 1994, 046203), blood (Geiser et al., 2005, 087362), and the brain (Wang et al., 2005, 193703; Wang et al., 2007, 090290; Wang et al., 2008, 157473).

Particles in the nasal cavity may enter the brain through: (1) the olfactory nerve (Elder et al., 2006, 089253; Oberdörster et al., 1994, 046203) [upper particle size limit: 200 nm (Elder et al., 2006, 089253)]; (2) the circulating blood and then crossing the blood-brain barrier (Oberdörster et al., 2004, 055639); and (3) the olfactory mucosa and through the ethmoid bone into cerebrospinal fluid (Illum, 2000, 157897). One of the most visually convincing demonstrations of olfactory nerve transport, as mentioned in Oberdörster et al. (2004, 055639), is a study by DeLorenzo (1970, 156391). DeLorenzo showed sequential 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, 055639).

Intranasal instillation of three sizes of nano-TiO_2 particles (approximately 20, 70, and 155 nm) at approximate 0.05 g/kg BW every other day for 30 days resulted in increased Ti concentrations in the olfactory bulb of mice (Wang et al., 2005, 193703). 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 at approximate 24 mg/kg BW every other day for 30 days (Wang et al., 2008, 157473). The authors noted that the fact that brain tissue Ti concentrations were higher than lung tissue concentrations suggested that the olfactory nerve was the route of transport to the brain in this study.

For respiratory exposure, the deposition pattern and concentration of particles in the respiratory tract can influence the health effects of these particles. Particles of various sizes can have different mechanisms of deposition (Gebhart, 1992, 157951; Heyder et al., 1985, 006919; Oberdörster et al., 2005, 087559). 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. (2005, 087559) 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, approximately 90% will be deposited in the nasal, pharyngeal, and laryngeal region; approximately 10% in the tracheobronchial region; and almost none in the alveolar region. These results contrast with the modeling of a 5-nm particle, which is deposited roughly equally in the three regions. Approximately 50% of larger, 20-nm particles are deposited in the alveolar region, with approximately 15% deposition in each of the other two

regions. Since these simulations are based on the International Commission on Radiological Protection (ICRP) model, which was designed for larger particles (supplement of Oberdörster et al., 2005, 087559), the performance of this model regarding particles at the lower end of the size distribution is unclear.

In contrast to these results, a Multiple Path Particle Dosimetry (MPPD) model that incorporated convective flow, axial diffusion , and convective mixing (dispersion) predicted that very few small nanoparticles would deposit in the alveolar area (Asgharian and Price, 2007, 093119). Nanoparticles less than 10 nm in diameter were predicted to deposit mainly in the tracheobronchial airway, and very few nanoparticles smaller than 5 nm were predicted to reach the alveolar region (Asgharian and Price, 2007, 093119). Depending on particle size, consideration of axial diffusion and dispersion could result in increased predicted deposition in the alveolar region of up to 10%. This modified MPPD model for nanoparticles found good agreement between predicted depositions of nanoparticles with measurements reported in the literature.

Inhaled nano-TiO_2 persisted in the lung longer than fine TiO_2 in rats (Oberdörster et al., 1994, 046203). 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, 046203).

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, 056707).

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

Species/Strain	Aerosol	Study Protocol	Observations	Reference
Fischer 344 rats, females (6 wk) B3C3F1 mice, females (6 wk) Hamsters, females (6 wk)	TiO_2: 1.29-1.44 µm MMAD (σg = 2.46-3.65), 21-nm primary particles	Animals exposed via inhalation 6 hr/day, 5 days/wk, for 13 wk 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) postexposure. 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 postexposure 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 × control groups) out to 52 wk without signs of recovery. Epithelial permeability was 3 to 4 × control in high exposure group rats through 4 wk postexposure, but approached control by 13 wk. Epithelial permeability was unaffected in all groups of hamsters.	Bermudez et al. (2004, 056707)
Wistar rats, 20 adult males, 250 ± 10 g	TiO_2 (22-nm CMD, σg = 1.7) Spark generated 0.11 mg/m^3 7.3 × 106 particles/cm^3 (SD 0.5 × 106 particles/cm^3)	Rats exposed 1 hr via endotracheal tube while anesthetized and ventilated at constant rate Lungs fixed at 1 or 24 hr postexposure	Distributions of particles among lung compartments (airspace, epithelium/endothelium, connective tissue, capillary lumen) were directly related to the volume fractions of compartments and did not differ significantly between 1- and 24 hr postexposure. 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, 087362)
			Re-evaluation of the data from Geiser et al. (2005, 087362) with a new statistical method in which a relative deposition index (RDI) was calculated for each compartment. When RDI=1, the particle number is the same as one would expect for the size of the compartment, if the particle distribution is random. RDI>1 suggests a preferential distribution. The new analysis suggested that at 1 hr postexposure, connective tissue was the preferential target for the nano-TiO_2, while capillary lumen was the preferential target at 24 hr postexposure. This study suggested pulmonary clearance via microvasculature, and does not exclude clearance through exhalation or mucociliary escalator.	Muhlfeld (2007, 091106)
WKY/NCrl (Charles River) rats, 5 young adult males, 250 ± 10 g	TiO_2 (22-nm CMD, σg = 1.7) Spark generated	Rats exposed 1 hr via endotracheal tube while anesthetized and ventilated at constant rate Lungs fixed immediately postexposure	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, σg = 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, 156624)

CMD – Count median diameter; MMAD – Mass median aerosol diameter; σg – Geometric standard deviation

Source: U.S. EPA (2009, 196063)

4.6.3. Dermal

Because sunscreen is used on the skin, human skin penetration of nano-TiO_2 (as particles in a solubility vehicle or in sunscreens) has been discussed in several reports and reviews (NanoDerm, 2007, 157660; Nohynek et al., 2007, 090619; TGA, 2006, 089202). Most dermal exposure studies reviewed used human skin and pig skin (Sadrieh et al., 2010, 594511); 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 approximately 60 μm to greater thickness on the plantar and palmar surfaces (Monteiro-Riviere et al., 1990, 625073). Other aspects of skin may also vary in different parts of the body (e.g., face versus forearm). Although published studies indicate the anatomy of stratum corneum of full-term infants and babies is comparable to that of adults (Fairley and Rasmussen, 1983, 193370), the physiology is not. Both the anatomy and physiology of pre-term infants' skin are not comparable to that of adults (Kalia et al., 1998, 196039). Skin studies include a range of experimental conditions, including in vivo and ex vivo/in vitro. With few exceptions discussed below (Kertesz et al., 2005, 180334; Menzel et al., 2004, 180361; Sadrieh et al., 2008, 157500), some of which were attributed to artifacts from sample preparation, most of these human and animal 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).

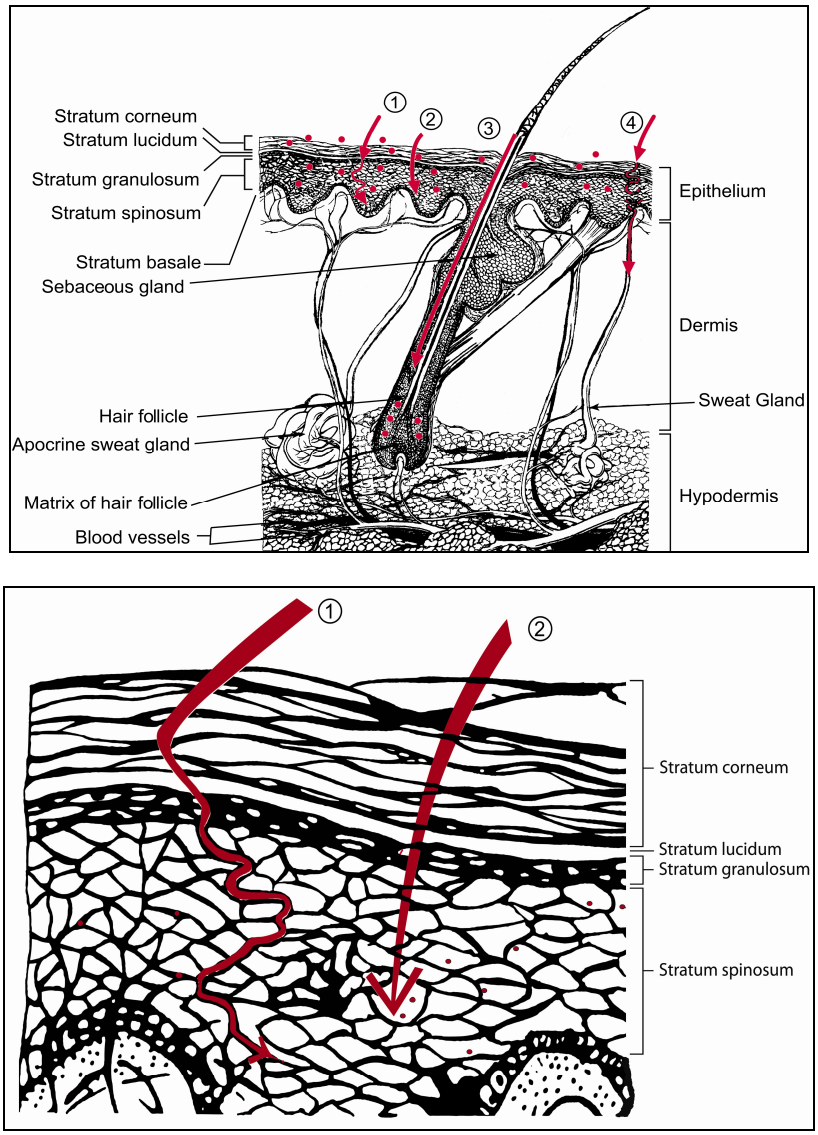
In healthy human skin, topically applied nano-TiO_2 penetrates only into the upper layers of the stratum corneum (Table 4-4). The pathways of skin penetration can include intracellular penetration, intercellular penetration, and penetration through hair follicles (Figure 4-1) (Nohynek et al., 2007, 090619). Penetration through sweat glands has not been reported, according to one source (NanoDerm, 2007, 157660). Although increased skin penetration of other nanomaterials has been reported in flexed porcine skin (Rouse et al., 2007, 157644) and flexed or abraded rat skin (Zhang and Monteiro-Riviere, 2008, 193735) and in UV-exposed murine skin in vivo (Mortensen et al., 2008, 155612), studies of skin penetration in healthy flexed human skin or damaged skin have not been identified for nano-TiO_2. Similarly, studies developing transcutaneous vaccine delivery detected the presence of nanoparticles in immune cells after topical application of nanoparticles on tape stripped skin (Mahe et al., 2009, 225307), but nano-TiO_2 has not been tested in these conditions.

Nano-TiO_2 was observed in some hair follicles (Lekki et al., 2007, 180280), but did not reach the living follicle cells (with the exception of one study in hairless mice (Wu et al., 2009, 193721), see below). 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, 157500) because the shaft of the hair is exposed to the surface but the 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 (Lademann et al., 2007, 157678), 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, 157758).

A recent in vitro and in vivo study using pig and hairless mice suggested that repeated in vivo dermal exposure may lead to nano-TiO_2 penetration into the living cells of epidermis and possibly systemic distribution (Wu et al., 2009, 193721). Similar to other studies, Wu and colleagues'(2009, 193721) 24 hour exposure in vitro of porcine skin to nano-TiO_2 did not show penetration beyond the stratum corneum. Thirty days of in vivo exposures to 4 nm nano-TiO_2, but not larger nano-TiO_2, on the ear skin of pigs, however, resulted in penetration deep into the basal cell layer of the epidermis. No nano-TiO_2 was observed in the dermis. After 60 days of in vivo dermal exposure to 10-60 nm nano-TiO_2, hairless mice showed increased Ti concentrations in multiple organs, including skin, subcutaneous muscle, heart, liver, spleen, as well as pathological changes in skin, liver, spleen and lung. The various tested sizes of nano-TiO_2 do not behave the same. For instance, in the heart, the increases in Ti concentration were similar in all nano-TiO_2 treatments, but the pathological changes were only seen in the 10 nm nano-TiO_2 group. Given that hairless mouse skin has a much thinner stratum corneum than human skin (Haigh and Smith, 1994, 625322) and other differences, it is

unclear to what extent the observed systemic distribution of nano-TiO_2 after repeated dermal exposure in the hairless mouse may occur in humans.

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 28-30 days, psoriatic skin cells mature in 3-4 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, 157748; Pinheiro et al., 2007, 180160). Psoriatic skin has a looser corneocyte organization than healthy skin due to the loss of stratum corneum cohesion (Pinheiro et al., 2007, 180160). In the Pinheiro et al. (2007, 180160) 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).



Source: Adapted from and used with permission from CRC Press, Monteiro-Riviere (1991, 157957; 2004, 157834); Used with permission from Informa Healthcare, Riviere and Monteiro-Riviere (1991, 625197); Used with permission from Informa Healthcare, Nohynek et al. (2007, 090619)

Figure 4-1. Possible pathways of nano-TiO_2 skin penetration. Top Graphic: Nanoparticles may penetrate into skin by passing through: (1) the intercellular space between cells; (2) transcellular space; (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 basal) at a high magnification showing simplified paths of nanoparticles passing: (1) between cells; and (2) through cells. Nanoparticles are not drawn to scale in either graphic.

Table 4-4. Overview of TiO_2 skin absorption/penetration studies

Test Material		Skin Model[a] (Sampling Technique)	Results	Reference
Sunscreen Formulations Containing Nano-TiO_2				
Nano-TiO_2 in a sunscreen formulation	Primary particle 17 nm (Kemira, 2000, 157896), rutile, Al_2O_3/stearic acid coated, aggregates 150-170 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, 090591)
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, 180160)
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, 090587)
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[b] 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, 180334)
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 hr	TiO_2 in stratum corneum, not in deeper layers of the skin.	Kiss et al. (2008, 157547)
Nano-TiO_2 in sunscreen formulation/sunscreen that contains nano-TiO_2	50-100 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, 193359)
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/wk for 4 wk (necropsy)	Increased Ti levels in epidermis in all TiO_2-treated groups. No penetration of TiO_2 particles into the dermis. No increases in Ti levels in lymph nodes or liver of any treated animals.	Sadrieh et al. (2010, 594511)
Photostable nano-TiO_2 in various formulations	Photostable nano-TiO_2, needle-like shape, 45-150 nm × 17-35 nm, coated with alumina and silica (Lodén et al., 2006, 157757), 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; and(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, 180361)

Test Material		Skin Model[a] (Sampling Technique)	Results	Reference
Photostable nano-TiO_2 in sunscreen formulations	(1) T-Lite SF-S: rutile, coated with SiO_2 and methicone; and (2) T-Lite SF: rutile, coated with methicone Both primary particles are needle-like: 30-60 nm × 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 hr (tape stripping)	No penetration beyond stratum corneum. Receptor solution recoveries of 0.8-1.4% of applied dose.	Gamer et al. (2006, 090588)
Other Nano-TiO_2 Formulations				
T 805 Degussa	Coated with trimethyloctylsilane; ~20 nm in diameter	Pig, in vivo	TiO_2 found exclusively in the outermost stratum corneum layer. Traces of TiO_2 were found in the upper part of the follicle, with no evidence of uptake into the follicular epithelium.	Pflucker et al. (1999, 644132)
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); and (3) 100-nm needles, coated with alumina and silica, hydrophilic surface (Tioveil AQ-10P, in dispersion, Solaveil)	Human forearm, in vivo, 6 hr (biopsy)	Penetration of particles into the upper layers of stratum corneum. No penetration into living skin.	Pflücker et al. (2001, 157887) and Schulz et al. (2002, 157872)
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, 157872)
Various TiO_2 and nano-TiO_2	14 nm-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, 092740)

Test Material		Skin Model[a] (Sampling Technique)	Results	Reference
Various nano-TiO_2	100% anatase, uncoated, nano-TiO_2 (Zhejiang Wanjin Material Technology Co., Ltd.): 4 nm, hydrophobic surface, measured particle size 5 ± 1 nm, surface area 200 m^2/g 10 nm, hydrophobic surface, measured particle size 10 ± 1 nm, surface area 160 m^2/g 75% anatase/25% rutile, uncoated nano-TiO_2 (P25 from Degussa, Germany): 21 nm, hydrophilic surface, surface area 50 m^2/g 100% rutile, uncoated, nano-TiO_2 (Zhejiang Hongsheng Material Technology Co., Ltd.): 25 nm, hydrophilic surface, measured particle size 25 ± 5 nm, surface area 80 m^2/g 60 nm, hydrophobic surface, measured particle size 60 ± 10 nm, surface area 40 m^2/g 90 nm, hydrophobic surface, measured particle size 90 ± 10 nm, surface area 40 m^2/g	(A) Porcine skin, in vitro, isolated pig ear skin (without and with tape stripping) on a modified Franz equipment, nano-TiO_2 suspension (4, 10, 25, 60, or 90 nm) on the skin for up to 24 hr (B) Porcine skin, in vivo, shaved pig ear starting at age of 4 wk, approximately 24 mg of test formulation containing 5% nano-TiO_2 (4 or 60 nm) and Tween 80 was topically applied in the marked test area on the right ear skin for 30 consecutive days. Punch biopsies collected at 24 hr after the last treatment for TEM (C) BALB/c hairless mice skin, in vivo, starting at age of 7-8 wk. Test formulation containing 5 % nano-TiO_2 (10 nm, 21, 25, 60, or 90 nm), carbopol 940, and triethanolamine was applied on the dorsal skin for 60 consecutive days at 8 mg emulation (or 400 µm nano-TiO_2) per cm^2 skin. 3 hr after application, the dressing was removed and residual nanomaterials were removed from the skin with lukewarm water and the skin was dried.	(A) No penetration beyond the stratum corneum (B) After 30 days, nano-TiO_2 was detected in all layers of epidermis (stratum corneum, stratum granulosum, prickle cell layer, and basal cell layers), but not in the dermis of porcine skin. Only 4 nm nano-TiO_2 penetrated into the deeper layer of the epidermis (basal cell layer). (C) After 60 days, hairless mice had increased Ti in the skin, subcutaneous muscle, liver, heart, and spleen, but not in the blood or subcutaneous saccus lymphaticus in 10-, 21-, 25-, and 60-nm groups. Almost negligible changes in the brain and kidney, with the exception of increased Ti in the brain after 21 nm nano-TiO_2 exposure. Increased Ti in the lung may be significant in the 21- and 60-nm groups.	Wu et al. (2009, 193721)
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 hr for 1 day or 2 hr daily for 3 days	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, 157928)
Nano-TiO_2 in various gels	For ion microscopy study: 20-nm × 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, 157555)	Porcine and human skins, for 30 min to 48 hr (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, 180280)

Test Material		Skin Model[a] (Sampling Technique)	Results	Reference
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 (2003, 193401)
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, 193398)
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 yr 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 was higher than concentration in cadavers (controls), after exclusion of one control outlier. No correlation between the duration of sunscreen application and Ti concentration.	Tan et al. (1996, 157933)
Various "microfine" TiO_2	Commercial microfine TiO_2 dispersions in octyl palmitate and in water (Tioxide Specialties Ltd, Billingham, U.K.)	Human abdominal skin, in vitro, and skin equivalents (keratinocytes and fibroblasts of native human origin), in vivo	Microfine TiO_2 penetrates into the human stratum corneum probably via sebum lipids of the hair follicles.	Bennat and Muller-Goymann (2000, 157403)

[a]Topical application unless specified.

[b]SCID = Severe combined immune deficiency.

Mortensen et al. (2008, 155612), 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. Though the size and chemical composition of the quantum dots differ from the nano-TiO_2 used in sunscreens, this increased susceptibility to penetration is of note. The authors qualified their results by noting that under no circumstances was there evidence for massive quantum dot penetration, and that quantum dots collected preferentially in the folds and defects in the stratum corneum and in hair follicles.

Using "microfine" TiO_2, Tan et al. (1996, 157933) 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, 180334) reported penetration of nano-TiO_2 into the stratum granulosum of grafted human foreskin in two samples (of an unknown total number).

Sadrieh et al. (2010, 594511) found elevated levels of Ti in the dermis and epidermis skin of minipigs exposed to coated and uncoated nanosize TiO_2. Investigators found no evidence of Ti penetration through expected routes such as the hair follicles, and concluded that the very few randomized Ti particles detected in living cells of the dermis were accounted for by contamination during sample preparation, possibly by small pieces of epidermis which contained 300- to 500-fold higher Ti concentrations than the dermis (Sadrieh et al., 2010, 594511). 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, 180361), 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, 090588; Lekki et al., 2007, 180280; Pflücker et al., 2001, 157887).

Some nanomaterials have been shown to penetrate deeper in damaged skin than in intact skin [quantum dots in UV-exposed murine skin (in vivo) (Mortensen et al., 2008, 155612) and abraded rat

skin (in vitro) (Zhang and Monteiro-Riviere, 2008, 193735); nano-silver coated with polyvinylpyrrolidone in abraded human skin-skin (ex vivo) (Larese et al., 2009, 193493)], but no experimental data on nano-TiO_2 dermal penetration in damaged skin were found. Preliminary 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, 193399; Gopee et al., 2009, 667592). 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, 193270; Simon and Maibach, 1998, 193647).

4.6.4. Ingestion

Currently only three toxicological studies of nano-TiO_2 through oral exposure have been reported (Section 5.3.1.2), and of these, only one (Wang et al., 2007, 090290) reported tissue concentrations of nano-TiO_2. In the Wang et al. (2007, 090290) study, male and female mice received a single oral gavage of a fixed large dose of 5,000 mg/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.

4.6.5. Blood Brain Barrier and Placental Transfer

The general potential for nanoparticles to cross the BBB has been investigated and developed primarily in relation to drug delivery systems (Beduneau et al., 2007, 193266; Emerich and Thanos, 2007, 193365). In addition to size (Sonavane et al., 2008, 193652), the surface properties of nanoparticles influence the potential for a nanomaterial to penetrate the BBB (Singh and Lillard, 2009, 193650). Nanoparticles developed for drug delivery often have ligands conjugated on the surface or other surface modifications to facilitate cellular uptake (Beduneau et al., 2007, 193266).

Table 4-5. Animal studies that measured Ti concentrations in brain after nano-TiO_2 exposures through injection or oral gavage

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. Fine TiO_2, 155 ± 33 nm TiO_2, anatase, uncoated, >10 wt% at <100 nm (from Zhonglina Chemical Medicine Co. (Chen, personal communication, 2008, 157588)	Single oral gavage at 5,000 mg/kg to male and female CD-1(ICR) mice Ti content was measured 2 wk 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. (2007, 090290)
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 µm/organ	TiO_2 was not detected in the brain at any tested time points.	Fabian et al. (2008, 157576)
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 min, 72 hr, and 1 mo 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, 157489)
Nano-TiO_2, 5 nm, anatase Conventional TiO_2 Both types of TiO_2 were made from controlled hydrolysis of titanium tetrabutoxide.	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, 193516)
Nano-TiO_2, 25-70 nm, anatase, surface area 20-25 m^2/g, purity 99.9% (from Sigma-Aldrich)	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 wk 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-wk-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-wk-old mice from nano-TiO_2-exposed dams.	Takeda et al. (2009, 193667)

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 dosage by oral gavage (Wang et al., 2007, 090290), and in mice following a 14-day exposure period of once-daily i.v. injections of nano-TiO_2 (Liu et al., 2009, 193516) (Table 4-5). No increase in Ti concentration in the brain was observed in rats or mice exposed to nano-TiO_2 via a single i.v. injection (Fabian et al., 2008, 157576; Sugibayashi et al., 2008, 157489). Due to the variations in nano-TiO_2 treatment regimens, 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 demonstrated presence of TiO_2 particles, and pathological changes, in the brain of 6-week-old mice born to nano-TiO_2 exposed dams (Takeda et al., 2009, 193667) (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, 193667). Although no evidence was identified from human studies for nano-TiO_2 passing through the placental barrier, 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, 187028). 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, 091407), whereas nano-TiO_2 does pass through the BBB in mice (Liu et al., 2009, 193516; Wang et al., 2007, 090290).

4.6.6. Dose Metrics

Quantitative risk assessment relies on dose-response relationships. Selecting a measurable characteristic of dosage that would be appropriate for predicting nanoparticle toxicity has drawn attention from both researchers and risk assessors. No single metric is recommended in this document, 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. Recently, the OECD developed a guidance document on sample preparation methods and dosimetry for safety testing with nanomaterials (OECD, 2010, 644192).

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, 625074; Liao et al., 2009, 157456; Oberdörster et al., 2005, 087559). 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 approximately 0.7 μm in diameter (Oberdörster et al., 1994, 046203). Hext et al. (2005, 090567) 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. (2006, 088436; 2007, 091305); by contrast, Sager and Castranova (2009, 193625) 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, 090567) – the increases in the numbers of pulmonary polymorphonuclear neutrophil (PMN) due to exposure to anatase fine and nano-TiO_2 (Oberdörster et al., 1994, 046203) and rutile fine TiO_2 (Cullen et al., 1999, 157905) – 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 intratracheal instilled rutile fine TiO_2 and 80% anatase/20% rutile nano-TiO_2 (Sager et al., 2008, 157499) 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, 157530). In the Lison et al. study (2008, 157530), 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.

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 nano-TiO_2 used for drinking 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 (NIOSH, 2005, 196072), comparatively less information is available on the effects of nano-TiO_2. Consistent with studies of other nanomaterials (Ostrowski et al., 2009, 193592), 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, 157569; Hassellöv et al., 2008, 157559; Powers et al., 2006, 088783). 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, 156609).

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

---

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

chapter is organized around environmental matrices 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, 090619). 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, 157547). 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. 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 germane to 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, 088436; Yamamoto et al., 2004, 157820), 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., 2007, 092297).

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, 2007, 195461; Powers et al., 2006, 088783; Powers et al., 2007, 090679; Warheit et al., 2007, 091075). 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, 157512; Attachment 5 to Appendix D of Taylor, 2008, 157487; Warheit et al., 2007, 091305). A compilation of characterization recommendations from a multi-stakeholder group can also be accessed at http://characterizationmatters.org.

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, 055639). 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, 036303). 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 approximately 20 nm in size, and the highest percentages of tracheobronchial deposition were observed in nanoparticles 1-10 nm in size (Oberdörster, 2000, 036303). 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, 089253). 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 Ti concentrations in the olfactory bulb in mice (Wang et al., 2005, 193703; Wang et al., 2007, 090290), 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., 2008, 157473).

Jiang et al. (2008, 156609) investigated the size effects of nano-TiO_2 on the generation of ROS 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, 156609).

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 ore rutile (Hidaka et al., 2005, 157804; Sayes et al., 2006, 090569; Uchino et al., 2002, 090568). 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, 156609), who tested 13 nano-TiO_2 particles of varying crystallinity, all within the size range of 42 to 102 nm. 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, 156609). 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, 180141). 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, 180141).

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, 090569). 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, 090568). Smaller particles that contain more anatase, however, are not always more toxic either in vitro (Sayes et al., 2006, 090569) or in vivo (Warheit et al., 2006, 088436) 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 (Section 2.2.2), coatings affect more than photoreactivity. In particular, the presence of a surface coating changes the nature of the interface between the nano-TiO_2 particle and the environment or an organism, and it is not clear whether the surface coating or the core material dominates particle-environment and particle-organism interactions. Coatings for nano-TiO_2 particles can be designed to reduce agglomeration/aggregation, which in turn affects the behavior of the particles in various matrices, including sedimentation behavior. This also affects the exposure conditions of organisms living in different parts of bodies of water 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, 157736) 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 intratracheally 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[1] did not "lead to remarkable differences in lung reaction" (Rehn et al., 2003, 090613).

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 (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 periodically been made.

For in vitro studies, Murdock et al. (2008, 193563) 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 human and environmental testing, a roundtable discussion at the 2005 Society of Toxicology Annual Meeting considered the following as essential parameters in nanomaterial physicochemical properties: size distribution, agglomeration state, crystalline structure, chemical composition, and shape (Holsapple and Lehman-McKeeman, 2005, 088087). For "hazard studies with nanoparticle-types," Warheit (2008, 193706) 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; (2) crystal structure/crystallinity; (3) aggregation status in the relevant media; (4) composition and surface coatings; (5) surface reactivity; (6) method of nanomaterial synthesis and/or preparation; and (7) purity of sample.

An expert working group convened by the International Life Sciences Institute (ILSI) Research Foundation/Risk Science Institute recommended both off-line (i.e., not using time-resolved continuous techniques) and on-line (continuous) measurement of mass, size distribution, surface area, and particle number for exposure characterization in inhalation studies (Table 5-1), and 17 off-line measurements/aspects for nanomaterial characterization for toxicological studies (Table 5-2) (Oberdörster et al., 2005, 090087).

[1] Silanization is the covering of a surface that has hydroxyl (OH) groups 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.

Table 5-1. Published recommendations for measuring nanomaterial parameters for exposure during characterization inhalation studies

Metric Measurement	Recommendation	
	Off-line (Discrete)[a]	On-line (Continuous)[b]
Mass	E (coupled with on-line)	E
Size distribution	E	E/D
Surface area	O	O
Particle Number	N	E

[a]Off-line: Collected and analyzed later.
[b]On-line: Real-time collection and analysis during the process.

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 with permission from BioMed Central, Oberdörster et al. (2005, 090087).

Table 5-2. Published recommendations for off-line nanomaterial characterization using noncontinuous techniques for toxicological studies

Characterization	Human Exposure	Toxicity Screening Studies		
		Supplied Material	Material as Administered	Material after Administration (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 nonessential information.
N – These characterizations are not considered to be of significant value to screening studies.

[a]As primary particle, secondary particle (primary particle agglomerates and self-assembled structures) and tertiary structure (assemblies of secondary structures). When possible, material agglomeration or de-agglomeration in different liquid media should also be characterized.
[b]Time and conditions, including temperature, humidity, exposure to light and atmosphere composition.
[c]Ratios of different components.

Source: Reprinted with permission from BioMed Central, Oberdörster et al. (2005, 090087).

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., 2005, 090087).

5.1.2. Experimental Conditions

Experimental conditions, particularly the choice of medium/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. For example, instability of nanoparticle suspensions may cause particle settling during the experiment, leading to the effective concentration being either higher or lower than the initial added concentration. This can result in

improper interpretation of the dose-response relationship. The advantages and disadvantages of various dispersion preparation methods are compared in a recent publication of nanomaterial ecotoxicity test methods (Crane et al., 2008, 157583).

5.1.2.1. Medium/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, 157403). Nano-TiO_2 did not penetrate into living cells of the skin in either aqueous dispersion or oily dispersion. 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. Although the stability could be improved by encapsuling the nano-TiO_2 into liposomes, liposome formulation increases nano-TiO_2 skin penetration (Bennat and Muller-Goymann, 2000, 157403). In another relevant phenomenon, the nanoparticles by themselves can act as a dispersant, forming so called Pickering emulsions and essentially acting as the surfactant that helps make liposomes. An in vivo study (Lansdown and Taylor, 1997, 157928) 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.

Different levels of free radical production were observed in cultured cells exposed to similar nano-TiO_2 but within different formulae of suspensions (Uchino et al., 2002, 090568). 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 (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 free 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, 090568). 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, 089620), 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 an increase in ionic strength, and the extent of aggregation can also be affected by the presence of NOM and other constituents such as divalent cations (Domingos et al., 2009, 193347; French et al., 2009, 193384; Kim et al., 2009, 635778). Aggregation was more severe in tap water than in nanopure water (Zhang et al., 2008, 157462), 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, intratracheal 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, 087145; Henderson et al., 1995, 002744). Sager et al. (2007, 091214) tried to disperse several types of nano-sized particles, including TiO_2, in several suspension media, including: PBS; rat and mouse BAL

fluid; and PBS containing 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. (2007, 091214) 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, 157684; Zhu et al., 2006, 157721). Entrapped or residual THF in the C_{60} and THF degradation products were suspected to have contributed to toxicity (Henry et al., 2007, 157684). Nevertheless, no difference in toxicity to daphnids was observed between nano-TiO_2 suspensions prepared with and without THF (Klaper, personal communication, 2008, 157546; Lovern and Klaper, 2006, 088040). Regardless of dispersion method, aggregation of nano-TiO_2 might be unavoidable. Several studies reported that nano-TiO_2 formed aggregates or agglomerates in water, and that these aggregates/agglomerates could not be separated into primary particles by ultrasound or chemical dispersants (Griffitt et al., 2008, 157565; Jeng and Swanson, 2006, 090085; Zhang et al., 2008, 157462). Furthermore, an unfiltered nano-TiO_2 suspension with aggregates/agglomerates has been reported to be less toxic to daphnia than a filtered nano-TiO_2 suspension that mostly contained primary particles (Lovern and Klaper, 2006, 088040). In contrast to Lovern and Klaper's (2006, 088040) reported difficulty of disagglomerating particles by sonication or chemical dispersants, Federici et al. (2007, 091222) reported effective dispersion of P25 by sonication in ultrapure water at final working concentrations up to 1 mg/L, although they did not evaluate potential agglomeration 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, 157593). For example, sonication with ultrasound has been used to decrease nano-TiO_2 agglomeration (Bihari et al., 2008, 157593) and has been shown to generate particles or agglomerates in the nanoparticle range (Maier et al., 2006, 090451). However, sonication alone could increase the size of nano-TiO_2 agglomerates, as reported by Porter et al. (2008, 157508) who found that the mean agglomerate size of P25 in PBS increased from 1,930 nm before sonication to 2,849 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., 2009, 157453), but also to decrease changes in enzyme activity caused by ingested nano-TiO_2 in isopods (Jemec et al., 2008, 157552). 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., 2007, 157449), 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 mediums. 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 (BAuA, 2007, 157694).

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, UV light exposure, purity of water (Zhang et al., 2008, 157462), and presence of organic matter (Domingos et al., 2009, 193347) have been investigated. Factors that affect nano-TiO_2 aggregation, such as pH value, ionic strength, and cation valence (Domingos et al., 2009, 193347; Dunphy Guzman et al., 2006, 090584; French et al., 2009, 193384), would influence not only nano-TiO_2 fate and transport (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 light 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 mammalian cells (Sayes et al., 2006, 090569) and fish cells (Reeves et al., 2008, 157506; Vevers and Jha, 2008, 157475) as well as microorganisms (Adams et al., 2006, 157782). Genotoxicity (Nakagawa et al., 1997, 157927) and clastogenicity (Nakagawa et al., 1997, 157927) of nano-TiO_2 to cultured mammalians cells were also increased by UV exposure. 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, 090569; Uchino et al., 2002, 090568). 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, 155612). In addition to UV, visible light was shown to increase the cytotoxicity 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, 157665).

Nano-TiO_2 was found to form aggregates more in pond water than in pure water (MilliQ 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, 157476). The adsorption of nano-TiO_2 onto certified reference material sediment did not increase the toxicity of the sediment (Blaise et al., 2008, 157592).

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, 193273; Zimmerer et al., 1986, 193744). 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 medium and vehicle effects on nano-TiO_2, the pH and chemistry of the water in which sunscreen may be mixed, e.g., in a swimming pool versus a lake or an ocean, might also modulate nano-TiO_2 effects. 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, 157526).

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, 157508; Sager et al., 2007, 091214; Sager et al., 2007, 090633). Because pH affects the electrostatic 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 acidic 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, vehicles, 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, 157552). The presence of UV and visible light often increase photocatalytic nano-TiO_2 activity and toxicity; other environmental factors, such as pH, ionic 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, 157598; Christian et al., 2008, 157586; Hassellöv et al., 2008, 157559; Navarro et al., 2008, 157517; Nowack and Bucheli, 2007, 092294) and conferences (such as the annual International Conference on the Environmental Effects of Nanoparticles and Nanomaterials) 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 in peer-reviewed articles (Christian et al., 2008, 157586; Crane et al., 2008, 157583; Handy et al., 2008, 157562; Hassellöv et al., 2008, 157559) and in several public databases, such as those sponsored by the OECD (International Council on Nanotechnology, 2010, 644440; OECD, 2009, 644433; Project on Emerging Nanotechnologies, 2010, 644439). Nonetheless, a brief review of ecological effects testing and the importance of the tests are presented at the beginning of each of the following sections 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 5.2.1.4. 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, 090635) and for protecting plastic from UV degradation (Warheit et al., 2007, 091075). 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, 157895), 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. In a study presented in a professional conference, nano-TiO_2 from a commercially available sunscreen has an outer hydrophobic polydimethylsiloxane coating and an intermediate AlOOH coating, and the coating was gradually degraded in water, as evidenced in one study by altered dispersion and release of silicon and Al from the coated nano-TiO_2 product (Botta et al., 2009, 625076). Additionally, the by-products penetrated into aquatic organisms and were toxic at high concentrations (Botta et al., 2009, 625076). 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, 180141; Evonik, 2007, 157696).

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) present in the final test suspension was available, it is also provided in Table 5-3. The environmental relevance of the tested concentrations is unclear due to limited information on nano-TiO_2 concentrations in the environment, although even the use of elevated concentrations in laboratory studies is informative in demonstrating the potential for an effect. It should be noted that several studies reported visible turbidity in nano-TiO_2 stock suspension (Velzeboer et al., 2008, 157476; Zhang et al., 2006, 157722; Zhang et al., 2008, 157462). 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

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, 157782)	66-nm powder, ~35% rutile:65% anatase, average 330-nm in water (Sigma product 634662) (Lyon, personal communication, 2008, 157524)	6-hr exposure to: (1) 50, 100, 500, 1,000, 2,000, 5,000 ppm in medium[a], in direct sunlight; or (2) 1,000 ppm in medium[a], in dark	In dark, similar growth inhibition for both bacteria In light, *B. subtilis*: 0%, 75%, and 99% growth inhibition at 500, 1,000, and 2,000 ppm, respectively *E. coli*: 0%, 15%, 44%, and 46% inhibition at 100, 500, 1,000, and 2,000 ppm, respectively
Bacterium (*Vibrio fischeri*) (Blaise et al., 2008, 157592)	<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, 193414)	25- to 70-nm powder mixture of anatase and rutile, ratio not disclosed (Sigma product 13463-67-7, Estonia) (Heinlaan, personal communication, 2008, 157558)	30-min exposure for up to 20,000 mg/L nano-TiO_2 and conventional TiO_2, 8-hr exposure to 20,000 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: 20,000 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, personal communication, 2008, 157558)		The highest concentration tested: 20,000 mg/L conventional TiO_2 (30-min and 8-hr exposure) did not decrease bacterial growth

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Bacterium (*Vibrio fischeri*) (Velzeboer et al., 2008, 157476)	<75-nm (primary particle) nano-TiO_2 in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer et al., 2008, 157476)	15 min, 1, 10, 100 mg/L, measure reduction of light output from bioluminescent bacteria (Microtox® method, which could be affected by turbidity of 100 mg/L TiO_2 suspension)[b]	EC_{50} >100 mg/L[b]
Bacteria (from a soil sample, species not identified) (Velzeboer et al., 2008, 157476)	<75-nm (primary particle) nano-TiO_2 in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer et al., 2008, 157476)	7 days (Biolog® test, gram positive), 100 mg/L[b]	EC_{50} >100 mg/L[b]
Bacteria and yeast (proprietary information) (Blaise et al., 2008, 157592; Dando, personal communication, 2008, 157582)	<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
Acute Exposure to Aquatic Organisms			
Alga (green alga, *Desmodesmus subpicatus*) (Hund-Rinke and Simon, 2006, 090607)	25-nm primary particle, 20% rutile:80% anatase (Degussa P25) (Baun et al., 2008, 157598) (photocatalytic)(Product 1)	72-hr growth inhibition, following the guidelines for EU standard algal assay (DIN 38412-33, 1991, 667415; ISA 8692, 2004, 667212; OECD 201, 2006, 199838) 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. subpicatus* growth Algal growth (without pre-illumination): 0, 3.1, 6.2, 12.5, 25, 50 mg/L (products 1 [P25] and 2 [UV100]) Shading effect: 0, 12.5, 25, 50 mg/L Algal growth (with pre-illumination): 12.5, 25, 50 mg/L (Product 1[P25])	EC_{50} and effects of additional particle cleaning: Product 1 (P25): 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 (UV100): 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 between algae and light source (but not in contact with algae) for 72 hr, 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 [P25]) did affect nano-TiO_2 effects on algal growth
	100-nm primary particle, 100% anatase; (Hombikat UV100) (Baun et al., 2008, 157598); photocatalytic (Mehrvar et al., 2002, 193541) (Product 2)		
Alga (green alga, *Pseudokirchneriella subcapitata*) (Velzeboer et al., 2008, 157476)	<75nm (primary particle) nano-TiO_2 in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer et al., 2008, 157476)	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[b]	EC_{50} >100 mg/L[b]
Alga (green alga, Pseudokirchneriella subcapitata) (Warheit et al., 2007, 091075)	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, 2008, 157470)	OECD 201 (2006, 199838) (72-hr growth), with light[a] 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 healthy 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 healthy cell number) EC_{50} 61 mg/L (based on inhibition of growth rate)

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Alga (green alga, Pseudokirchneriella subcapatitata) (Huang et al., 2005, 157801)	Photocatalytic nano-TiO_2: Degussa P25 (75% anatase, 25% rutile, 30 nm)	Short-term chronic toxicity – Adsorbsion onto surface of algae; concentrations not specified.	Algae carried 2.3 times their own weight in TiO_2 particles on their surface Cellular weight increased by >130%
Ala (green alga,) (Aruoja et al., 2009, 193254)	Nano-TiO_2	OECD 201 (2006, 199838) (algal growth inhibition test) Algal cell culture; formation of agglomerates.	Nano-TiO_2 formed large agglomerates with almost all algal cells entrapped. EC50 = 5.83 mg/L NOEC = 0.98 mg/L
	Conventional (Bulk) TiO_2		Conventional TiO_2 formed small agglomerates with some algal cells entrapped and some remained free. EC50 = 35.9 mg/L NOEC = 10.1 mg/L
Invertebrate (water flea, *Daphnia magna*) (Hund-Rinke and Simon, 2006, 090607)	25-nm primary particle, 20% rutile:80% anatase (Degussa P25) (2008, 157598) (photocatalytic); ultrasonic dispersion	ISO 6341 (1996, 667232), OECD 202 (2004, 667207) and DIN 38412-30 (1989, 667416) (48-hr immobility), exposure to up to 3 mg/L, 16:8 hr light:dark cycles, compare the effects of pre-illuminated and nonilluminated 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 nonilluminated nano-TiO_2
	100-nm primary particle, 100% anatase; (Hombikat UV100) (2008, 157598); photocatalytic (Mehrvar et al., 2002, 193541); ultrasonic dispersion		Pre-illumination showed a trend of increasing toxicity No dose-response relationship with either pre-illuminated or nonilluminated nano-TiO_2
Invertebrate (water flea, *Daphnia magna*) (Lovern and Klaper, 2006, 088040)	Primary particle <25 nm (smallest 5-nm), anatase, uncoated (photocatalytic) (Klaper, personal communication, 2008, 157546); 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, 1991, 667211) (mortality) Filtered nano-TiO_2: 0.2, 1, 2, 5, 6, 8, and 10 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, personal communication, 2008, 157546) sonicated, unfiltered, secondary particle 100-500 nm in deionized water	Sonicated, unfiltered nano-TiO_2: 50, 200, 250, 300, 400, and 500 ppm	LC_{50} >500 mg/L
Invertebrate (water flea, *Daphnia magna*) (Wiench et al., 2007, 090635)	20-30 nm, 80% anatase, 20% rutile, no surface coating, BET surface area 48.6 m^2/g	OECD 202 (2004, 667207), 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 × 10 nm, rutile, surface coating aluminum hydroxide, dimethicone/methicone copolymer, BET 100 m^2/g (T-LiteTM SF) (photostable UV filter)		EC_{50} >100 mg/L
	50 nm × 10 nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethicone/methicone copolymer, BET 100 m^2/g (T-LiteTM SF-S) (photostable UV filter)		EC_{50} >100 mg/L
	50 nm × 10 nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethoxydiphenylsilane/ triethoxycaprylsilane crosspolymer, BET 100 m^2/g (T-LiteTM 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
Invertebrate (water flea, *Daphnia magna*) (Lovern et al., 2007, 091069)	30 nm, anatase	1-hr exposure to 2.0 mg/L	No changes in heart rate or behaviors

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Invertebrate (water flea, *Daphnia magna*) (Warheit et al., 2007, 091075)	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, 2008, 157470)	OECD 202 (2004, 667207) (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, 157565)	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, 157476)	<75 nm (primary particle) nano-TiO_2 in water suspension (Sigma product 643017, the Netherlands), mixture of rutile and anatase, ratio not reported (Velzeboer et al., 2008, 157476)	48-hr mortality, 17:7 hr light:dark cycle (Chydotox test) [b]	EC_{50} >100 mg/L [b]
Invertebrates (water flea, *Daphnia magna*; fairy shrimp, *Thamnocephalus platyurus*) (Heinlaan et al., 2008, 193414)	25- to 70-nm powder mixture of anatase and rutile, ratio not disclosed (Sigma product 13463-67-7, Estonia) (Heinlaan, personal communication, 2008, 157558)	48-hr mortality for D. magna 24-hr immobilization for T. platyurus	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, personal communication, 2008, 157558)	Up to 20,000 mg/L for both nano- and conventional TiO_2	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, 157592)	<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
Invertebrate (freshwater hydra, *Hydra attenuata*) (Blaise et al., 2008, 157592)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	96-hr morphological changes, tested concentrations not specified	EC_{50} in 10 to 100 mg/L range
Fish cell (trout primary hepatocytes) (Blaise et al., 2008, 157592)	<100-nm powder (Sigma product 634662, France), characteristics in water not reported	48-hr cytotoxicity, tested concentrations not specified	TEC in 1 to 10 mg/L range
Fish (zebrafish, *Danio rerio*), embryo and larvae (Zhu et al., 2008, 193742)	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 Milli Q water): mean measured size 230 nm, measured size range 100 to 550 nm, secondary particles formed by primary particles have irregular shapes	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; and (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.
	Conventional TiO_2: anatase, purity >99.0%, published size: 10,000 nm (Third Chemical Regent Factory of Tianjin, Tianjin, China); in suspension (in Milli Q water): mean measured size 1,100 nm, measured size range 330 to 2,250 nm, neither primary nor secondary particles have a uniform shape		
Fish (zebrafish, *Danio rerio*) (Griffitt et al., 2008, 157565)	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

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Fish (rainbow trout, *Oncorhynchus mykiss*) (Warheit et al., 2007, 091075)	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, 2008, 157470).	OECD 203 (1992, 667208) (96 hr acute toxicity) 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, 157782)	66-nm powder, ~35% rutile:65% anatase, average 330 nm in water, (Sigma product 634662) (photocatalytic) (Lyon, personal communication, 2008, 157524)	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, 090635)	50 nm × 10 nm, rutile, surface coating aluminum hydroxide, hydrated silica, dimethicone/ methicone copolymer, BET surface area 100 m^2/g (T-LiteTM SF-S) (photostable UV filter)	OECD 211 (2008, 667210) (21-day 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
		OECD 202 (2004, 667207) (Chronic toxicity, 21-day immobility)	LC_0 = 30 mg/L
Fish (rainbow trout, *Oncorhynchus mykiss*) (Federici et al., 2007, 091222)	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, 157592)	<100-nm powder (Sigma product 634662, Canada or France), characteristics in water not reported	15 min, tested concentrations not specified, measure the decrease in chlorophyll fluorescence emitted from the enzyme complexes as an indicator of inhibition of photosynthetic efficiency (Luminotox assay) (Bellemare et al., 2006, 157779)	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, 157534)	Nano-TiO_2: 5 nm, anatase, not coated Conventional TiO_2	Soak the seeds in 0.25% nano-TiO_2 or conventional TiO_2 for 48 hr, and spray 0.25% nano-TiO_2 or conventional TiO_2 onto the leaves from 2-leaf stage to 8-leaf stage at 0.25%	**Nano-TiO_2:** Enhanced growth (size, single plant fresh weight, single plant dry weight) Increased chlorophyll content Increased net photosynthetic rate Increased mRNA, protein concentration, and activity of Rubisco activase **Conventional TiO_2:** No significant changes
Plant (spinach, *Spinacia oleracea*) (Zheng et al., 2005, 157784)	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)
Plant (willow trees) (Seeger et al., 2009, 644124)	Nano-TiO_2: Degussa P25 (20/80% rutile/anatase, average diameter 25 nm); Hombikat UV100 (100% anatase, average diameter <10 nm)	190-hr exposure to 0, 1, 10, and 100 mg/L (TiO_2) and 0, 1, 20, and 50 mg/L (TiO_2) for Degussa, and 100 mg/L (TiO_2) for Hombikat UV100. Solutions shaken. Adsorption measured by light microscopy; growth, transpiration, and water use efficiency measured using standard willow tree acute toxicity test	No statistically significant reduction in growth, transpiration or water use efficiency rates, therefore no findings of toxicity. Adsorption of Hombikat UV100 particles more apparent than Degussa particles.

Test Species (Reference)	Material	Protocol (No UV illumination, unless specified)	Study Outcome
Plant (maize, *Zea mays L.*) Asli and Neumann (2009, 193771)	Nano-TiO_2: Degussa P25 (mean diameter 30 nm) Bentonite clay (1-60nm)	5-hr root exposure to 0.3 and 1.0 g/L of either nanomaterial in hydroponic solution over 5-day period; 6-wk root exposure to 1.0g/L of either nanomaterial in clay soil	Accumulation at the cell wall surfaces of primary roots and subsequent inhibition of cell wall pore size, water transport capacity, leaf growth and transpiration. No statistically significant effect observed on shoot growth rate when exposed to nanomaterials in soil.
Invertebrate (isopod, [woodlouse] *Porcellio scaber*) (Jemec et al., 2008, 157552)	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). 780- to 970-nm aggregates in nonsonicated dispersion, 350- to 500-nm aggregates in sonicated dispersion, sizes on dry leaves not reported	3-day dietary exposure to nonsonicated 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 nonsonicated 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 nonsonicated nano-TiO_2, but not in middle doses of nonsonicated nano-TiO_2 or any doses of sonicated nano-TiO_2 No changes in feeding rate, defecation rate, food assimilation efficiency, weight, or mortality were noted up to 3,000 µg/g
Invertebrate (nematode, *Caenorhabditis elegans*) (Wang et al., 2009, 193696)	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 to 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 to 687 nm (medium 494 nm), zeta potential at pH 7.0 = -33.8 mV (ACROS, Acros Organics)	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

N/A – Not applicable

[a]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.

[b]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.

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., 2003, 157862; Gabrielson et al., 2003, 157863)

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 × LOEC)1/2

TEM – Transmission electron microscopy

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

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, 089849); surface coatings and paints (Kuhn et al., 2003, 090597; Tsuang et al., 2008, 157483); and food

packaging (Chawengkijwanich and Hayata, 2007, 157708). 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 nonpathogenic 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, 196069). Additionally, some bacteria and fungi form a symbiotic relationship with plants. A well-known example is the nitrogen-fixing bacteria (genus *Rhizobium*) that live on 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 (Long, 1989, 644893). 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, 157782) 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 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, 157782). The different dose-response relationships of gram-positive and gram-negative bacteria to nano-TiO_2 suggest 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 (Kuhn et al., 2003, 090597; Neal, 2008, 196069), such as lipid peroxidation (Maness et al., 1999, 193538). 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, 157782; Coleman et al., 2005, 089849). Because TiO_2 generates ROS (mainly highly reactive hydroxyl radicals, ·OH) in the presence of UV light and oxygen (Reeves et al., 2008, 157506), mechanisms other than oxidative stress might also contribute to nano-TiO_2 toxicity in the dark and possibly also under UV light. 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, 193433).

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., 2007, 091075; Wiench et al., 2007, 090635). For other aspects of U.S. EPA Tier I aquatic toxicity testing (e.g., estuarine and marine organism acute toxicity, whole sediment acute toxicity, and bio-availability/bio-magnification toxicity) (U.S. EPA, 2008, 157481), studies have not yet been reported.

Algae

Algae are primary producers of chemical energy 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 (2008, 157481). 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., 2007, 091075). In contrast, exposure to concentrations of 0.01 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., 2007, 091075). 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, 047938; Davis and Svendsgaard, 1990, 048278). Fine (approximately 380 nm) TiO_2 showed almost no inhibition in growth rate (or cell number) at up to 1 mg/L, and EC_{50} values of 16mg/L (based on decreases in healthy cell numbers) and 61 mg/L (based on inhibition of growth rate) (Warheit et al., 2007, 091075). Hartmann et al. (2010, 196322) also studied green algal growth inhibition, testing three different sizes of nano-TiO_2 and observing toxicity in all three cases. The authors' primary discussion, however, was on the difficulty of reproducing results due to the complex interactions of the systems; the determination of a dose-response relationship was complicated by the effects of concentration-dependent aggregation of the nanoparticles, subsequent sedimentation, and possible attachment to vessel surfaces. Hartmann et al (2010, 196322) concluded that their research underlines the potential for interactions with existing environmental constituents to affect the toxicity of nanoparticles.

For photocatalytic nano-TiO_2, the EC_{50} values determined for 72-hour growth inhibition in green algae ranged from approximately 30 mg/L to > 50 mg/L (Blaise et al., 2008, 157592; Hund-Rinke and Simon, 2006, 090607). Hund-Rinke and Simon (2006, 090607) 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, 157801). 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., 2008, 644828). As discussed earlier (Section 5.1.1), the ionic strength due to 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, 157801). If an increase in weight forces surface algae into deeper water, photosynthesis could be decreased[1] due to less sunlight available in deeper water than at the surface. The reduced light available to algae was also suggested to be the cause of more growth inhibition seen in algal cells treated with nano-TiO_2, compared to conventional TiO_2 (Aruoja et al., 2009, 193254). In Aruoja's

[1] 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, 157534; Zheng et al., 2005, 157784).

study (2009, 193254), nano-TiO_2 formed large agglomerates with almost all algal cells entrapped. Conventional TiO_2, on the other hand, formed small agglomerates with some algal cells entrapped and some algal cells free (Aruoja et al., 2009, 193254). Since these studies were both conducted in algal culture medium, it cannot be ruled out that some of the observed growth inhibition may be due to absorption of zinc and phosphate by nano-TiO_2, decreasing the availability of these nutrients to the algae (Kuwabara et al., 1986, 625577).

Aquatic 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, 193296; Löhr et al., 2007, 193518). 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, 193297). 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 (*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, 157697).

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 Wiench et al. (2007, 090635). 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, 090635). In the chronic exposure study, photostable coated T-Lite SF-S was given to *D. magna* at up to 100 mg/L for 21 days, and the authors reported that the LC_{50} 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, 090635). In a different study that used photostable nano-TiO_2 intended to protect plastics against UV-induced degradation, 48-hour exposure to 100 mg/L of the nano-TiO_2 induced 10% immobility in *D. magna* (Warheit et al., 2007, 091075).

The effects of photocatalytic nano-TiO_2 toxicity have been studied by several research teams in four types of water fleas (*D. magna*, *D. pulex*, *Ceriodaphnia dubia*, and *Chydorus sphaericus*), one type of fairy shrimp (*T. platyurus*), and one type of freshwater hydra (*H. attenuata*). For water fleas, the 48-hour mortality or immobility EC_{50} was generally greater than 100 mg/L (Lovern and Klaper, 2006, 088040 [unfiltered]) (Velzeboer et al., 2008, 157476; Wiench et al., 2007, 090635), 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, 157565). 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, 088040). 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, 088040). Chronic exposure for 8 days caused 40% mortality at 20 mg/L in daphnids (Adams et al., 2006, 157782). For fairy shrimp, the 24-hour mortality or immobility LC_{50} was higher than 100 mg/L (Blaise et al., 2008, 157592; Heinlaan et al., 2008, 193414). In the only study of hydra, the EC_{50} of 96-hour morphological changes was <100 mg/L (Blaise et al., 2008, 157592). 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 *D. 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, 157598). Baun et al. (2008, 157598) 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, 088054). Because nano-TiO_2 primary particles are smaller than the size range of particles daphnids feed on (400 to 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, 157598). Whether nano-TiO_2 is taken up by other tissues, excreted, or transformed in daphnids is unclear (Baun et al., 2008, 157598). Even if nano-TiO_2 is not absorbed into tissues, the presence of nano-TiO_2 in the digestive tract of daphnids could still contribute to bioaccumulation in the food web (Section 4.6.1.2.).

The behavior and heart rate of *D. magna* were evaluated in daphnids exposed to photocatalytic nano-TiO_2 at 2.0 mg/L for 1 hour (Lovern et al., 2007, 091069). In this study, nano-TiO_2 had an average particle diameter of 30 nm, and tetrahydrofuran, the 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 *D. magna* mortality after 48-hour exposure (Lovern and Klaper, 2006, 088040). 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, 091069).

Fish

Fish are used in toxicity tests to represent secondary energy consumers in aquatic systems. Commonly used fish species in ecotoxicity tests include freshwater 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 (Section 4.6.1.1, "Bioaccumulation"); mortality; behavioral markers (e.g., fatigue, abnormal buoyancy control, and swimming); and pathology.

The toxicological studies of photostable nano-TiO_2 in fish are very limited. The 96-hour 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., 2007, 091075). 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, 157565; Zhu et al., 2006, 157721) and chronic effects (Federici et al., 2007, 091222). Bioaccumulation (Zhang et al., 2006, 157722) and interaction with other heavy metals were discussed previously (Table 4.2). In the acute exposure study, the LC_{50} for a 48-hour exposure to an anatase/rutile mixture of uncoated nano-TiO_2 was >10 mg/L for zebrafish (in both female adults and <24-hour post-hatch fry) (Griffitt et al., 2008, 157565). 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, 193742). In the Zhu et al. (2008, 193742) study, nano-Al_2O_3 and conventional Al_2O_3 at up to 1,000 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, 091222). 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 Ti accumulation in the internal organs, suggesting no nano-TiO_2 accumulation, as discussed earlier in Section 4.6.1.1. 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, 091222) 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.

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, 090635), 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, 091222). 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, 157476; Zhang et al., 2006, 157722; Zhang et al., 2008, 157462). Given that natural organic matter in the environment can affect the extent of aggregation and deposition of nanoparticles or modify nanoparticle surface charges (Navarro et al., 2008, 157517)(Kim et al., 2009, 635778), 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

Plants

Information on nano-TiO_2 interactions with plants is currently available for photocatalytic uncoated nano-TiO_2 in spinach and willow trees (Table 5-3). Photocatalytic uncoated nano-TiO_2 has been shown to enhance the growth of spinach in several studies (Lei et al., 2008, 157540; Linglan et al., 2008, 157534; Mingyu et al., 2007, 157667; Mingyu et al., 2007, 157666; Yang et al., 2006, 157723; Zheng et al., 2005, 157784). 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, 157784). 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, 157723), and anti-oxidative stress response (Lei et al., 2008, 157540). 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, 157534; Zheng et al., 2005, 157784).

Seeger et al. (2009, 644124) exposed willow tree roots to two types of TiO_2 nanoparticles (Degussa P25 and Hombikat UV100) suspended in deionized water at various concentrations. There were no statistically significant changes in transpiration rates, growth, or water use efficiency after 190 hours of exposure to nano-TiO_2 in solution. Investigators found that roots exposed to the solution with smaller nano-TiO_2 particles (<10 nm average diameter) had nanoparticles compactly

attached all over the roots' surface, while roots in the solution with larger particles (average 25 nm diameter) showed minimally attached particles. However, the researchers did not determine whether the nanoparticles entered the trees through the xylem. The investigators concluded that these two types of nano-TiO_2 are not toxic to willow trees, under the experimental conditions used (Seeger et al., 2009, 644124).

In contrast, Asli and Neumann (2009, 193771) found that colloidal suspensions of TiO_2 nanoparticles interfered with water transport capacity, leaf growth, and transpiration in maize (*Zea mays* L.) seedlings. The authors exposed maize roots to colloidal suspensions of inorganic bentonite clay (particle size 1-60 nm) and Degussa P-25 TiO_2 nanoparticles (mean diameter of 30 nm) in hydroponic solutions and in soil. The authors found statistically significant reductions in hydraulic conductivity (i.e. water flow through roots) when adding either material at low concentrations (1 g/L) to hydroponic solutions surrounding maize roots over a 5-hour period. Also, transpiration was rapidly inhibited (over a 3-hour period), when both materials were added to the solution at the same concentration. Colloidal nanoparticles of both materials suspended in water flowing to roots appeared to attach to root cell walls, thereby reducing cell wall pore diameters and root hydraulic conductivities. However, when the authors grew maize for 6 weeks in clay soil irrigated with either bentonite or TiO_2, they found no statistically significant effect on shoot growth; they hypothesized that this apparent lack of effect may be due to the fact that the total number of roots increased during this time, thereby increasing the plants' water supply capacity, which could counterbalance possible pore clogging actions by the nanoparticles.

Terrestrial 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, 157552), and a study on nematodes, *Caenorhabditis elegans* (Wang et al., 2009, 193696). 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, 157552) investigated the effects of photocatalytic anatase nano-TiO_2 on the terrestrial isopod *Porcellio scaber,* known as woodlouse. Woodlice, approximately 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, 157552) study, woodlice ate dry leaves that had been soaked in nano-TiO_2 dispersions (sonicated or nonsonicated). The sonication process decreased the mean agglomerate size from 780-970 nm in a nonsonicated dispersion to 350-500 nm. The activities of catalase and glutathione-S-transferase (GST), two anti-oxidative stress enzymes in the digestive gland (hepatopancreas) were measured. The activities of both enzymes were decreased at 0.5, 2,000, and 3,000 µg/g of nonsonicated nano-TiO_2, but not at middle concentrations (1, 10, 100, and 1,000 µg/g) of nonsonicated nano-TiO_2 or at any concentration (1,000, 2,000, and 3,000 µg/g) of sonicated nano-TiO_2 (Jemec et al., 2008, 157552). No changes in feeding rate, defecation rate, food assimilation efficiency, weight, or mortality were noted at concentrations up to 3,000 µg/g of either sonicated or nonsonicated 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. (2009, 193696) 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. (2009, 193696) 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., 2009, 193696).

5.2.1.4. Indirect and Interactive Ecological Effects

In addition to the direct toxicity of nano-TiO_2, indirect and potentially synergistic effects of nano-TiO_2 could also be important. Nano-TiO_2 could adsorb pollutants (Nagaveni et al., 2004, 090578; Pena et al., 2006, 090573), carry the pollutants into areas in an organism that the pollutants alone would not naturally appear (Moore, 2006, 089839), 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 4.6.1.3, co-exposure to nano-TiO_2 in water increased the uptake of arsenic (Sun et al., 2007, 193662) and cadmium (Zhang et al., 2007, 090114) 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, 157592). The effects of 11 nanomaterials on sediment toxicity (as measured in 2 direct contact assays, the Microtox solid phase assay[1] and the Luminotox solid phase assay[2]) and sediment elutriate toxicity (as measured with the MARA assay[3]) were studied 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, 157592).

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 macro-organisms, 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.

---

[1] 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.

[2] 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.

[3] 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.

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, 196337). Most health effects studies used pure nano-TiO_2, and therefore their characteristics and effects may differ from nano-TiO_2 as used in commercial products or products containing nano-TiO_2. For instance, nano-TiO_2 in sunscreen may include mostly agglomerates, instead of perfectly dispersed primary particles. As discussed in Section 5.1, many other factors also influence the effects. When available, data on factors with potential influence on health effects are provided in Tables 5-4 through 5-10. The health effects evidence is organized by human and laboratory animal studies and route of exposure, with noncarcinogenic effects discussed in Section 5.3.1 and carcinogenic effects discussed in Section 5.3.2.

5.3.1. Noncarcinogenic Effects

This section summarizes in vivo studies of nano-TiO_2 noncarcinogenic 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 4.6.3. Commercial sunscreens were tested in dermal exposure studies only. 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, 090139; Mohr et al., 2006, 097493; Nemmar et al., 2008, 157514; Oberdörster et al., 1992, 045110; Pott and Roller, 2005, 157790; Wang et al., 2007, 090290; Wang et al., 2007, 157616; Warheit et al., 2007, 091075; Warheit et al., 2007, 090594).

5.3.1.1. Studies in Humans

No epidemiological studies or case reports are available for nano-TiO_2 noncarcinogenic effects. A few case reports described noncarcinogenic 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, 193728), pulmonary fibrosis and bronchopneumonia (Moran et al., 1991, 157956), and pulmonary alveolar proteinosis (Keller et al., 1995, 157938). TiO_2 or Ti accumulation in the lung, sometimes years after workplace exposures, and Ti-loaded macrophages have also been reported in workers (Keller et al., 1995, 157938; Määttä and Arstila, 1975, 157979; Yamadori et al., 1986, 193728), as have Ti particles in the lymph nodes (Määttä and Arstila, 1975, 157979; Moran et al., 1991, 157956) and in the liver and spleen (Moran et al., 1991, 157956). 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, 193312) 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 noncancer effects 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, 180141; Dunford et al., 1997, 157929).

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, 157660; Scientific, 2007, 196826). Photocatalytic nano-TiO_2, however, sometimes is used in sunscreens (Barker and Branch, 2008, 180141; Dunford et al., 1997, 157929). Photocatalytic nano-TiO_2 can generate ROS when exposed to UV light and can cause oxidative stress and cytotoxicity in cells (cultured human fibroblasts) and in cell-free in vitro experiments (Dunford et al., 1997, 157929; Lu et al., 2008, 157526). 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 (Section 4.6.3), 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 photostable nano-TiO_2, laboratory rabbits showed no skin irritation 4 hours after application or sensitization 3 days after application (Warheit et al., 2007, 091075). Furthermore, although some sunscreens containing TiO_2 (size not specified) increased mouse skin absorption of herbicides and pesticides (2,4-D, paraquat, parathion or malathion), TiO_2 alone actually decreased the mouse skin absorption of the tested herbicide, 2,4-D (Brand et al., 2003, 157866). 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, 157866).

Some researchers, such as Nohynek et al. (2007, 090619), 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 four investigations reviewed, only three report in vivo studies of health effects after dermal exposure to TiO_2 (pages 16, 17, and 41-43 of NanoDerm, 2007, 157660; Warheit et al., 2007, 091075; Wu et al., 2009, 193721), and only one of those three used nano-TiO_2 intended for sunscreen (pages 16, 17, 41, and 43 of NanoDerm, 2007, 157660). Warheit et al. (2007, 091075) used ultrafine particles, roughly 100 nm in size. Three studies used a single application, and the longest exposure was only 3 days. The NanoDerm report (2007, 157660) concluded that "TiO_2 exposure did not modify the viability, proliferation, apoptosis, and differentiation [or] adhesive properties of skin cells." As discussed previously (Section 4.6.3), skin penetration studies have shown that some nano-TiO_2 can stay in hair follicles for up to 10 days.

The only report with repeated dermal exposure included 30 days of daily dermal exposure on porcine skin and 60 days of daily dermal exposure on hairless mouse skin (Wu et al., 2009, 193721). As discussed in Section 4.6.3, the 30-day exposure of 4-nm nano-TiO_2, but not larger nano-TiO_2, resulted in nano-TiO_2 particles in the basal cells of epidermis, but not in dermis, of pigs. In addition, morphological changes at the subcellular level were seen in the basal cells in the 4 nm TiO_2 group. The authors also tested 60-day dermal exposures to 10-, 21-, 25-, 60-, and 90-nm nano-TiO_2 with various anatase and rutile ratios in hairless mice for nano-TiO_2 penetration in skin, as well as distribution, signs of oxidative stress, and pathological changes in various organs (Table 5-4). Most

changes were seen in the 10-, 21-, and 25-nm nano-TiO_2 groups, and none was seen in control or 90-nm nano-TiO_2 group. Increased Ti concentrations were seen in skin, subcutaneous muscle, heart, liver, and spleen. Signs of oxidative stress were seen in skin and liver. Pathological changes were seen in the skin, liver, heart (only 10-nm group), spleen, and lung. While hairless mice are commonly used as a model for skin studies, hairless mice study results should be evaluated with care for human relevance. For instance, the thicknesses of stratum corneum and epidermis of hairless mice are approximately half and two thirds of that in humans, respectively; skin permeability was higher in hairless mice than in humans (Haigh and Smith, 1994, 625322). Furthermore, hairless mice (BALB/c nu/nu) are deficient in T cells (Ku and Lee, 2006, 625354), and their immune function deficiency may render them more susceptible to nano-TiO_2-induced changes than other animals or humans.

With relatively few in vivo dermal exposure studies investigating nano-TiO_2 skin absorption and penetration (Table 4-4) 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, 157644; Zhang and Monteiro-Riviere, 2008, 193735) and compromised skin (Zhang and Monteiro-Riviere, 2008, 193735), including UV-exposed skin (Mortensen et al., 2008, 155612), have been shown to allow nanoparticles (other than nano-TiO_2, which was not tested) to penetrate deeper than healthy nonflexed 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 (Lee et al., 2009, 157457), 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 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

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Mouse Female hairless CRL:SKH1 In vitro exposure Single and repeated exposures	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 min 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 was applied. 2,4-D was also applied. Both were applied on skin, and then again 4.5 hr after the first application. d) TiSilc untinted sunscreen and pesticides: Paraquat, Malathion, and Parathion	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 pesticides (Paraquat, Malathion, and Parathion) was also increased in skin pretreated with sunscreen TiSilc.	Brand et al. (2003, 157866)
Human foreskin grafts on SCID mice In vivo exposure Single exposure	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 hr 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.	(NanoDerm (2007, 157660)
Rabbit New Zealand White In vivo exposure Single exposure	Nano-TiO_2 (identified as uf-C , a pre-commercial version of DuPont Light Stabilizer 210), 79% anatase/21% rutile, not coated, approximately 90 wt% TiO_2, 7% alumina, and 1% amorphous silica, average particle size 140.0 ± 44 nm in water, average BET surface area 38.5 m^2/g	For testing acute dermal irritation Doses – 0 or 0.5 g Single exposure for 4 hr (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 hr after exposure	No dermal irritation effects, no clinical signs of toxicity, and no BW loss. Not considered a skin irritant.	Warheit et al. (2007, 091075)
Mouse Female, CBA/JHsd In vivo exposure Repeated exposure		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 Diluting vehicle: N,N-Dimethyl formamide	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. (2007, 091075)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Pig Male In vivo exposure Repeated exposure	100% anatase, uncoated, nano-TiO_2 (Zhejiang Wanjin Material Technology Co., Ltd.): 4 nm, hydrophobic surface, measured particle size 5 ± 1 nm, surface area 200 m^2/g 10 nm, hydrophobic surface, measured particle size 10 ± 1 nm, surface area 160 m^2/g 75% anatase/25% rutile, uncoated nano-TiO_2 (P25 from Degussa, Germany): 21 nm, hydrophilic surface, surface area 50 m^2/g 100% rutile, uncoated, nano-TiO_2 (Zhejiang Hongsheng Material Technology Co., Ltd.): 25 nm, hydrophilic surface, measured particle size 25 ± 5 nm, surface area 80 m^2/g 60 nm, hydrophobic surface, measured particle size 60 ± 10 nm, surface area 40 m^2/g 90 nm, hydrophobic surface, measured particle size 90 ± 10 nm, surface area 40 m^2/g	Porcine skin, in vivo, shaved pig ear starting at age of 4 wk, approximately 24 mg of test formulation containing 5% nano-TiO_2 (4 or 60 nm) and Tween 80 was topically applied in the marked test area on the right ear skin for 30 consecutive days. Punch biopsies collected at 24 hr after the last treatment for TEM.	Pig: After 30 days of treatment Nano-TiO_2 was detected in all layers of epidermis (stratum corneum, stratum granulosum, prickle cell layer, and basal cell layers), but not in the dermis of porcine skin. Only 4 nm nano-TiO_2 penetrated into the deeper layer of the epidermis (basal cell layer). Subcellular changes (extended intercellular space, impairment of desmosome, and vacuoles around nucleus in basal cells) were seen. No gross lesions (such as erythema or edema).	Wu (2009, 193721)
Mouse Male and female BALB/c (hairless) In vivo exposure Repeated exposure		BALB/c hairless mice skin, in vivo, dorsal region starting at age of 7-8 wk. Test formulation containing 5% nano-TiO_2 (10 nm, 21, 25, 60, or 90 nm), carbopol 940, and triethanolamine was applied on the dorsal skin for 60 consecutive days at 8 mg emulation (or 400 µg nano-TiO_2) per cm^2 skin. 3 hr after application, the dressing was removed and residual nanomaterials were removed from the skin with lukewarm water and the skin was dried.	Hairless mice: After 60 days of treatment Mice treated with 10, 21, and 25 nm nano-TiO_2 had decreased BW and increased relative liver weight to BW. Mice in the 10- and 21-nm groups also had increased relative spleen weight to BW. Decreased SOD activities (indicator of antioxidant defense) in the skin and liver (10 and 21 nm). Increased lipid peroxidation (as measured by malondialdehyde) in the skin and liver (10, 21, 25 nm) (in skin only - 60 nm). Decreased collagen content of skin (as measured by hydroxyproline) (10, 21, 25, and 60 nm). Increased Ti in the skin, subcutaneous muscle, liver, heart, and spleen, but not in the blood or subcutaneous saccus lymphaticus in the 10-, 21-, 25-, and 60-nm groups. Almost negligible changes in the brain and kidney, with the exception of increased Ti in the brain after 21 nm nano-TiO_2 exposure. Increased Ti in the lung may be significant in the 21- and 60-nm groups. Pathological changes in skin (excessive keratinization, thinner dermis) (particularly in the 10- and 21-nm groups, also in the 25- and 60-nm groups), liver (focal necrosis – 21-, 25-, and 60-nm groups; liquefaction necrosis – 10-nm group), heart (small trace of white blood cells – 10-nm group), spleen (minor increase in local macrophages – 10-, 21-, 25-, and 60-nm groups), and lung (slight alveolar thickening -- in 10, 21-, 25-, and 60-nm groups). No pathological changes in the brain. 90-nm group showed no changes.	

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
SOD – superoxide dismutase
TUNEL –Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling

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., 2007, 090290; Warheit et al., 2007, 091075), and the other investigated genomic instability after nano-TiO_2 exposure through drinking water for 5 or 10 days (Trouiller et al., 2008, 157484).

The Warheit et al. study (2007, 091075) 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 5,000 mg/kg photostable nano-TiO_2 (uf-C) (3 rats per dose). The authors reported "no biologically important BW 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 5,000-mg/kg nano-TiO_2 was not lethal as tested.

In the Wang et al. study (2007, 090290), male and female mice received a single oral gavage of 5,000 mg/kg TiO_2 as 25-nm rutile spindles, 80-nm rutile spindles, or 155-nm anatase octahedrons (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, 157484) study showed increased DNA and chromosomal damage in various tissues of adult mice given 60-600 µg/mL photocatalytic nano-TiO_2 (P25) in drinking water for 5 days. In a separate experiment, the offspring of mice that were given nano-TiO_2 in drinking water for ten days in the second half of the pregnancy showed increases in DNA deletions in the eye-spot assay (Trouiller et al., 2008, 157484), 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, 157857). 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 (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

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat Female, strain/stock not specified	Nano-TiO_2 (identified as uf-C, a pre-commercial version of DuPont Light Stabilizer 210), 79% anatase/21% rutile, not coated, approximately 90 wt% TiO_2, 7% alumina, and 1% amorphous silica, average particle size 140.0 ± 44 nm in water, average BET surface area 38.5 m^2/g	For testing acute effects Doses – 175, 550, 1,750, or 5,000 mg/kg (3 rats per dose) Single oral gavage Observation for 14 days postexposure	No mortality, no biologically important BW losses, and no gross lesions present in the rats at necropsy. Grey colored feces were observed in rats dosed at 1,750 mg/kg (1 of 3 rats) and 5,000 mg/kg (All 3 rats). Oral LD_{50} >5,000 mg/kg for female rats.	Warheit et al. (2007, 091075)
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, personal communication, 2008, 157588) 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, personal communication, 2008, 157588) 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, personal communication, 2008, 157588)	Single oral gavage (acute effects) Dose – 5,000 mg/kg 10 female and 10 male mice per TiO_2 size group Necropsy at 2 wk after the gavage	**Hepatic Toxicity:** Increases in coefficients (wet organ weight/BW) 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).[a] Decreases in AST in males in the 155-nm group (Chen, personal communication, 2008, 157588). 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, personal communication, 2008, 157588).[a] 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, personal communication, 2008, 157588). **Possible Myocardial Damage:** Increase in serum LDH[a] (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, personal communication, 2008, 157588). Based on the data in this study alone, it cannot be ruled out that LDH and α-HBDH were from kidney or liver. **Pathological Results:** 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. **Distribution:** TiO_2 distribution in female mice: increased Ti concentrations in liver (80-nm group), spleen (25-, 80-, and 155-nm groups), kidney (25- and 80-nm groups), lung (80-nm group) and brain (25-, 80-, and 155-nm groups). For the 80-nm group, highest Ti concentration was in liver (3,970 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. (2007, 090290)

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 adult mice:** 60, 120, 300 and 600 µg/mL in drinking water for 5 days (Based on 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 BW) **C57BL/6Jp^{un}/p^{un} mice** for eye-spot assay: 10-day exposure, pregnant mice were given nano-TiO_2 in drinking water from 8.5-18.5 days post conception. Offspring were sacrificed at 20 days old.	**Increased genomic instability (adult 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 (offspring):** Increases in DNA deletions at the pink-eyed unstable (pun) locus which result from homologous recombination or double strand breaks between the DNA fragments that contain duplicated pink-eyed dilution (p) gene (Reliene and Schiestl, 2003, 157857) 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, 157484)

[a]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 (MedlinePlus, 2009, 193814).

α-HBDH – Alpha-hydroxybutyrate dehydrogenase
γH2AX – Phosphorylated form of histone H2AX (phosphorylation of H2AX at serine 139)
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
BET – Brunauer, Emmett, Teller method of calculating surface area
BUN – Blood urea nitrogen
HPLC – High performance liquid chromatography
IFN-γ – Interferon-gamma
IL-4 – Interleukin-4
IL-8 (KC) – IL-8 stands for interleukin-8 and KC for chemokine (CXC motif) ligand 1 (CXCL1)
IL-10 – Interleukin-10
LDH – Lactate dehydrogenase, a general marker of cell injury (Ma-Hock et al., 2009)
LD50 – Lethal dose 50; the dosage that is lethal to 50% of the tested population
RT-PCR – Reverse transcription polymerase chain reaction
TGF-β – Transforming growth factor-beta
TNF-α – Tumor necrosis factor-alpha

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, 011376). 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, 011376; Osier et al., 1997, 086056). 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, 011376). Furthermore, studies have shown that exposure to the same particle through intratracheal 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, 086056). Also, results from instillation cannot be extrapolated quantitatively for estimating inhalation results (Driscoll et al., 2000, 011376).

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, 157491). 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: "Because of exposure to smaller SWCNT structures by inhalation of a dry aerosol versus 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 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 (ISLI Risk Science Institute Workshop Participants, 2000, 002892). Thus, under particle overload conditions, exposure-response relationships and even the type of responses produced can be unreliable. However, the nanoparticle-specific 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, 036303). Toxicity of nano-TiO_2 could be decreased by cross-tolerance to oxidative stress, because nano-TiO_2 given through an intratracheal 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. (2007, 093170) 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., 2007, 093170; Warheit et al., 2007, 091075), especially when the crystal form and other factors are not the same. In the Grassian et al. (2007, 093170) 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, 086056), acute intratracheal inhalation of high levels (125 mg/m^3) of fine and nano-TiO_2 caused less severe pulmonary response than intratracheal instillation. Intratracheal 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., 2007, 090606; Ma-Hock et al., 2009, 193534).

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, 048642; Bermudez et al., 2002, 055578; Bermudez et al., 2004, 056707; Hext et al., 2002, 157878; Hext et al., 2005, 090567; Oberdörster et al., 1994, 046203). 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, 048642; Bermudez et al., 2002, 055578; Bermudez et al., 2004, 056707; Hext et al., 2002, 157878; Hext et al., 2005, 090567; Oberdörster et al., 1994, 046203). 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, 157963; Gallagher et al., 1994, 045102; Heinrich et al., 1995, 076637) and mice (Heinrich et al., 1995,

076637), 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, 157963), 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 three 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, 157963).

Systemic Effects and Specific 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 intratracheal 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, 157514). 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., 2008, 157473).

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, 156816). This microvascular dysfunction was attributed 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, 191961). 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, 191961).

Effects in Brain

Since 1970, scientists have known that inhaled ultrafine air pollutants and engineered nanoparticles translocate into the brain (Oberdörster et al., 2004, 055639). Inflammatory responses, altered neurotransmitter levels, and pathological changes have been observed in rodent brains after inhalation of manganese oxide (Elder et al., 2006, 089253); instillation of nano carbon black (Tin Tin Win et al., 2008, 157486); and inhalation of ultrafine elemental ^{13}C particles (Oberdörster et al., 2004, 055639). A few recent studies showed that anatase and rutile nano-TiO_2 translocate into the brain following intranasal instillations (Wang et al., 2007, 157616; Wang et al., 2008, 157474).

The only available studies of nano-TiO_2 effects on the central nervous system are from a research group that has administered high doses of nano-TiO_2 to mice using intranasal instillation (Wang et al., 2007, 157616; Wang et al., 2008, 157474; Wang et al., 2008, 157473). 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 2 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., 2007, 157616). 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 Ti concentrations in the brain were in the hippocampus and olfactory bulb, the two regions where most pathological changes were also seen

(Wang et al., 2008, 157474; Wang et al., 2008, 157473). The hippocampus and astrocytes seem to be the targets of nano-TiO_2 toxicity in the brain (Wang et al., 2008, 157474; Wang et al., 2008, 157473). 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, 089584; Wang et al., 2008, 157473). 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., 2008, 157474; Wang et al., 2008, 157473).

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., 2007, 157616; Wang et al., 2008, 157474; Wang et al., 2008, 157473). 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., 2008, 157474). Additionally, the activity of cholinesterase, which inactivates the neurotransmitter acetylcholine, increased (Wang et al., 2008, 157474). 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

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 hr 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 hr 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 hr 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 hr 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 hr 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 hr after exposure)	Grassian et al. (2007, 093170)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
		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 hr 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	
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 intratracheal instillation and intratracheal inhalation Intratracheal inhalation exposure for 2 hr at 125 mg/m^3 Intratracheal instillation exposure to the equivalent amount of TiO_2 as in the lung at day 0 of intratracheal inhalation (500 μg fine TiO_2 or 750 μg nano-TiO_2 in 0.2 mL saline) Necropsy 0, 1, 3 or 7 days postexposure (3 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 intratracheal instillation, intratracheal 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 intratracheal inhalation of fine TiO_2; on days 1, 3, and 7 after intratracheal instillation of nano-TiO_2; and days 0 and 1 after intratracheal inhalation of nano-TiO_2. Decreases in macrophage inflammatory protein-2 levels in BAL supernatant on days 0, 1, and 3 after intratracheal inhalation of nano-TiO_2; and day 1 after intratracheal instillation of nano-TiO_2. Increases in macrophage inflammatory protein-2 levels in BAL cell pellets on days 1, 3, and 7 after intratracheal instillation of nano-TiO_2; and on days 0 and 1 after intratracheal inhalation of nano-TiO_2. Increases in TNF-α protein was detected by immunocytochemistry (but not by ELISA) on days 0 and 1 after intratracheal inhalation of water (control); days 1 and/or 3 after intratracheal instillation of fine or nano-TiO_2 and intratracheal inhalation of fine TiO_2; and at all time points after intratracheal 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, 086056)
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 mo 6- or 12-mo 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 mo, 12 mo after exposure, respectively. 6 mo 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 mo 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, 048642)

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 wk 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) wk 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 wk of recovery, while hamsters retained only 10% initial burden after 26 wk 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-wk recovery, while hamsters had a biphasic fashion decrease to 3% after 48-wk 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 wk postexposure. 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 wk postexposure. 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, 055578) Nano-TiO_2: Bermudez et al. (2004, 056707) Comparison of fine and nano-TiO_2 data reported in Bermudez et al. (2002, 055578) and Bermudez et al. (2004, 056707): Hext et al. (2002, 157878; 2005, 090567)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
			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 wk of recovery, hamsters showed normal neutrophil % and macrophage number; within 46 wk 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 wk 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 had pulmonary inflammation. Nano-TiO_2 exposure: Rats and mice, but not hamsters, in the 10 mg/m^3 groups had increased numbers of macrophage and neutrophil and concentrations of LDH and protein in BAL fluid. Pulmonary lesions were most severe in rats, and least in hamsters. Fine TiO_2 exposure: Alveolar cell proliferation was seen in rats (0 week postexposure at mid- and high-dose groups, 4 and 13 wk postexposure at high-dose group) and mice (13 and 26 wk postexposure 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 wk postexposure, 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 postexposure, 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 wk to 10 mg/m^3 nano-TiO_2 or to 50 mg/m^3 fine TiO_2 were similar for all three species.	

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 mo exposure: 7.2 mg/m^3 for the first 4 mo, followed by 14.8 mg/m^3 for 4 mo, 9.4 mg/m^3 for 16 mo, and clean air for 6 mo (concentration sometimes are reported as 7.5, 15, 10 mg/m^3) 18 or 19 hr/day, 5 days/wk in whole body chamber Mice: 13.5-mo exposure: Same treatment as in rats for the first 8 mo, followed by 9.4 mg/m^3 for 5.5 mo, and clean air for 9.5 mo	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 postexposure (from 40 mg/lung after 18 mo of nano-TiO_2 exposure to 3.3 mg/lung at 4 mo postexposure). Increased half-time of pulmonary clearance of tracer particles **For inhaled 0.35 µm labeled tracer particles** After 3-, 12-, 18-mo nano-TiO_2 exposure and 18-mo exposure plus 3-mo 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-mo nano-TiO_2 exposure and 18-mo exposure plus 3-mo recovery, clearance half times were 1,222, 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-mo 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, 157963)
			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, 045102)
			Rats: Increased mortality (60% vs. 42% in control) and lung wet weight, decreased mean lifetime and BW. Increased incidence of lung tumors [18-mo exposure: 5 out of 20 rats exposed to TiO_2 (0 out of 18 in control) had lung tumors. 24-mo 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 BW. Carcinogenic in rats, but not in mice.	Heinrich et al. (1995, 076637)

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 hr 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. (2007, 090606)
		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 postexposure but not at wk 3 postexposure. Macrophages in BAL fluid were loaded with TiO_2 particles and less so at wk 3 postexposure.	
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 to 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/ for 5 days, head-nose exposures to dust aerosols No recovery (immediately after the last exposure [0 days]), 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 1,635 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 1,340 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, 193534)

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/wk, for 12 wk Recovery for 4, 8, 12, 29 or 64 wk before sacrifice	Nano-TiO_2 caused more severe and prolonged (~1 yr) 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 mo 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 yr 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, 046203)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
Rat Male Wistar, 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: 2,025/1,547 µg TiO_2 in lung, 2.2/8.5 µg TiO_2 in mediastinal lymph nodes. Fine TiO_2: 9,182/7,257 µg TiO_2 in lung, 8.2/108 µg TiO_2 in mediastinal lymph nodes. Quartz DQ 12: 2,190/1,975 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 disagglomeration 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 neutrophilic inflammation in lung; inflammation declined by 14 days of recovery; lymphoreticulocellular hyperplasia in the mediastinal lymph nodes. Fine TiO_2 group: Reversible increases in absolute lung weight; particle-loaded macrophages in the mediastinal lymph nodes. Quartz: Increase absolute lung weight, which maintained throughout recovery; multifocal infiltration of granulocytes in lung; after recovery time, pulmonary histological changes increased severity, and mediastinal lymph nodes had increased macrophage number and granulomatous inflammation.	van Ravenzwaay et al. (2009, 193689)

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, 157499) (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, 157499); 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 which do not alter BAL markers of pulmonary inflammation or lung damage Exposure to fine TiO_2: aerosol concentration × exposure time (actual deposition in lung) 15 mg/m^3 × 480 min (90 µg) 16mg/m^3 × 300 min (67 µg) 12 mg/m^3 × 240 min (36 µg) 6 mg/m^3 × 240 min (20 µg) 3 mg/m^3 × 240 min (8 µg) Exposure of nano-TiO_2: aerosol concentration × exposure time (calculated/actual deposition in lung) 10 mg/m^3 × 720 min that took place over 3 days (38 µg) 12 mg/m^3 × 240 min (19 µg) 6 mg/m^3 × 240 min (10 µg) 3 mg/m^3 × 480 min (10 µg) 12 mg/m^3 × 120 min (10 µg) 3 mg/m^3 × 240 min (6 µg) 1.5 mg/m^3 × 240 min (4 µg) Sham exposure (control): 0 mg/m^3 × 240 min 24 hr postexposure, sample collection, including exteriorizing spinotrapezius 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 sham-exposed rats. Anuclear alveolar macrophages are presumed to be an apoptotic change. Endothelium-dependent arteriolar dilation as measured after intraluminal infusion of the Caionophore A23187 in exteriorized spinotrapezius 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-TiO_2 was approximately 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-TiO_2 surface area delivered, since no agglomeration was considered). Additional nano-TiO_2 exposure conditions (12 mg/m^3 × 2 hr; 4 mg/m^3 × 6 hr; 8 mg/m^3 × 3 hr) yielded the same level of impairment of systemic arteriolar dilation, suggesting the response is dependent on the exposure concentration (of product) × time.	Nurkiewicz et al. (2008, 156816)
		Same exposure conditions as above (Nurkiewicz et al., 2008, 156816) for endogenous microvascular NO production tests, but only three groups in all other tests: aerosol concentration × exposure time (actual deposition in lung) Sham exposure (control): 0 mg/m^3 × 240 min Fine TiO_2: 16mg/m^3 × 300 min (67 µg) Nano-TiO_2: 6 mg/m^3 × 240 min (10 µg) 24 hr postexposure, sample collection, including and exteriorizing spinotrapezius muscle as described in Nurkiewicz et al. (2008, 156816) and excising spinotrapezius muscles from separate groups of rats for measurement of NO, microvascular oxidative stress, and nitrotyrosine staining	Same impairment of arteriolar dilation at 67 µg fine TiO_2 and 10 µg nano-TiO_2more than 50% decrease compared to sham 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 sham 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 sham control, the NO production was sensitive to nitric oxide synthase inhibition caused by NG-monomethyl-L-arginine. 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, 191961)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
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 intratracheal 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 wk 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, 090139)
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 intratracheal instillation Instilled doses: 5 instillations × 3 mg 5 instillations × 6 mg 10 instillations × 6 mg	Increased primary benign tumors and malignant cancers in lung in all tested doses.	Mohr et al. (2006, 097493) Pott and Roller (2005, 157790)[a]
	Nano-TiO_2 (Degussa T805 /P805),[a] crystal form not specified, coated with an organic silicon compound; 21 nm; 32.5 m^2/g	Repeated weekly intratracheal instillation Instilled doses: 15 instillations × 0.5 mg 30 instillations × 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 intratracheal instillation Instilled doses: 10 instillations × 6 mg 20 instillations × 6 mg	Increased primary benign tumors and malignant cancers in lung in all tested doses.	
Rat Male Wistar	Nano-TiO_2, rutile, primary particle diameter 4-6 nm, rod shape (synthesized in the lab by a soft chemistry technique); BET surface for instilled nano-TiO_2 rods was 14.64 cm^2 for dose of 1 mg/kg, 82.30 cm^2 for 5 mg/kg. Aggregates appeared to be in a radial arrangement and usually less than 1 µm.	Single intratracheal instillation (acute effects) 1 or 5 mg/kg nano-TiO_2 or vehicle only (150 µL) Single intratracheal instillation nano-TiO_2 was suspended in saline containing 0.01% Tween 80 (a surfactant and emulsifier) Blood collection and necropsy at 24 hr 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, 157514)

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 intratracheal instillation (acute effects) 500 µg of either anatase nano-TiO_2 or anatase fine TiO_2 A single intratracheal 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, 045110)
	Nano-TiO_2, ~20 nm, anatase (free anatase nano-TiO_2) Alveolar macrophage collected 24 hr after donor-rat received 200 µg anatase nano-TiO_2 via intratracheal 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 intratracheal instillation (acute effects) Free anatase nano-TiO_2, 104 µg Phagocytized anatase nano-TiO_2 104 µg + 9.5 × 106 alveolar macrophages + 3.9 × 106 polymorphonuclear neutrophils Alveolar macrophages 6.8 × 106 Polymorphonuclear neutrophils 2.2 × 106 Serum-exposed anatase nano-TiO_2 100 µg A single intratracheal 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 intratracheal instillation of 500 µg each; anatase fine TiO_2 was also tested at 1,000 µg; anatase nano-TiO_2 was also tested at 65, 107, 200, and 1,000 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.	

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 Intratracheal instillation (acute effects) Nano-TiO_2: 30, ~150, 500 µg Fine TiO_2: ~150, 500, 2,000 µg	Pulmonary inflammation (neutrophil % in lung lavage) was seen at 24 hr postexposure. 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, 036303)
	Nano-TiO_2 Polytetrafluoroethylene (PTFE) (Teflon) fume, count median diameter ~18 nm	Repeated inhalation of PTFE fume (5 × 105 particles/cm^3 = ~50 µg/cm^3, 5 min/day for 3 days) followed by a single intratracheal 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 3 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 intratracheal 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 min of inhalation of LPS or saline, intratracheal instillation of nano-or fine TiO_2 24 hr of recovery	LPS alone: mild pulmonary inflammation (~10% neutrophil in lung lavage at 24 hr postexposure). 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 intratracheal 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, 090613)
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 intratracheal instillation (acute effects) 0, 125, and 500 µg particles in saline 24 hr 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, 056067)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
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 × 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 to 3 µm), crystalline silica, BET surface area 4 m^2/g	Single intratracheal 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 wk, 1 mo, and 3 mo postexposure (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 postexposure only; increases in LDH by 5 mg/kg nano-TiO_2 rods at 24 hr postexposure 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 wk postexposure (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 mo and 3 mo postexposure). Absolute lung weight was increased at 5 mg/kg at 1 wk, 1 mo, and 3 mo postexposure. Increased tracheobronchial cell proliferation at 5 mg/kg (not measured in low dose) at 24 hr postexposure only.	Warheit et al. (2006, 088436)
Rat Crl:CD®(SD)IGS BR	Nano-TiO_2 (DuPont), photostable, rutile, coated with alumina, (~98% TiO_2, ~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 % TiO_2, ~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% TiO_2 and ~1% alumina), an average particle size 382 nm in water, average BET surface area 5.8 m^2/g Quartz	Single intratracheal instillation (subchronic effects) 0, 1, or 5 mg/kg 90-day 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. (2007, 090594)

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 every 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 instillations), increased AST (80-nm group after 5 instillations) and increase ALP (155-nm group after 1 instillation). Pathological changes in kidney: atrophy of renal glomerulus, infiltration and dwindling of interstitially inflammatory cells in the lumen of Bowman's capsules. No changes in organ weight. No pathological changes in heart, liver, spleen, cerebral cortex or cerebellum. No change in proinflammatory cytokine TNF-α in serum. Brain: Oxidative stress: GSH-Px and GST activities and GSH levels were increased in the 80-nm group after 5 instillations, but not in other groups or other time points. Malondialdehyde levels (indicator for lipid peroxidation) and soluble protein carbonyl content (indicator for protein oxidation; measured only after 15 instillations) were increased in both the 80- and 155-nm groups after 15 instillations. SOD activity was decreased in 155 nm after 15 instillations. Catalase activity (measured only after 15 instillations) was increased in the 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 the 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 the 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 the 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. (2008, 157474) Wang et al. (2008, 157473)

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 2 days Blood and brain were collected from anesthetized mice after 2, 10, 20, or 30 days	No changes in water and food consumption or BW. 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. (2007, 157616)

[a]According to Pott and Roller (2005, 157790): "Titanium dioxide T805 from Degussa was ordered from Sigma-Aldrich, but the supplier only offered an amount of at least 40 kg P805. 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 Pott and Roller (2005, 157790). 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, personal communication, 2008, 157529).

ALP – Alkaline phosphatise, a marker of type II epithelial cell toxicity (Ma-Hock et al., 2009, 193534) 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 rat strain
GFAP – Glial fibrillary acidic protein
GGT – gamma-γ–glutamyltransferase, a marker for damage to Clara and type II epithelial cells (Ma-Hock et al., 2009, 193534)
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, 193534)
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

Toxicity by Other Exposure Routes

Ocular exposure, i.v., and subcutaneous (s.c.) 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, 157648). A single ocular exposure to a photostable nano-TiO_2 caused conjunctival redness for 1 or 2 days in rabbits (Warheit et al., 2007, 091075).

One journal article and two professional meeting abstracts were identified on the effects of injected nano-TiO_2 in rats and mice. In the Fabian et al. (2008, 157576) 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., 2007, 157668; Weaver, personal communication, 2008, 157467). 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, personal communication, 2008, 157467).

Prenatal exposure to nano-TiO_2 has been reported to affect the offspring in mice in one journal article (Takeda et al., 2009, 193667) and one poster at a scientific meeting (Trouiller et al., 2008, 157484).

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

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 <1,000 nm, with 10 wt% <100 nm Necropsy at 1, 14, and 28 days after the injection (12 rats total for 4 treatment groups) Ti concentrations were measured in lung, liver, kidney, spleen, brain, blood cells, plasma, and popliteal lymph nodes at 1, 14, and 28 s 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 the 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 was 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, 157576); van Ravenzwaay et al. (2009, 193689)
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, 2007, 157577; Llames, personal communication, 2008, 157528; Weaver, personal communication, 2008, 157467)	i.v. 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. (2007, 157668)
Mouse Slc:ICP, pregnant female and male offspring	Nano-TiO_2, 25-70 nm, anatase, surface area 20-25 m^2/g, purity 99.9% (from Sigma-Aldrich)	s.c. injections of 100 µL of 1 mg/mL nano-TiO_2 (i.e., 0.1 mg nano-TiO_2) into each time-pregnant Slc:ICP mouse once per day at 3, 7, 10 and 14 days post-mating Male offspring were weighed, and sacrificed at 4 days or 6 wk of age for evaluation.	In 6-wk-old male offspring from nano-TiO_2-exposed dams Nano-TiO_2 particles were seen in the testis and brain (olfactory bulb and the cerebral cortex – frontal and temporal lobes) Decreased daily sperm production, epididymal sperm motility, and the number of Sertoli cells. Abnormal testicular morphology (seminiferous tubules) Markers of apoptosis (activation of caspase-3 and crescent-shaped cells), occlusion of small vessels, and perivascular edema observed in the brain	Takeda et al. (2009, 193667)

Animal	Testing Material	Treatment Conditions	Summary of Major Effects	Reference
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, 2007, 157577; Llames, personal communication, 2008, 157528; Weaver, personal communication, 2008, 157467)	Subcutaneous injections: total 0 or total 5,600 mg/kg over 2 days Intravenous injections: total 0 or total 560 mg/kg over 2 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, 193713)
Ocular exposure				
Rabbit Male New Zealand White	Nano-TiO_2 (identified as uf-C , a pre-commercial version of DuPont Light Stabilizer 210), 79% anatase/21% rutile, approximately 90 wt% TiO_2, 7% alumina, and 1% amorphous silica, average particle size 140.0 ± 44 nm in water, average BET surface area 38.5 m^2/g	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 hr following administration of the nano-TiO_2	Reversible conjunctival redness in the treated eye (normal by 24 or 48 hr after administration of nano-TiO_2). No corneal injury evident, no clinical signs observed, and no BW loss occurred.	Warheit et al. (2007, 091075)

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 Noncarcinogenic Effects

Some of the noncarcinogenic 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, 157717; IARC, 2010, 157762) 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, 157774).

In a 2005 NIOSH draft evaluation, TiO_2 was not designated as a "potential occupational carcinogen," due to insufficient evidence (NIOSH, 2005, 196072). For nano-TiO_2, NIOSH expressed concern in the 2005 draft about the potential carcinogenicity of ultrafine TiO_2 (primary particle <0.1 μm) 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, 196072). Based on an assessment of the lung tumor response in the rat and supported by consideration of the other pulmonary effects of TiO_2, NIOSH draft recommended exposure limits are 1.5 mg/m^3 for fine TiO_2 (primary particle <10 μm)[1] and 0.1 mg/m^3 for ultrafine (nano) TiO_2. As mentioned in Section 4.2.2, these numbers were derived from converting particle mass to surface area dose (6.68 m^2/g for fine TiO_2 and 48 m^2/g for ultrafine TiO_2), and the risk estimates will vary for other particle sizes and surface areas (pages 60-61 of NIOSH, 2005, 196072), as well as crystal form and other factors not considered by NIOSH (Section 5.1)

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 tumors through inhalation in rats at 250 mg/m^3 (6 hours/day, 5 days/week for 24 months) (Lee et al., 1985, 193501; Lee et al., 1985, 067628), but not at 50 mg/m^3 or below (Lee et al., 1985, 193501; Lee et al., 1985, 067628; Muhle et al., 1991, 063996). No increases in tumors were observed in mice receiving a single intratracheal 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 intratracheal instillation weekly for 15 weeks (Baan, 2007, 157717). Similarly, epidemiological studies did not show increased lung cancer in people exposed to conventional TiO_2 (Boffetta et al., 2001, 157891; Chen and Fayerweather, 1988, 193312; Fryzek et al., 2003, 157864; Ramanakumar et al., 2008, 157507; Siemiatycki, 1991, 157954). The carcinogenicity studies of conventional TiO_2 are not discussed in detail in this document, and readers are referred to studies cited here and in the IARC monographs Working Group report (Baan, 2007, 157717).

5.3.2.1. Studies in Humans

Several epidemiological studies of TiO_2 carcinogenicity have been reported: two population-based case-control studies (one for lung cancer (Boffetta et al., 2001, 157891) and the other for 20 types of cancer (Siemiatycki, 1991, 157954); two retrospective cohort mortality studies (Boffetta et al., 2004, 157849; Fryzek et al., 2003, 157864); one mortality, morbidity, and case-control study (lung cancer and chronic respiratory diseases) (Chen and Fayerweather, 1988, 193312); and a case-control study (lung cancer) (Ramanakumar et al., 2008, 157507). Based on these studies, IARC (2010, 157762), the CCOHS (2006, 157774), and NIOSH (2005, 196072) 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. Even if the TiO_2 in these studies included some particles in the nanoscale range, 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, 041486; Heinrich et al., 1995, 076637; Pott and Roller, 2005, 157790). Increased lung tumor incidences were observed in rats (Borm et al., 2000, 041486;

[1] "Fine" particles in the NIOSH draft (2005, 196072) are defined as all particle sizes that are collected by respirable particle sampling, i.e., 50% collection efficiency for particles of 4 μm, with some collection of particles up to 10 μm.

Heinrich et al., 1995, 076637; Pott and Roller, 2005, 157790), but not in mice (Heinrich et al., 1995, 076637), exposed to P25 through inhalation or intratracheal instillation. Photocatalytic nano-TiO_2 given through intraperitoneal injections did not increase tumors in the abdominal cavity in rats (Pott et al., 1987, 029823). 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, 090611). Data specifically on photostable nano-TiO_2 carcinogenicity are inconclusive (Pott and Roller, 2005, 157790). As mentioned in Chapter 4, because internal transport of the materials will influence the ultimate dose to the organism, it should be noted that multiple routes of exposure will be considered that may not be likely or possible primary exposure routes but that could have relevance when internal transport is considered (e.g., i.v., i.p., and i.m.).

Intratracheal Instillation

Female Wistar CRP/WU rats received fine and nano-TiO_2 via intratracheal instillations, and the tumor incidence and pulmonary inflammation were measured 2.5 years after administration (Borm et al., 2000, 041486). 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, personal communication, 2008, 157591). 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, 041486) 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, 046203).

Pott and Roller (2005, 157790) 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 intratracheal 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 intratracheal instillation in Pott and Roller (2005) study

Treatment	Crystal form; primary particle size; specific surface area (BET)	Photo-stability	Dose (number of instillations × mg per instillation)	Rats at start/at risk[a]	Survival 50% (wk)	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 × 3.0	48/42	114	21.4	31.0	52.4	14.3
			5 × 6.0	48/46	114	17.4	50.0	67.4	15.2
			10 × 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 × 0.5	24/11	86	0.0	0.0	0.0	9.1
			30 × 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 × 6.0	48/44	108	15.9	13.6	29.5	11.4
			20 × 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 to 400 nm

[a]Rats at risk were "sufficiently examined rats which survived at least 26 wk after first instillation" according to Pott and Roller (2005, 157790).

[b]Regarding particle characteristics, Pott and Roller (2005, 157790) 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, 157790).

[c]According to Pott and Roller (2005, 157790): "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, 157790) 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, 157790) 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 intratracheal 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, 157790) 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, 157790). In contrast to the high acute toxicity of hydrophobic nano-TiO_2 reported in the Pott and Roller (2005, 157790) study, very low toxicity of hydrophobic nano-TiO_2 was reported in an earlier study by Rehn et al. (2003, 090613). Rehn et al. (2003, 090613) reported that a single intratracheal 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, 090613). The reasons for the discrepancy in the toxicity of hydrophobic nano-TiO_2 (P805 versus T805 manufactured by Degussa) remain unclear.

Inhalation

Heinrich et al. (1995, 076637) reported increased lung tumor rates 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 MMAD of 0.80 μm, with a geometric standard deviation of 1.80.

For female Naval Medical Research Institute (NMRI) 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% in the natural lifespan of female NMRI mice (Lohrke et al., 1984, 157978); 12% broncho-alveolar lung adenoma and 10% bronchiolo-alveolar lung carcinoma in female Han:NMRI mice up to 104 weeks old (Rittinghausen et al., 1997, 157924), 13.8% would not be considered as an increase even 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, 076637). The lung particle loading was 23.2 mg/lung after 6 months, and 39.2 mg/lung after 24 months (Gallagher et al., 1994, 045102). The exposure to nano-TiO_2 did not increase the levels of DNA adducts in the lung (Gallagher et al., 1994, 045102). 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, 157963).

The aerosol concentrations used in the Heinrich et al. (1995, 076637) 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.

Intraperitoneal Injection

Pott et al. (1987, 029823) intraperitoneally injected Wistar and Sprague-Dawley rats with photocatalytic nano-TiO_2 (P25)[1] 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, 029823) concluded there were no increases in tumor incidence (in the abdominal cavity) in nano-TiO_2 treated rats.

[1] Data from Pott et al. (1987, 029823) 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, personal communication, 2008, 193844).

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 yr after treatment		
Wistar rat, 9-wk old	i.p. injection of 18 mg/rat, once per wk for 5 wk (total dose 90 mg/rat)	6 of 113 rats examined (5.3%)
Sprague-Dawley rats, 8-wk old	i.p. injection of 5 mg/rat	2 of 52 rats examined (3.8%)
Wistar rats, 4-wk old	i.p. injection of 5 mg/rat	0 of 47 rats examined (0%)
Wistar rats, 5-wk old	i.p. injections of 2, 4, and then 4 mg/rat (total dose 10 mg/rat)	0 of 32 rats examined (0%)
Preliminary results at 28 mo after i.p. injection		
Wistar rats, 8-wk old	i.p. injection of 20 mg/rat, once per wk for 5 wk (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, 029823).

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, 090611). Each of the 10 male Sprague-Dawley rats was surgically implanted with conventional TiO_2 (a 9-mm × 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; noncancerous 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. 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[1] (Dankovic et al., 2007, 157704).

With the exception of mutation, all the events described in the previous paragraph (Baan et al., 2006, 186864; Muhle and Mangelsdorf, 2003, 157859) have been reported in rats exposed to both

[1] 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.

fine TiO_2 and photocatalytic nano-TiO_2 through inhalation or instillation (Borm et al., 2000, 041486; Heinrich et al., 1995, 076637; Hext et al., 2002, 157878; Pott and Roller, 2005, 157790). 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 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 intratracheal instillation (Driscoll et al., 1997, 053253). 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, 053253). 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, 053253). 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 hours/day, 5 days/week) of exposure (Hext et al., 2002, 157878). 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, 076637).

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[1] rather than overload (Borm et al., 2000, 041486). 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.

[1] High interstitialization can be a part of the process of particle overload, but high interstitialization may not lead to overload.

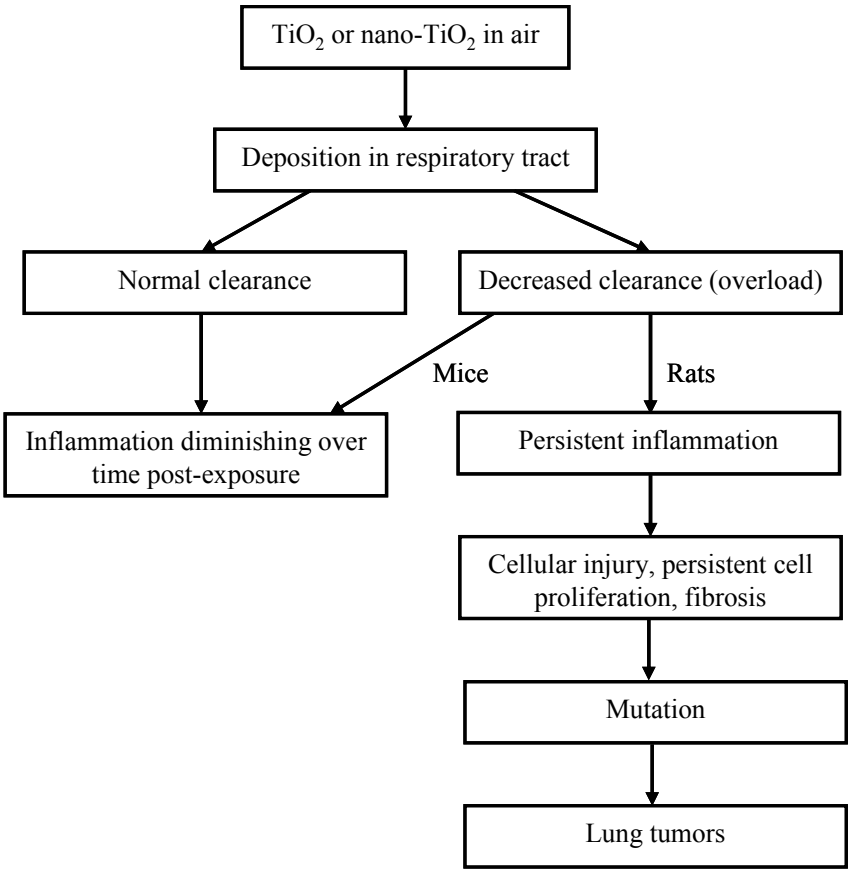


Figure 5-1. The pulmonary effects of TiO_2 or nano-TiO_2 exposure through inhalation or instillation.

Although the carcinogenicity of TiO_2 and nano-TiO_2 in rats at high doses has been shown 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, 157859). 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, 090567). In human exposures, people working in dusty environments, such as coal miners, could encounter high concentrations of particles and may have impaired lung clearance (Baan et al., 2006, 186864). 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, 157938; Moran et al., 1991, 157956; Yamadori et al., 1986, 193728).

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, 157717), and "carcinogenic" (Class D2A) by WHMIS (CCOHS, 2006, 157774). NIOSH (2005, 196072) 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 for 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				
Intratracheal instillation	Wistar rats, female	Increased lung tumors (benign and malignant)	5 instillations at 6.0 mg/instillation	Borm et al. (2000, 041486)
			5 instillations at 3.0 mg/instillation	Pott and Roller (2005, 157790)
Inhalation	Wistar rats, female	Increased lung tumors	Approximately 12 mg/m^3 for 24 mo[a]	Heinrich et al. (1995, 076637)
	NMRI mice, female	No increases in lung tumors	(Approximately 10 mg/m^3 for 13.5 mo)[b]	Heinrich et al. (1995, 076637)
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, 029823)
Nano-TiO_2 with unspecified photoreactivity[c]				
Intratracheal instillation	Wistar rats, female	No conclusion[d]	(30 instillations at 0.5 mg/instillation)	Pott and Roller (2005, 157790)
Intramuscular implantation	Sprague-Dawley rats, male	No increases in tumor at implantation sites	(not specified)	Hansen et al. (2006, 090611)

NMRI = Naval Medical Research Institute

[a]7.2 mg/m^3 for 4 mo, followed by 14.8 mg/m^3 for 4 mo and then 9.4 mg/m^3 for 16 mo

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

[c]Nano-TiO_2 particles not specified or have questionable identification

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

Chapter 6. Summary

This chapter briefly summarizes information from the preceding chapters, describing case studies of nano-TiO_2 for arsenic removal in drinking water treatment and for topical sunscreens. It also highlights information gaps and research questions identified in these case studies as they might relate to future risk assessment efforts.

The case studies were developed using the CEA framework, which, as described in Chapter 1, combines a product life-cycle perspective with the risk assessment paradigm to provide a more holistic examination of a material's potential environmental impacts. However, the goal of this document is not to provide an actual CEA or to state conclusions regarding possible ecological or health risks related to nano-TiO_2, but to provide a foundation for a process to identify and prioritize research directions to support future efforts, and, eventually, provide input to policy and regulatory decision-making.

Given that the CEA is both a framework and a process, these case studies were used as a starting point for a formal collective judgement method known as a "nominal group technique" (NGT) to identify and prioritize research questions related to nano-TiO_2. The NGT process was conducted at a workshop held on September 29-30, 2009, and a summary report is available that describes the workshop and summarizes the collective prioritization results (U.S. EPA, 2010, 625483). A brief description of this process is provided in Section 6.2.1. Section 6.2.2 considers the information in the case studies in light of research themes identified in the U.S. EPA's *Nanomaterial Research Strategy Document* (U.S.EPA, 2009, 625484) and discusses the potential for using this information together with collective priority ranking results to inform refined nanomaterial research strategies, not only within EPA but in the broader scientific community. Section 6.2.3 discusses ways in which information from the case studies, collective prioritization results, and emerging research can be integrated to support future assessment efforts for nanomaterials.

6.1. Case Study Highlights

This section summarizes what is known, as well as what is unknown, regarding life cycle stages (feedstocks, manufacturing, distribution, storage, use, and disposal/recycling), fate and transport in the environment, exposure and dose characterization for biota and humans, and ecological and health effects of two uses of nano-TiO_2: (1) for arsenic removal from drinking water; and (2) in topical sunscreens. For each topic area, readers are referred back to the specific sections of the document where detailed discussion of the evidence and associated references are presented. In some cases, the findings and research questions are specific to nano-TiO_2, while in others, they pertain to nanomaterials in general. These issues may be immediately useful to scientists engaged in ongoing nanomaterial research, as well as for those involved in shaping future research and assessment efforts.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

6.1.1. Analytical Methods

Sensitive and accurate analytical methods for physicochemical characterization of nanomaterials are critical tools for CEA (Section 1.6). Measurement and characterization of nanomaterials, alone and in various media, are required for properly monitoring fate and transport, assessing exposure, conducting toxicological studies, estimating dose-response relationships, and understanding the behavior and effects of nanomaterials. Minimum characterization requirements for toxicological studies have been recommended to facilitate interpretation and comparison of studies. Physicochemical characterization of nano-TiO_2, as with other nanomaterials, is extremely important at each stage of its life cycle, because these properties change depending on characteristics of the surrounding matrix (e.g., pH, ionic strength, presence of natural organic matter or large biomolecules, soil or sediment composition). In conducting laboratory studies, researchers must account for changes in the properties of the raw material from the point of production during their transport, storage, and preparation for testing. These physicochemical properties are also likely to change when nanomaterials enter the environment, making it difficult to predict their behavior based on laboratory studies. Additional challenges of characterizing nano-TiO_2 in the environment include low expected concentrations and the difficulty of distinguishing naturally occurring materials in the nanoscale size range from manufactured nanomaterials. As a further complication, the critical properties or suite of properties that have the most influence on environmental behavior, exposure, and ecological and health effects are unknown, necessitating the use of multiple methods to characterize nanomaterials. This need for complex characterization distinguishes nano-TiO_2 and other nanomaterials from chemical compounds typically studied in environmental assessments, which often may be characterized by mass concentration alone and maintain their chemical structure in different matrices.

Properties of nano-TiO_2 and other nanomaterials include particle size and size distribution, mass, surface area, particle number, crystal structure, chemical composition, and zeta potential. Concomitant measurements of relevant properties of liquid or solid media are also necessary for characterizing nanomaterials in those matrices, such as pH, salinity, or the presence of organic matter. Methods developed for measurements of aerosol properties are available for measuring nanomaterials in the air, including the condensation particle counter (CPC) for measuring particle number; the scanning mobility particle sizer (SMPS) and electrical low pressure impactor (ELPI) for measuring number, surface area, and mass; the optical particle counter (OPC) for number; direct number counts and size distribution by electron microscopy, which can be combined with EDS for elemental composition; and size-selective personal and static samplers for mass measurements of different size fractions. The BET method of gas adsorption to determine particle surface area has also been useful in characterizing nano-TiO_2 and other nanomaterials.

Liquid-phase techniques for particle size, as presented in Table 1-3 in Section 1.6.1, include dynamic light scattering (DLS) and static light scattering, size exclusion chromatography (SEC), acoustic techniques, SPM, and centrifugal sedimentation. Some of these techniques can also provide information on number and zeta potential (e.g., DLS) and mass or density (e.g., centrifugal sedimentation). Crystal structure has been measured by HRTEM and XRD. Field flow fractionation (FFF) to determine particle size has been combined with inductively coupled plasma atomic emission spectroscopy (ICP-AES) for detection and characterization of nano-TiO_2 in commercial sunscreen, providing information on mass, size distribution, and Ti content of extracted nano-TiO_2. Specific applications of other techniques to nano-TiO_2 in water treatment processes and sunscreens have not been identified.

Methods for characterizing nanomaterials in soil and sediment would ideally be performed in situ to avoid changes in nanoparticle properties caused by sample handling, but this requires portable instrumentation and techniques that can operate in complex environmental media. Therefore, liquid-phase techniques are used following sample extraction. As presented in Table 1-4 in Section 1.6.2,

these include centrifugation, ultrafiltration, FFF, and SEC for size fractionation; electron microscopy and DLS for size distribution; the BET method for surface area, and spectroscopic techniques for phase and crystalline structure. As with nanomaterials suspended in gas and liquid phases, the use of multiple techniques is recommended to provide more complete characterization.

Although characterization of nanomaterials is recognized to be a critically important aspect of evaluating their fate and effects, no consensus has been reached on a critical set of properties. Remaining questions include standardization of methodology and terminology, the potential need for improvements to existing methods or entirely new methods, the influence of coatings and other formula components on nano-TiO_2 properties and behavior in sunscreen, information on agglomeration of particles in drinking water treatment processes and sunscreens, and correlation among parameters measured in different phases. These questions were reflected in the collective prioritization results from the workshop (U.S. EPA, 2010, 625483); three of the four highest-priority topics were directly related to characterization, including the need for improved physico-chemical characterization throughout life cycle stages, method development and evaluation, and product-specific characterization needs. Research to address these questions may be an important part of future investigations into the ecological and health effects of nano-TiO_2 and other nanomaterials.

6.1.2. Life Cycle Characterization

Feedstocks used in TiO_2 production include ilmenite and rutile ores, with ilmenite accounting for approximately 90% of worldwide production of Ti minerals (Section 2.1). The presence of low levels of radioactive materials in these ores raises the question of the potential for risk associated with ore mining and processing. It is not clear at this time whether certain feedstocks are more suitable and widely used for production of nano-TiO_2 compared to conventional TiO_2. Information is also lacking on the nature and magnitude of contaminant release associated with mining and processing of Ti-containing ores. The production quantity of conventional TiO_2 for use in pigments and other applications is far larger than production of nano-TiO_2, with 2005 production of conventional TiO_2 and nano-TiO_2 estimated at 4.5 million metric tons and 2,000 metric tons, respectively. The production volume of nano-TiO_2 is expected to grow rapidly over the next few decades, with widely varying estimates of the rate of growth.

A variety of techniques are available for commercial production of nano-TiO_2, many of which are adapted from manufacturing processes for conventional TiO_2 (Section 2.2). These include CVD, flame hydrolysis, sol-gel, calcination, aerosol pyrolysis, and colloidal synthesis. One commercially available product (P25) is produced by flame hydrolysis using $TiCl_4$ as a feedstock, resulting in agglomerated TiO_2 particles with a mean diameter of approximately 3,600 nm, with the smallest 4% of particles having an average diameter of 160 nm (Section 2.2). This gas-phase chloride method generates hydrogen chloride as a by-product, with the potential for some residual chloride ions adsorbed onto the TiO_2 particles. Specific information was not identified on processes for preparing or formulating nano-TiO_2 for use in arsenic removal from drinking water, although information is available on nano-TiO_2 formulations used in sunscreens. Rutile is a more photostable crystalline form of TiO_2 than anatase, which should make it preferable for use in sunscreen applications, although anatase/rutile mixes are also common. The potential for photocatalytic action of nano-TiO_2 is mitigated by surface coatings applied to the particles, such as silica, alumina, dimethicone, or other compounds; these coatings also improve particle behavior in formulation of sunscreen dispersions. Other components of these dispersions include emulsifiers, emollients, other physical or chemical UV blockers, and ingredients to improve characteristics such as spreadability, water resistance, and viscosity. Among the questions regarding the manufacturing component of the life cycles for these nano-TiO_2 applications are the influence of different manufacturing techniques on physicochemical properties of nano-TiO_2; the specificity of certain manufacturing techniques for

nano-TiO_2 used in either drinking water treatment or sunscreen, and the potential for new techniques to emerge; the potential for release of nanoscale and larger-sized waste products from nano-TiO_2 manufacturing; and the potential for general population exposure to nano-TiO_2 in the vicinity of manufacturing facilities.

Limited information was identified specifically relating to nano-TiO_2 distribution and storage (Section 2.3). Raw nano-TiO_2 in powdered form is shipped in paper bags, in some cases lined with polyethylene film, and in glass bottles enclosed in sealed bags. Dispersions of nano-TiO_2 are shipped in pails, drums, or totes. No specific information was identified on distribution and storage of nano-TiO_2 formulated for drinking water treatment or sunscreen use. Some general information is available from the sunscreen industry on shipping and handling of topical sunscreens, with approximately two-thirds of sunscreen retail sales in the U.S. occurring in supermarkets, drugstores, and mass merchandise outlets. Accidental releases could occur to air, water, or soil at a variety of points along the distribution chain. Inclusion of distribution and storage information in life cycle and comprehensive assessments will require additional data regarding shipping modes, distances, and quantities for nano-TiO_2 in various packaging, modes of storage prior to use in drinking water treatment and sunscreens, and estimates of releases under various scenarios of distribution and storage.

The drinking water treatment case study considers only the application of nano-TiO_2 for arsenic removal (Section 2.4.1), although it is possible that it can be used to remove other biological or chemical contaminants. Approximately 13 million people in the U.S. use water that is treated to remove arsenic. No information was identified on current use of nano-TiO_2 in community water systems to remove arsenic, but future use could affect a substantial population. At least two commercial technologies are known to be capable of using nano-TiO_2 in oxidative processes for water treatment, although currently they are not known to be used in this way. One process uses nano-TiO_2 in a fixed membrane, while the other uses nano-TiO_2 in a slurry. In bench-scale studies, slurry applications have been shown to produce higher arsenic oxidation rates compared to fixed-matrix nano-TiO_2; however, immobilized applications presumably would result in less release to finished water than slurries, which require filtration. A bench-scale simulation of a conventional drinking water treatment process found that more than 20% of an initial 10 mg/L concentration of nano-TiO_2 (15-40 nm particle size, 200-500 nm aggregate size) remained in water following coagulation, flocculation, and sedimentation (prior to filtration). Filtration through a 0.45 μm membrane reduced residual TiO_2 to 1-8% of the initial concentration, although this level of filtration is not typical in drinking water treatment plants or whole-house filtration systems. Another factor that could be important, but for which information is lacking, is the effect of drinking water treatment chemicals on the solubility, particle size, and behavior of nano-TiO_2. Information is also limited on the potential volume of nano-TiO_2 required for arsenic removal in the U.S., details on different treatment processes and the likelihood of their use to serve populations of various sizes, release of nano-TiO_2 to finished water or process waste, and the effect of nano-TiO_2 on biofilms and corrosion in distribution systems.

As discussed in Section 2.4.2, sunscreen use is substantial in the U.S., with most surveys reporting that 30-50% of respondents use sunscreen regularly. Parents report more frequent use of sunscreen on their children than on themselves. The amount and use of nano-TiO_2 in sunscreens is unknown, in part because available survey data do not distinguish between conventional and nano-TiO_2, although conventional TiO_2 is likely to contain nanoscale TiO_2. Production estimates of nano-TiO_2 indicate that a substantial fraction (65%) is used in personal care products such as sunscreen and cosmetics. The lack of specific information on nano-TiO_2 use in sunscreens represents an important gap in knowledge for life cycle and exposure assessment. Questions identified as priorities during the collective prioritization workshop (U.S. EPA, 2010, 625483) include the potential for release of nano-TiO_2 to various media through different use patterns, the need for better characterization of nano-TiO_2 as used in specific products, and the stability and behavior of surface

treatments during sunscreen use. Removal of surface coatings may increase the photocatalytic activity of the nano-TiO_2 particles and have implications for its effects on biota and humans.

Disposal of nano-TiO_2 after use is likely to vary between drinking water treatment processes and sunscreen (Section 2.5). Some fraction of nano-TiO_2 used in drinking water treatment is likely to be associated with sludge, which may be taken to a landfill or applied to agricultural lands (U.S. EPA, 2010, 635678). To the extent that nano-TiO_2 reaches finished water, it would then be expected to enter wastewater treatment facilities. Disposal of used sunscreen containers, presumably with some residual product, would also result in introduction of nano-TiO_2 to municipal landfills or incinerators. Recycling of sunscreen containers is also possible, potentially introducing nano-TiO_2 into recycled materials. Remaining disposal issues for which little information is available include disposal procedures for packaging containing nano-TiO_2 and substandard product at manufacturing facilities, the behavior of nano-TiO_2 in landfills, the exact nature of waste streams from water treatment facilities, and the circumstances that might result in release of nano-TiO_2 from discarded sunscreen products.

6.1.3. Fate and Transport

Information is currently unavailable regarding the transport and transformation of nano-TiO_2 specifically from drinking water treatment processes and sunscreens in air, water, ground water, soil, or sediment (Chapter 3). As mentioned in Chapter 1 and Section 6.1.1, physicochemical properties are likely to change when nanomaterials enter the environment, making it difficult to predict their behavior based on laboratory studies. One aspect of nano-TiO_2 that is heavily influenced by local conditions is agglomeration of particles to form larger clusters. These agglomerates would tend to behave differently in the environment than individual particles. Degree of agglomeration is affected by ionic strength and presence of organic matter in water, as demonstrated in laboratory studies. Agglomeration is also pH-dependent, with minimum particle attraction at the pH_{pzc}. Researchers have also demonstrated in bench-scale studies that free particles or agglomerates with diameters less than 100 nm can be present even when the predominant form of nano-TiO_2 is larger clusters (Chapter 3), a finding that could have implications for agglomeration in natural waters. Surface modifications to maintain dispersion may also contribute to the presence of nanoscale particles under conditions normally considered to promote agglomeration.

Nano-TiO_2 that reaches wastewater treatment plants, such as through washing off of sunscreens, has the potential to pass through the facility in the liquid phase and reach receiving waters. Studies of other metal oxide nanomaterials in model wastewater treatment plants indicated that surfactants stabilized dispersions even at high ionic strength, although most nanoparticles were removed by agglomeration with microorganisms and subsequent sedimentation. The low solubility of nano-TiO_2 compared with other metal and metal oxide nanoparticles suggests that it is likely to remain in the solid phase, although researchers have found that crystalline form, morphology, manufacturing method, and particle size can influence saturation concentration. Another aspect of nano-TiO_2's behavior in aqueous media that should be kept in mind is its photocatalytic generation of ROS in the presence of UV light, which may be a factor in surface waters exposed to sunlight.

The lack of information on environmental behavior specific to nano-TiO_2 used in drinking water treatment processes and sunscreens represents an open question. Substantial transformation of nano-TiO_2 is not expected due to its physical and chemical stability, so the processes likely to be relevant are transport and accumulation in various environmental compartments. Chapter 3 describes scenarios through which nano-TiO_2 used for arsenic removal could enter the finished water distribution system and thereby end up in surface water or the subsurface via leaks, or it could become part of sedimented sludge and enter the subsurface through landfilling. Likewise, nano-TiO_2 from sunscreens could be released to natural bodies of waters through wear-off, and to wastewater

via bathing or laundry following sunscreen use. This nano-TiO_2 could migrate to sediment through agglomeration with natural organic matter or microorganisms or could remain in the water column. Studies have observed other sunscreen constituents in natural waters, providing plausibility for this scenario. However, no studies were identified that have documented the occurrence of nano-TiO_2 specifically from sunscreens in wastewater or natural waters.

Laboratory studies have measured transport of nano-TiO_2 in synthetic porous media or model soil columns, and found nano-TiO_2 to be mobile in these model systems, where large soil particles and low ionic strength favored increased mobility, while high clay content, dissolved organic carbon, and salinity favored retention (Section 3.2). Particle size also affected mobility, with smaller particles passing through the columns to a greater extent than larger particles. However, pH had an unanticipated effect in one model system, with high transport observed at pH values for which retention was expected. This indicates that current transport theory may not accurately predict transport of nanomaterials and agglomerates. Specific information was not identified regarding pathways for nano-TiO_2 from arsenic removal or sunscreen use to end up in soil, although one possible scenario is accumulation in wastewater treatment sludge which is then applied to agricultural land as a soil amendment.

Information is not available on the fate of nano-TiO_2 that may be emitted to the atmosphere by manufacturing or distribution facilities (Section 3.3). It is unclear whether its atmospheric fate and transport behavior would be similar to that of ultrafine particulate matter emitted from combustion sources, which tends to agglomerate rapidly and undergo phase change (condensation/volatilization) near the source, due to the differing physicochemical characteristics of nano-TiO_2 from combustion emissions.

The collective prioritization process at the workshop identified three priority topics with fate and transport aspects (U.S. EPA, 2010, 625483). These include: (1) the role of physicochemical properties, including surface treatments and agglomeration, in environmental behavior of specific nano-TiO_2 products; (2) identification of pathways that pose the greatest exposure potential to nano-TiO_2; and (3) the spatial and temporal distribution of nano-TiO_2 in the environment. Other questions relating to fate and transport of nano-TiO_2 from drinking water treatment and sunscreen use include: what the effect is of environmental factors (e.g., pH, natural organic matter type and concentration) on nano-TiO_2 mobility and fate; whether existing theory and models of particle transport are applicable to nano-TiO_2 and other nanomaterials; the extent to which knowledge regarding fate and transport of other nanomaterials may be applicable to nano-TiO_2; the potential for nano-TiO_2 to influence the behavior of other water and soil constituents and to bioaccumulate; whether photocatalytic activity of nano-TiO_2 is of concern in water treatment, wastewater treatment, or the environment; and the effect of co-occurring sunscreen constituents on nano-TiO_2 fate and transport.

6.1.4. Exposure and Dose Characterization

6.1.4.1. Exposure Characterization

The term exposure refers to contact between an individual and a pollutant, combining information on activity patterns and time spent in various microenvironments with concentration data in multiple environmental media. Biota and humans may be directly exposed to nano-TiO_2 used in drinking water treatment or sunscreen, or may receive indirect exposure through contact with nano-TiO_2 in air, water, soil, or sediment. Transfer of nano-TiO_2 between these media is also likely to occur. As described in Chapter 4, limited evidence is currently available regarding environmental exposures of biota and humans to nano-TiO_2, although some information is available on occupational exposures associated with nano-TiO_2 manufacturing.

Aquatic organisms may be exposed to nano-TiO_2 present in the water column or in sediment, with exposure depending on the distribution of nano-TiO_2 between water and sediment as well as the tendency of the organism to feed or otherwise spend time near the bottom of water bodies. The propensity of nano-TiO_2 to agglomerate may also result in deposition to the surfaces of aquatic organisms, including the gills of fish. This could increase the concentration of nano-TiO_2 relative to the water and may result in surface toxicity even when uptake is not observed (Section 4.1.1). For terrestrial organisms, exposure scenarios may include contact between material spilled during shipping or storage and microbial, invertebrate, and vertebrate species, as well as potential contact with nano-TiO_2 in environmental media. No specific evidence has been identified regarding actual exposures of biota to nano-TiO_2 in the environment.

Human exposure to nano-TiO_2 may occur either in occupational settings or among the general population (Section 4.2). The general population may be exposed through use of nano-TiO_2 sunscreens or by drinking water with residual nano-TiO_2, as well as through contact with nano-TiO_2 from these applications that ends up in environmental media. The use of nano-TiO_2 for arsenic removal in drinking water appears to be limited at present, although implementation of this technology could result in substantial exposure given the sizeable population receiving finished water that has been treated for arsenic. If nano-TiO_2 were present in potable water, exposures could involve pathways other than ingestion, such as dermal contact and inhalation of droplets during bathing and showering. Potential exposures may be of greater concern for infants and children, who consume more water per body weight than adults. Sunscreen-related exposure to nano-TiO_2 may occur through skin contact, although dermal uptake has been found to be relatively low; other potential pathways include inhalation of spray products and ingestion via hand-to-mouth contact (particularly for children). Based on one series of assumptions, the amount of applied nano-TiO_2 per sunscreen application was estimated to range from 12 to 55 mg/kg body weight for a three-year-old child and 8-37 mg/kg for adults (Table 4-2). This higher exposure combined with parent reports of greater sunscreen use on their children than on themselves could indicate an important role for exposure to nano-TiO_2 and related sunscreen constituents in children.

Nearly every stage of the life cycle for the applications considered in these two case studies presents some potential for occupational nano-TiO_2 exposure. Most available information pertains to manufacture of nano-TiO_2 rather than product formulation, shipping, or use by operators of drinking water treatment facilities (Section 4.2.2). Manufacturers have reported workplace inhalation exposures of less than 0.3-0.5 mg/m^3, although concentrations in some areas were higher. As a frame of reference, the NIOSH has proposed a draft occupational exposure limit of 1.5 mg/m^3 for fine TiO_2 and 0.1 mg/m^3 for ultrafine TiO_2 based on relative surface area. Independent measurements in a facility producing nano-TiO_2 found lower mass concentrations, ranging from 0.004 to 0.042 mg/m^3; personal sampling in this facility found a concentration of 0.010 mg/m^3. Number concentrations in the facility ranged from 15,000 to 29,000 particles/cm^3, with 60% of the particles in the 20 to 30 nm size range; airborne TiO_2 concentrations outside the plant were measured at 13,000 particles/cm^3. Surface area concentrations were 13-50 $\mu m^2/cm^3$. A modeling study using these and other data estimated that packers were exposed to 0.39 m^2 TiO_2 per 300 m^3 air, while surface treatment workers had lower exposure at 0.17 m^2 per 300 m^3. These preliminary data indicate that there is a wide range of concentrations and exposures among nano-TiO_2 manufacturing and handling facilities and that exposure may vary by occupation. In addition, dermal exposure may be relevant depending on the type and usage rate of personal protective equipment.

As described in Chapter 4, both aggregate exposures (representing exposure to nano-TiO_2 through multiple routes, such as dermal, ingestion, and inhalation) and cumulative exposures (representing exposures to multiple substances associated with the use of nano-TiO_2 in drinking water treatment and sunscreen) are relevant to the consideration of nano-TiO_2 exposure. However, limited evidence specific to nano-TiO_2 is currently available. Cumulative exposure may involve other sunscreen constituents, transformation products of reactions catalyzed by TiO_2 (in drinking water or sunscreen), or pollutants adsorbed to nano-TiO_2 and carried into the body as co-

contaminants. This latter phenomenon has been observed for arsenic and cadmium, as summarized in the following section on dose characterization. Although models have not been specifically developed for characterizing exposure to nano-TiO_2, EPA has various models that have been used in assessments of other chemicals to provide screening-level estimates of aquatic exposure, general population exposure, and consumer exposure. Adaptation of these models for use with nanomaterials would likely be necessary prior to their quantitative use for nano-TiO_2. Researchers in Switzerland have developed models to predict environmental concentrations of nano-TiO_2 and compared their estimates to no-effect concentrations in aquatic toxicity studies, although an explicit exposure component was not included.

The collective priority ranking process identified several exposure-related questions (U.S. EPA, 2010, 625483), including:

- which properties of nano-TiO_2 are most relevant for exposure characterization, and whether available methods are adequate to characterize exposure in air, water, soil, and other media;
- which pathways pose the greatest exposure for biota and human exposure to nano-TiO_2 used in drinking water treatment and sunscreens; and
- whether certain populations of biota and humans have greater exposure potential.

Other questions include how exposure models can be developed or adapted to estimate nano-TiO_2 exposure, and the degree of exposure to secondary contaminants associated with nano-TiO_2 used in drinking water treatment and sunscreens. Occupational exposure can be further characterized as well, leading to questions such as:

- the size of the potentially exposed population;
- additional information on concentrations and durations of exposure in various job classifications;
- which monitoring methods and properties are appropriate for measuring workplace exposure; and
- which management practices and protective equipment are appropriate for controlling exposure by various routes.

6.1.4.2. Dose Characterization

Dose is defined as the amount of a substance that enters an organism by crossing a biological barrier. Various exposure routes have been investigated for uptake of nano-TiO_2 in studies of fish, laboratory animals, and humans (Section 4.6). Two studies investigating accumulation of nano-TiO_2 in fish following multi-day exposures have found mixed results, possibly due to differences in species (bottom feeder versus pelagic) and other aspects of study design. Two additional fish studies have indicated the potential for nano-TiO_2 to increase uptake of arsenic and cadmium, presumably by adsorption and facilitated transport. In addition to this example of cumulative dose to multiple pollutants, bioaccumulation in the food web is also a possibility, although no studies were identified that demonstrated multi-species bioaccumulation of nano-TiO_2.

In animal toxicological studies relevant to terrestrial biota and humans, various exposure routes have been evaluated to determine the uptake of nano-TiO_2 and other nanomaterials, including respiratory (inhalation and instillation), dermal, and ingestion. Animal studies have shown that inhaled or inspired nano-TiO_2 can translocate into the interstitium of the lung, the lymph nodes, blood, and the brain (Section 4.6.2). Deposition patterns in the respiratory tract depend on several factors, including particle size and breathing pattern. Model results of human lung deposition

indicate that very few nanoparticles reach the alveolar region, having been removed by diffusive deposition in the upper airways or tracheobronchial region. Studies in rats indicate that the retention half-life of inhaled nano-TiO_2 was approximately three times as long as that of fine TiO_2.

Dermal uptake of nano-TiO_2 is particularly relevant for sunscreens containing nano-TiO_2, and both human and animal studies are available (Section 4.6.3). These studies predominantly indicate that nano-TiO_2 does not penetrate beyond the stratum corneum or hair follicles into living cells of healthy skin. In a study comparing psoriatic and healthy skin, nano-TiO_2 in a sunscreen formulation penetrated into deeper areas of the stratum corneum of psoriatic skin, but still did not reach living cells. No studies have been identified that evaluated nano-TiO_2 penetration in damaged skin (e.g., from sunburn), although preliminary results indicate greater penetration of quantum dots and nano-silver in damaged skin compared to healthy skin. The extent and duration of nano-TiO_2 accumulation on the skin via reapplication of sunscreen and the ultimate fate of nano-TiO_2 from sloughed skin cells are both open questions at this time.

Evidence for accumulation of nano-TiO_2 following ingestion is extremely limited, with a single study reporting elevated concentrations in the liver, spleen, kidney, lung, and brain of female mice following oral gavage (Section 4.6.4).

The potential for nanoparticles to cross the blood-brain barrier (BBB) has been investigated for medical applications, where in many cases the particle surfaces have been modified to enhance translocation (Section 4.6.5). Mixed evidence is available for translocation of nano-TiO_2 across the BBB following injection or gavage, with some studies finding increased Ti concentrations in the brain and others finding no evidence of an increase. A recent study showed TiO_2 particles and pathological changes in the brain of mouse offspring following maternal exposure during gestation, although it is not clear whether nano-TiO_2 crossed the placenta or entered the milk to result in lactational exposure.

Various metrics are possible for characterizing nanoparticle dose, such as mass, surface area, or particle number, as well as crystalline form, shape, and surface modifications (Section 4.6.6). Studies comparing mass and surface area to evaluate dose-response curves provide mixed results, with some evidence indicating that surface area provides a more consistent dose-response relationship for both fine and nano-TiO_2. Composite metrics of two or more properties may also be useful, as suggested by a study indicating that separate surface-area-based dose-response curves for anatase and rutile TiO_2 would better fit the data than a single dose-response curve.

Questions highlighted during the collective prioritization process (U.S. EPA, 2010, 625483) for future research on dosimetry of nanomaterials in general and nano-TiO_2 in particular include:

- whether certain populations (e.g., children) may be particularly susceptible to receiving high doses of nano-TiO_2 from its use in drinking water or sunscreens; and
- which dose metrics are most relevant for characterizing nano- TiO_2 dosimetry.

Other questions include:

- what modifications need to be made to physiologically-based pharmacokinetic models so that they are appropriate for understanding absorption, distribution, metabolism, and excretion of TiO_2;
- how to extrapolate received dose from animal toxicological studies to humans; the extent to which nano-TiO_2 may bioaccumulate and biomagnify in food webs; and
- whether increased uptake of copollutants in the presence of nano-TiO_2 indicates the need for consideration of other substances in nano-TiO_2 monitoring and exposure studies.

6.1.5. Ecological and Health Effects

Several factors influence the ecological and health effects of nano-TiO_2, including physicochemical characteristics, experimental conditions, and environmental conditions (Section 5.1). The need for thorough characterization of nanomaterials used in toxicity studies is now well recognized. Important properties considered part of a minimum set of characteristics include: particle size, size distribution, and aggregation status; chemical composition and crystal structure; surface chemistry and charge; specific surface area; particle shape; and production method. Studies have found these variables to be important in determining the chemical and biological behavior of nanomaterials. Experimental conditions also modify the effects of nano-TiO_2 and are therefore important to measure and report in detail. For example, skin penetration of nano-TiO_2 increased for an oily dispersion compared with an aqueous dispersion, although nano-TiO_2 did not reach living skin cells. Suspension media used in laboratory studies, such as deionized water, tap water, saline solutions, and BAL fluid, each lead to different states of agglomeration which can affect the uptake and effects of nano-TiO_2. Different levels of in vitro OH radical production have been observed in different sunscreen formulations containing similar nano-TiO_2, indicating that the other components of the mixture can affect the observed results. Similar issues exist for environmental conditions, such as differential effects due to changes in the aquatic chemistry of surface or ground water or the presence of natural organic matter. UV radiation is well known to increase the toxicity of nano-TiO_2; in addition, it may make the skin more permeable by causing sunburn. Other issues that are potentially important include the influence of temperature and water saturation on skin penetration, but no studies were identified that have investigated these parameters.

6.1.5.1. Ecological Effects

Ecological effects on microorganisms, aquatic species, and terrestrial species are discussed in Section 5.2, and key studies are summarized in Table 5-3. Most studies have tested photocatalytic nano-TiO_2 as would be used in water treatment, with only a few studies evaluating photostable nano-TiO_2 intended for use in sunscreen. However, coatings used to increase the photostability of nano-TiO_2 could be removed in the environment through weathering or biotransformation, yielding nano-TiO_2 with photocatalytic properties. Studies of acute effects in microorganisms and higher aquatic species generally provide little evidence of toxicity at concentrations below 10 mg/L, with several studies finding no effects at concentrations of 100 mg/L or higher. However, a longer-term (14-day) study found respiratory toxicity, injury to the gill and intestine, and evidence of oxidative stress in the gill and intestine in juvenile rainbow trout following exposure to photocatalytic nano-TiO_2 at concentrations as low as 0.1 mg/L. Studies evaluating terrestrial invertebrates found no effect on behavior or mortality for *P. scaber* and decreased reproduction without change in body length for *C. elegans*. Spinach growth was enhanced by nano-TiO_2 in several studies, possibly due to increases in the activity of enzymes responsible for photosynthesis, nitrogen metabolism, and oxidative stress response. Incorporation of nano-TiO_2 into sediment was not found to increase toxicity of sediment or elutriate, even at a 1:1 ratio. In general, the focus of these studies on growth and mortality provides little information on mode of action of nano-TiO_2 ecotoxicity.

One of the highest priority areas identified in the collective prioritization results from the workshop (U.S. EPA, 2010, 625483) included the question of whether standard ecotoxicity tests are appropriate for nanomaterials in general and nano-TiO_2 in particular. Changes in nano-TiO_2 properties in different matrices (raw materials, products containing nano-TiO_2, environmental media, and biological systems) may lead to differing behavior and make extrapolation of test results difficult. It is not currently clear whether a suite of physicochemical properties can be used for a structure-activity relationship to predict biological effects. In addition, the interplay between physicochemical properties of nano-TiO_2 and changes in environmental variables (e.g., pH, oxygen

level) is not well understood and could result in changes to both the ecotoxicity of nano-TiO_2 and underlying soil or aquatic chemistry. Other issues potentially relevant to changes in physicochemical properties include the effect of in vivo biochemical processes on nano-TiO_2 and the potential for interaction between nano-TiO_2 and associated substances resulting in increased uptake and effects of either nano-TiO_2 or copollutants. The collective priority ranking results also included questions surrounding the mechanism or mode of action of nano-TiO_2 and whether different modes of action are important at low and high concentrations.

6.1.5.2. Health Effects

Health effects of nano-TiO_2 are discussed in Section 5.3. Both noncarcinogenic and carcinogenic effects have been examined. Noncarcinogenic effects have been investigated in animal toxicological studies for several exposure routes, including dermal, oral, and respiratory; however, no epidemiologic studies or case reports were identified pertaining specifically to nano-TiO_2. Limited evidence from acute in vivo dermal exposure studies does not indicate skin irritation or skin cell toxicity following exposure to photocatalytic nano-TiO_2; as discussed in Section 6.1.4, uptake of nano-TiO_2 through healthy skin was not observed. No studies were identified that evaluated either effects in flexed or abraded skin or long-term effects of any kind, which would be relevant to typical sunscreen usage patterns. Of the three animal studies identified that evaluated toxicity following oral intake of nano-TiO_2, two studies found no evidence of lethality or obvious acute toxicity following a single dose of 5,000 mg/kg, although brain morphological changes were observed in one of these studies. The third study found DNA damage in mice in both mothers and offspring following exposure to 60-600 μg/mL nano-TiO_2 in drinking water for 5 days; this is also relevant to carcinogenic effects. A larger group of studies focused on respiratory effects following inhalation or instillation, and found pulmonary inflammation and impaired particle clearance, with effects generally most severe in rats, followed by mice, and least severe in hamsters. Nano-TiO_2 effects were often more severe or persistent than conventional TiO_2 at the same doses. Preliminary evidence has also been observed for systemic effects outside the lung following respiratory exposure, including changes in inflammatory cell and platelet counts in the blood, renal pathology, potential hepatic toxicity, and changes in brain morphology and neurotransmitter levels. Carcinogenic effects of nano-TiO_2 have been examined in several studies due to the classification of TiO_2 (size unspecified) as a possible human carcinogen by IARC. The evidence indicates that inhalation or instillation of photocatalytic nano-TiO_2 increases lung tumor incidence in rats, but not mice. This raises the question of the human health relevance of rat-specific nano-TiO_2 carcinogenicity due to increased susceptibility to pulmonary inflammation and poor particle clearance in the rat strains studied. No carcinogenic effects were observed following intraperitoneal or intramuscular administration of photocatalytic nano-TiO_2.

Another of the highest priority areas identified in the collective prioritization results from the workshop (U.S. EPA, 2010, 625483) included the question of whether current EPA test guidelines and assays are appropriate for determining the health effects of nano-TiO_2, and, if not, which modifications, additional assays and standard reference materials would be useful. Additional priority questions from the workshop included: what the fundamental biological responses are for nano-TiO_2interactions at the cellular level; what the important modes and mechanisms of action are for nano-TiO_2 effects and whether the mode of action differs for different types of nano-TiO_2 (e.g., photocatalytic versus photostable) or different organs (e.g., lung versus brain); and what the long-term effects of nano-TiO_2 may be. Other potentially relevant issues included: which properties are necessary and desirable for proper characterization of nano-TiO_2 during assays; whether nano-TiO_2 has the potential to penetrate compromised skin; and whether nano-TiO_2 has reproductive, developmental, or carcinogenic effects.

6.2. Role of Case Studies in Research Planning and Assessment Efforts

These two case studies are designed to benefit ongoing nanomaterial research efforts, the research planning process, and potential future assessment efforts on the environmental (ecological and health) effects of nano-TiO_2 and other nanomaterials. The currently available information presented here, along with gaps in knowledge that have been identified, should be useful in the interpretation of newly available data as well as planning for future research and assessments. As stated previously, the case studies are not intended to represent completed assessments or to serve as the basis for near-term risk management decisions regarding the use of nano-TiO_2 in drinking water treatment or sunscreen. In addition, other scenarios for potential use of nano-TiO_2, such as in coatings or as a component of a solid matrix, may involve separate issues not considered in this document. This section describes how the case studies may be used in informing ongoing and planned research on nanomaterials and in developing information useful for future assessments. It also highlights some of the information gaps identified through the case studies and the associated workshop on research priorities, described in Section 6.2.1.

6.2.1. Workshop on Research Priorities for Nano-TiO_2

As part of the process of identifying areas where additional knowledge may be useful, NCEA held a workshop to identify and prioritize research directions. The workshop used a formal group decision method known as the "nominal group technique (NGT)," which is a process for a group of selected individuals to identify and rank a series of choices. Each individual presents a brief statement outlining the rationale for assigning a high priority to a particular choice. The group then discusses the priorities, with the opportunity to consolidate similar choices, and votes for the highest priority items. The result is a rank order of priorities based on the collective judgment of the individual participants. Research questions identified in the nano-TiO_2 case studies were prioritized using this technique.

A summary report (U.S. EPA, 2010, 625483) describes the workshop and summarizes the main outcomes of the ranking process. The reader is referred to this summary report, which provides more detailed information regarding the specific questions used to develop research priorities and the rationale for prioritizing each of the research needs; this information is not repeated here. The NGT process identified several high-priority topic areas, with the top-ranked priorities addressing whether existing human and ecological toxicity test protocols are appropriate for use with nano-TiO_2, as well as questions regarding characterization of the physicochemical properties of nano-TiO_2 at each stage of the product life cycle, in the environment, and in biological systems. Other priority topics included: determining what effect surface coatings and product formulations have on physic-chemical properties and biological activity of nano-TiO_2; evaluating exposure pathways and populations of greatest concern, and whether available methods are appropriate for characterizing exposure to nano-TiO_2; developing a database of information on environmental concentrations of nano-TiO_2 in various media, including biological systems; research into the mode of action of nano-TiO_2, both at high and low doses; and determining the effects of long-term exposure to nano-TiO_2. Many, if not all, of these topics are relevant to other nanoparticles, although separate lines of research may be useful in characterizing diverse particle types (e.g., metal oxides and carbon-based nanoparticles).

6.2.2. Implications for Research Planning

The U.S. EPA's Nanomaterial Research Strategy document (U.S.EPA, 2009, 625484) outlined research themes and science questions relating to sources, fate, transport, and exposure to nanomaterials; human health and ecological effects, and nanomaterial risk assessment and risk management. These science questions are guiding ongoing research in EPA's ORD and will form the basis for research planning efforts within ORD. The findings of these case studies are consistent with the themes emphasized in the Nanomaterial Research Strategy and should further inform interpretation of current results and planning for future research, both within EPA and among the broader scientific community. For example, the case studies and workshop highlighted the question of the appropriateness of health and ecological toxicity testing protocols for use with nano-TiO_2. This concept is included in the research strategy document as background for the key science questions on health and ecological effects of nanomaterials, and may form an overarching theme that can be used to integrate results from individual experiments using different study designs, protocols, and endpoints. Development of methods for physicochemical characterization of nanomaterials under controlled conditions, in environmental matrices, and in biological systems is also a common priority. This highlights the potential for integrated transdisciplinary research, a focus area for ORD, to utilize contributions from materials science, engineering, and biology to fully understand nanomaterial properties and effects.

One area of missing information identified in the case studies was research into the long-term effects of nano-TiO_2. Consideration of longer-term chronic effects in the context of existing research strategy themes and science questions would help address this gap in knowledge. Research into modes of action of nano-TiO_2, both at low and high doses, is another priority area identified by the case studies and workshop. The research strategy references the importance of mode of action information, and specifically discusses the potential utility of determining the physical and chemical properties responsible for biological effects of nanomaterials. Integration of critical properties with their associated modes of action could help create a more complete understanding of the health and ecological effects of nanomaterials, as well as differences between nanoscale and conventional materials and effects unique to nanomaterials. Linkages such as these between the case study findings and research planning efforts may result in more focused and effective nanomaterial research, as well as providing information useful for future risk assessments of nano-TiO_2 and other nanomaterials.

6.2.3. Implications for Future Assessment Efforts

The Nanomaterial Research Strategy (U.S.EPA, 2009, 625484) and EPA's Nanotechnology White Paper (U.S. EPA, 2007, 090564) both highlight the importance of improved information and research results to support future assessment efforts. Their recommendations on research directions to support risk assessment for environmental fate and transport, as well as health and ecological effects, resonate with the priorities identified by the case studies and workshop. A variety of information will likely figure into future risk assessments, including characterization of nano-TiO_2 in multiple matrices; information on the magnitude of potential releases, environmental concentrations, and exposure pathways for nano-TiO_2; appropriateness of methods for evaluating human and ecological toxicity; information on the interaction between physicochemical properties, dose, and mode of action; and both short-term and long-term health and ecological effects of nano-TiO_2. Information on the life cycle of nano-TiO_2 and products incorporating nano-TiO_2, including their fate and transport in the environment, can be combined with these data to support a CEA of nano-TiO_2. These case studies have summarized what is known on these and other topics, as well as what remains unknown, and the accompanying workshop has presented a set of priorities that can be used to guide future research and assessment efforts.

An important function of environmental assessments is the integration and synthesis of information from multiple lines of evidence. This is difficult at this stage for nano-TiO_2 or other nanomaterials due to the limited and somewhat scattered evidence in many areas along with the near-total lack of evidence in other areas. Risk assessments for human health effects of environmental chemicals typically bring together evidence from toxicological, epidemiological, and controlled human exposure studies, which is then integrated to evaluate the likelihood of a causal relationship between the pollutant and a particular category of health effects. The CEA framework expands upon this by considering the impact of a material's life cycle and environmental fate on health and ecological effects. CEA is particularly appropriate for engineered nanomaterials because it facilitates the transdisciplinary integration of information from materials science, engineering, and biology that is required to fully understand nanomaterial effects. CEA also can consider the multiple impacts expected to result from introduction of a new technology, compare those impacts with those from conventional technologies, and provide information relevant for evaluating the sustainability of new nanomaterials. Much work remains before this will be possible for nano-TiO_2 to the same degree as it has been accomplished for other pollutants. The information presented in these case studies of nano-TiO_2 in drinking water treatment and sunscreen provides a starting point for this important work.

REFERENCES

Adams LK; Lyon DY; McIntosh A; Alvarez PJ (2006). Comparative toxicity of nano-scale TiO2, SiO2 and ZnO water suspensions. Water Sci Technol, 54: 327-334. 157782

Adams WA; Bakker MG; Macias T; Jefcoat IA (2004). Synthesis and characterization of mesoporous silica films encapsulating titanium dioxide particles: Photodegradation of 2,4-dichlorophenol. J Hazard Mater, 112: 253-259. http://www.sciencedirect.com/science/article/B6TGF-4CPVNC3-2/2/65122ceca6d79b38b540c9a537344977. 193250

Aitken RJ; Creely KS; Tran CL (2004). Nanoparticles: An occupational hygiene review. Edinburgh, UK: Institute of Occupational Medicine for the Health and Safety Executive. 090566

Alrousan DMA; Dunlop PSM; McMurray TA; Byrne JA (2009). Photocatalytic inactivation of E. coli in surface water using immobilized nanoparticle TiO2 films. Water Res, 43: 47-54. 157461

Andreeva VA; Unger JB; Yaroch AL; Cockburn MG; Baezconde-Garbanati L; Reynolds KD (2009). Acculturation and sun-safe behaviors among U.S. Latinos: Findings from the 2005 Health Information National Trends Survey. Am J Public Health, 99: 734-741. http://dx.doi.org/10.2105/AJPH.2007.122796 193252

Aruoja V; Dubourguier HC; Kasemets K; Kahru A (2009). Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata. Sci Total Environ, 407: 1461-1468. http://dx.doi.org/10.1016/j.scitotenv.2008.10.053 193254

Asgharian B; Price OT (2007). Deposition of ultrafine (nano) particles in the human lung. Inhal Toxicol, 19: 1045-1054. 093119

Asli S; Neumann PM (2009). Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant Cell Environ, 32: 577–584. 193771

Auffan M; Pedeutour M; Rose J; Masion A; Ziarelli F; Borschneck D; Chaneac C; Botta C; Chaurand P; Labille J; Bottero J-Y (2010). Structural degradation at the surface of a TiO2-based nanomaterial used in cosmetics. Environ Sci Technol, 44: 2689-2694. 625063

AWWA Staff (2003). Principles and practices of water supply operations: Water treatment. Denver, CO: American Water Works Association. 193818

Baan R; Straif K; Grosse Y; Secretan B; El Ghissassi F; Cogliano V (2006). Carcinogenicity of carbon black, titanium dioxide, and talc. Lancet Oncol, 7: 295-296. 186864

Baan RA (2007). Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. Inhal Toxicol, 19 Suppl 1: 213-28. 157717

Baggs RB; Ferin J; Oberdorster G (1997). Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. Vet Pathol, 34: 592-597. 048642

Balmer ME; Buser HR; Muller MD; Poiger T (2005). Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environ Sci Technol, 39: 953-962. http://dx.doi.org/10.1021/es040055r 157817

Barker PJ; Branch A (2008). The interaction of modern sunscreen formulations with surface coatings. Progr Org Coating, 62: 313-320. http://dx.doi.org/10.1016/j.porgcoat.2008.01.008 180141

BASF (2008). Guide to safe manufacture and for activities involving nanoparticles at workplaces in BASF AG. Germany: BASF. http://basf.com/group/corporate/en/function/conversions:/publish/content/sustainability/dialogue/in-dialogue-with-politics/nanotechnology/images/BASF_Guide_to_safe_manufacture_and_for_activities_involving_nanoparticles.pdf. 193811

Basu R; Woodruff TJ; Parker JD; Saulnier L; Schoendorf KC (2004). Comparing exposure metrics in the relationship between PM25 and birth weight in California. J Expo Sci Environ Epidemiol, 14: 391-396. 087896

Battin TJ; Kammer FV; Weilhartner A; Ottofuelling S; Hofmann T (2009). Nanostructured TiO2: Transport behavior and effects on aquatic microbial communities under environmental conditions. Environ Sci Tech, 43: 8098-8104. http://dx.doi.org/10.1021/es9017046 201604

BAuA (2007). Nanotechnology: Health and environmental risks of nanomaterials - research strategy. Germany: Federal Institute for Occupational Safety and Health (BAuA); Federal Institute for Risk Assessment (BfR); Federal Environmental Agency (UBA). http://www.baua.de/nn_49456/en/Topics-from-A-to-Z/Hazardous-Substances/Nanotechnology/pdf/research-strategy.pdf. 157694

Baun A; Hartmann NB; Grieger K; Kusk KO (2008). Ecotoxicity of engineered nanoparticles to aquatic invertebrates: A brief review and recommendations for future toxicity testing. Ecotoxicology, 17: 387-395. 157598

Beduneau A; Saulnier P; Benoit JP (2007). Active targeting of brain tumors using nanocarriers. Biomaterials, 28: 4947-4967. http://dx.doi.org/10.1016/j.biomaterials.2007.06.011 193266

Bellemare F; Rouette ME; Lorrain L; Perron E; Boucher N (2006). Combined use of photosynthetic enzyme complexes and microalgal photosynthetic systems for rapid screening of wastewater toxicity. Environ Toxicol, 21: 445-449. http://dx.doi.org/10.1002/tox.20205 157779

Benavides F; Oberyszyn TM; VanBuskirk AM; Reeve VE; Kusewitt DF (2009). The hairless mouse in skin research. J Dermatol Sci, 53: 10-18. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18938063. 193270

Bennat C; Muller-Goymann CC (2000). Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. Int J Cosmet Sci, 22: 271-283. 157403

Bennett I (2005, June). Recent developments in the physical characterisation of ultra fine particles. Talk presented at Environmental Nanoparticles - Exploring the links between Vehicle Emissions and Ambient Air: A meeting of the Automation and Analytical Management Group of the Royal Society of Chemistry in collaboration with the National Physical Laboratory, Birmingham, UK. 193820

Benson HA (2005). Transdermal drug delivery: Penetration enhancement techniques. Curr Drug Deliv, 2: 23-33. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16305405. 193273

Berges M (2007, August). Workplace exposure characterization at TiO2 nanoparticle production. Talk presented at 3rd International Symposium on Nanotechnology, Occupational and Environmental Health, Taipei. http://www.dguv.de/ifa/de/fac/nanopartikeln/taipei.pdf. 157594

Berges M (2008, June). Workplace exposure characterization at TiO2 nanoparticle production. Talk presented at 11th International Inhalation Symposium: Benefits and Risks of Inhaled Engineered Nanoparticles, Hanover, Germany. 193274

Bermudez E; Mangum JB; Asgharian B; Wong BA; Reverdy EE; Janszen DB; Hext PM; Warheit DB; Everitt JI (2002). Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol Sci, 70: 86-97. 055578

Bermudez E; Mangum JB; Wong BA; Asgharian B; Hext PM; Warheit DB; Everitt JI (2004). Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci, 77: 347-357. http://dx.doi.org/10.1093/toxsci/kfh019 056707

Bianco Prevot A; Vincenti M; Banciotto A; Pramauro E (1999). Photocatalytic and photolytic transformation of chloramben in aqueoussolutions. Appl Catal B-Environ, 22: 149-158. 193278

Bihari P; Vippola M; Schultes S; Praetner M; Khandoga AG; Reichel CA; Coester C; Tuomi T; Rehberg M; Krombach F (2008). Optimized dispersion of nanoparticles for biological in vitro and in vivo studies. Part Fibre Toxicol, 5: 14. http://dx.doi.org/10.1186/1743-8977-5-14 157593

Bird S (2002). Sense and stability. Soap, Perfum Cosmet, 75, 42-44. 093306

Blaise C; Gagne F; Ferard JF; Eullaffroy P (2008). Ecotoxicity of selected nano-materials to aquatic organisms. Environ Toxicol, 23: 591-598. http://dx.doi.org/10.1002/tox.20402 157592

Boffetta P; Gaborieau V; Nadon L; Parent MF; Weiderpass E; Siemiatycki J (2001). Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal. Scand J Work Environ Health, 27: 227-232. 157891

Boffetta P; Soutar A; Cherrie JW; Granath F; Andersen A; Anttila A; Blettner M; Gaborieau V; Klug SJ; Langard S; Luce D; Merletti F; Miller B; Mirabelli D; Pukkala E; Adami HO; Weiderpass E (2004). Mortality among workers employed in the titanium dioxide production industry in Europe. Cancer Causes Control, 15: 697-706. 157849

Boncagni NT; Otaegui JM; Warner E; Curran T; Ren J; de Cortalezzi MM (2009). Exchange of TiO2 nanoparticles between streams and streambeds. Environ Sci Tech, 43: 7699-7705. http://dx.doi.org/10.1021/es900424n 634454

Borm PJA; Hohr D; Steinfartz Y; Zeittrager I; Albrecht C (2000). Chronic inflammation and tumor formation in rats after intratracheal instillation of high doses of coal dust, titanium dioxides and quartz. Inhal Toxicol, 3: 225-231. 041486

Botta C; Labille J; Feng J; Rose J; Chaurand P; Auffan M; Garric J; Vollat B; Abbaci K; Noury P; Masion A; Bottero J-Y (2009). Physical-chemical characterization and ecotoxicity of residues from alteration of engineered nanomaterials. Geochim Cosmo Act, 73: A144. http://adsabs.harvard.edu/abs/2009GeCAS..73Q.144B. 625076

Boxall A; Chaudhry Q; Sinclair C; Jones A; Aitken R; Jefferson B; Watts C (2007). Current and future predicted environmental exposure to engineered nanoparticles. York, UK: Central Science Laboratory. 196111

Boxall AB; Tiede K; Chaudhry Q (2007). Engineered nanomaterials in soils and water: How do they behave and could they pose a risk to human health? Nanomed, 2: 919-927. 157712

Brand RM; Pike J; Wilson RM; Charron AR (2003). Sunscreens containing physical UV blockers can increase transdermal absorption of pesticides. Toxicol Ind Health, 19: 9-16. http://dx.doi.org/10.1191/0748233703th169oa 157866

Brausch JM; Cox S; Smith PN (2006). Pesticide usage on the Southern High Plains and acute toxicity of four chemicals to the fairy shrimp Thamnocephalus platyurus (Crustacea: Anostraca). Tex J Sci, 58: 309-324. 193296

Brausch JM; Smith PN (2009). Pesticide resistance from historical agricultural chemical exposure in Thamnocephalus platyurus (Crustacea: Anostraca). Environ Pollut, 157: 481-487. 193297

BSI (2007). Terminology for nanomaterials (Report No. PAS 136:2007). London, UK: British Standards Institution. 202162

Calabrese EJ; Baldwin LA (1998). Hormesis as a biological hypothesis. Environ Health Perspect, 106: 357-362. 047938

CCOHS (2006). Titanium dioxide classified as possibly carcinogenic to humans. Retrieved October 30, 2008 from http://www.ccohs.ca/headlines/text186.html. 157774

CDC (2000). Weight for age tables, infants, ages birth to 36 months selected percentiles. Retrieved October 22, 2008 from http://www.cdc.gov/growthcharts/html_charts/wtageinf.htm. 157982

CEN (1993). Workplace atmospheres-size fraction definitions for measurement of airborne particles (Report No. BS EN 481:1993). London, England: European Committee for Standardization (CEN), British Standards Institute. http://legacy.library.ucsf.edu:8080/i/e/m/iem52d00/Siem52d00.pdf. 078032

Chandler M (2006). Innovative UV Protection. Creating your advantage in sunscreen products. Talk presented at Croda Educational eSeminar, Edison, NJ. http://www.cosmeticsandtoiletries.com/networking/eventcoverage/3729901.html. 193834

Chaudhuri RK; Majewski G (1998). Amphiphilic microfine titanium dioxide: Its properties and application in sunscreen formulations. Drug Cosmet Ind, 162: 24-31. 093308

Chawengkijwanich C; Hayata Y (2007). Development of TiO2 powder-coated food packaging film and its ability to inactivate Escherichia coli in vitro and in actual tests. Int J Food Microbiol, 123: 288-292. 157708

Chen D; Ray AK (2001). Removal of toxic metal ions from wastewater by semiconductor photocatalysis. Chem Eng Sci, 56: 1561-1570. http://www.sciencedirect.com/science/article/B6TFK-42HFNY7-1G/2/21935a6ea4a8f93551b2770acc69dcf7. 193310

Chen H-W; Su S-F; Chien C-T; Lin W-H; Yu S-L; Chou C-C; Chen JJW; Yang P-C (2006). Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. FASEB J, 20: 2393-2395. 090139

Chen JL; Fayerweather WE (1988). Epidemiologic study of workers exposed to titanium dioxide. J Occup Med, 30: 937-42. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3230444. 193312

Chen X; Mao SS (2007). Titanium dioxide nanomaterials: Synthesis, properties, modifications, and applications. Chem Rev, 107: 2891-2959. 193313

Christian P; Von der Kammer F; Baalousha M; Hofmann T (2008). Nanoparticles: Structure, properties, preparation and behaviour in environmental media. Ecotoxicology, 17: 326-343. 157586

Cleasby JL; Logsdon GS (1999). Granular bed and precoat filtration. In R.D. Letterman (Ed.), Water quality and treatment: A handbook of community water supplies (pp. 8.1-8.99). New York, NY: McGraw-Hill. 091181

Cokkinides VE; Johnston-Davis K; Weinstock M; O'Connell MC; Kalsbeek W; Wingo PA (2001). Sun exposure and sun-protection behaviors and attitudes among U.S. youth, 11 to 18 years of age. Prev Med, 33: 141-151. 193321

Coleman HM; Marquis CP; Scott JA; Chin SS; Amal R (2005). Bactericidal effects of titanium dioxide-based photocatalysts. Chem Eng J, 113: 55-63. 089849

Collier DE; Brown SA; Blagojevic N; Soldenhoff KH; Ring RJ (2001). Thorium in Mineral Products. Radiat Prot Dosimetry, 97(2): 177-180. 155617

Comparative study on cosmetics legislation in the EU and other principal markets with special attention to so-called borderline products. (2004). Norfolk, U.K.: Risk and Policy Analysts Limited. http://ec.europa.eu/enterprise/newsroom/cf/itemshortdetail.cfm?item_id=3620. 157826

Contado C; Pagnoni A (2008). TiO2 in commercial sunscreen lotion: Flow field-flow fractionation and ICP-AES together for size analysis. Anal Chem, 80: 7594-608. http://dx.doi.org/10.1021/ac8012626 157585

Conti JA; Killpack K; Gerritzen G; Huang L; Mircheva M; Delmas M; Harthorn BH; Appelbaum RP; Holden PA (2008). Health and Safety Practices in the Nanomaterials Workplace: Results from an International Survey. Environ Sci Technol, 42: 3155-3162. 155619

Costa M; Silva A; Levizzani V (2004). Aerosol characterization and direct radiative forcing assessment over the ocean. Part I: Methodology and sensitivity analysis. J Appl Meteorol, 43: 1799-1817. 190006

Crane M; Handy RD; Garrod J; Owen R (2008). Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. Ecotoxicology, 17: 421-437. 157583

Creutzenberg O; Bellmann B; Heinrich U; Fuhst R; Koch W; Muhle H (1990). Clearance and retention of inhaled diesel exhaust particles, carbon black, and titanium dioxide in rats at lung overload conditions. J Aerosol Sci, 21: S455-S458. 157963

Croda (2006). Croda invests in growth: Acquires Uniqema [brochure]. Cosmetics & Toiletries. 193851

Croda (2007). Product Trade Name: SOLAVEIL CT-100 [brochure]. 193875

Croda (2008). Chemical Composition of Solaveil CT-200 [brochure]. 193878

Croda (2009). Weightless Morning Dew with Sun Protection (SC-383-1) [brochure]. 193880

Cullen RT; Tran CL; Buchanan D; Davis JMG; Jones AD; Searl A; Donaldson K (1999). Investigations into the pulmonary effects of low toxicity dusts Part I: Relative toxicological potency of dusts+. Sudbury, UK: HSE Books. 157905

Dankovic D; Kuempel E; Wheeler M (2007). An approach to risk assessment for TiO2. Inhal Toxicol, 19 Suppl 1: 205-212. 157704

Davis DA (1993). Defining the sunscreen market. Drug Cosmet Ind, 153: 28-30. 157949

Davis DA (1994). Sunscreen oddities. Drug Cosmet Ind, 155: 20-24. 157946

Davis JM (2007). How to assess the risks of nanotechnology: Learning from past experience. J Nanosci Nanotechnol, 7: 402-409. 089803

Davis JM; Svendsgaard DJ (1990). U-shaped dose-response curves: Their occurrence and implications for risk assessment. J Toxicol Environ Health, 30: 71-83. http://dx.doi.org/10.1080/15287399009531412 048278

Davis JM; Thomas VM (2006). Systematic approach to evaluating trade-offs among fuel options: The lessons of MTBE. Ann N Y Acad Sci, 1076: 498-515. 089638

Degussa (2003). Titanium dioxide P25: Manufacture, properties, applications (Report No. Technical Bulletin Fine Particles Number 80). Germany: Degussa AG. 193916

Degussa (2007). Aeroxide® TiO2 P 25: Hydrophilic fumed titanium dioxide: Product information. Retrieved April 24, 2007 from http://www.aerosil.com. 090576

Degussa (2007). Degussa Science Newsletter. Retrieved April 24, 2007 from http://www.degussa.com/NR/rdonlyres/6783C90B-0F91-4BC3-BFA4-CE6B44FED1AB/0/elements_08_en.pdf. 193917

Degussa (2009). Degussa nanotechnology: Safe production. Retrieved June 02, 2009 from http://www.degussa-nano.com/nano/en/sustainability/safeproduction/. 193919

DeLorenzo AJD (1970). The olfactory neuron and the blood-brain barrier. In GEW Wolstenholme; J Knight (Eds.), Taste and Smell in Vertebrates: A Ciba Foundation symposium (pp. 151-175). London: Churchill Livingstone. 156391

Delrieu P; Shao Y; Schlossman D (2007). Particle size measurement of attenuation grade titanium dioxide in dispersion and in sunscreen lotion [brochure]. South Plainfield, NJ: Kobo Products Inc. 157449

Demirbilek Z; Smith J; Zundel A; Jones R; MacDonald N; Davies M (2005). Particle Tracking Model (PTM) in the SMS: III. Tutorial with examples [brochure]. Vicksburg, MS: U.S. Army Engineer Research and Development Center. 193887

Department for Environment, Food and Rural Affairs (2007). Characterising the potential risks posed by engineered nanoparticles. A second UK government research report. Retrieved from: http://www.defra.gov.uk/environment/quality/nanotech/reports.htm#risks. 195461

Diebold U (2003). The surface science of titanium dioxide. Surf Sci Rep, 48: 53-229. 193342

DIN (1989). 38412-30. German standard methods for the examination of water, waste water and sludge; bio-assays (group L); determining the tolerance of Daphnia to the toxicity of waste water by way of a dilution series (L 30). 667416

DIN (1991). 38412-33. German standard methods for the examination of water, waste water and sludge; bio-assays (group L); determining the tolerance of green algae to the toxicity of waste water (Scenedesmus chlorophyll fluorescence test) by way of dilution series (L 33). 667415

Domingos RF; Baalousha MA; Ju-Nam Y; Reid MM; Tufenkji N; Lead JR; Leppard GG; Wilkinson KJ (2009). Characterizing manufactured nanoparticles in the environment: Multimethod determination of particle sizes. Environ Sci Technol, 43: 7277-7284. http://dx.doi.org/10.1021/es900249m 193346

Domingos RF; Tufenkji N; Wilkinson KJ (2009). Aggregation of titanium dioxide nanoparticles: Role of a fulvic acid. Environ Sci Technol, 43: 1282-1286. http://dx.doi.org/10.1021/es8023594 193347

Dransfield G (2005). Manufacture of novel, transparent TiO2 based sunscreens. Retrieved May 09, 2008 from http://www.wun.ac.uk/nanomanufacturing/archive/05_06_series/documents/dransfield.pdf. 157809

Driscoll KE; Costa DL; Hatch G; Henderson R; Oberdorster G; Salem H; Schlesinger RB (2000). Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: Uses and limitations. Toxicol Sci, 55: 24-35. 011376

Driscoll KE; Deyo LC; Carter JM; Howard BW; Hassenbein DG; Bertram TA (1997). Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis, 18: 423-430. 053253

Driscoll KE; Lindenschmidt RC; Maurer JK; Higgins JM; Ridder G (1990). Pulmonary response to silica or titanium dioxide: Inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. Am J Respir Cell Mol Biol, 2: 381-390. 087145

Dunford R; Salinaro A; Cai L; Serpone N; Horikoshi S; Hidaka H; Knowland J (1997). Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Lett, 418: 87-90. http://dx.doi.org/10.1016/S0014-5793(97)01356-2 157929

Dunphy Guzman KA; Finnegan MP; Banfield JF (2006). Influence of surface potential on aggregation and transport of titania nanoparticles. Environ Sci Technol, 40: 7688-7693. 090584

DuPont (2007). Nanomaterial Risk Assessment Worksheet DuPont(TM) Light Stabilizer. Retrieved June 18, 2008 from http://www.edf.org/documents/6913_TiO2_Worksheet.pdf. 157699

Dussert AS; Gooris E (1997). Characterisation of the mineral content of a physical sunscreen emulsion and its distribution onto human stratum corneum. Int J Cosmet Sci, 19: 119-129. 193359

Dutta PK; Ray AK; Sharma VK; Millero FJ (2004). Adsorption of arsenate and arsenite on titanium dioxide suspensions. J Colloid Interface Sci, 278: 270-5. 157845

Effects of nanoparticles on the wastewater treatment industry (Report No. P08002E). (2008). Alexandria, VA: Water Environment Federation. http://www.e-wef.org/timssnet/products/tnt_products.cfm?primary_id=P08002E&Action=LONG&subsystem=ORD. 195800

Elder A; Gelein R; Silva V; Feikert T; Opanashuk L; Carter J; Potter R; Maynard A; Ito Y; Finkelstein J; Oberdorster G (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect, 114: 1172-1178. http://dx.doi.org/10.1289/ehp.9030 089253

Emerich DF; Thanos CG (2007). Targeted nanoparticle-based drug delivery and diagnosis. J Drug Target, 15: 163-183. http://dx.doi.org/10.1080/10611860701231810 193365

Englert BC (2007). Nanomaterials and the environment: Uses, methods and measurement. J Environ Monit, 9: 1154-61. http://dx.doi.org/10.1039/b705988d 193367

Environmental Defense-DuPont Nano Partnership (2007). Nano risk framework. New York: Environmental Defense Fund. http://www.environmentaldefense.org/go/nano. 090565

Environment Canada (2007). The freshwater hydra (Hydra attenuata): Useful in ecotoxicology. Retrieved October 05, 2009 from http://www.ec.gc.ca/stl/default.asp?lang=En&n=91EAEB1F-1. 157697

European Commission (2008). Follow-up to the 6th meeting of the REACH competent authorities for the implementation of Regulation (EC) 1907/2006 (REACH) (Report No. CA/59/2008 rev. 1). Brussels: European Commission. http://ec.europa.eu/environment/chemicals/reach/pdf/nanomaterials.pdf. 196378

European Chemicals Bureau (2003). Technical guidance document on risk assessment (Report No. EUR 20418 EN/1). Italy: European Commission; Institute for Health and Consumer Protection. http://ecb.jrc.it/documents/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart2_2ed.pdf. 196375

Evonik (2007). Aerodisp® W740X: Aqueous dispersion of hydrophilic fumed titanium dioxide. Retrieved August 19, 2008 from http://www.aerosil.com/product/aerosil/en/Pages/default.aspx#7_I_3EN. 157577

Evonik (2007). AEROXIDE® TiO2 T 805. Retrieved October 05, 2009 from http://www.aerosil.com/wps/portal/p9/kcxml/bcnRCoIwGEDhZ-kB5LcyzcvpVqlUI9I2b2RYDCm3ylXm02d0EUR3h-9ADhxyJe6VFKbSSpyAQe4WUTGa-O_1SRf4jw0p_mPh15YLXR_6w70exmQFW2CzUrdYI4kRRqarHKM962EfWeoSHYQyCGLr3qmD4wh-lNgO57O4jEh9U1VDbN5eG79r1_2lGXKM7zcZ9XY4UhezNzpY7dv1lZZit0hcGTOixQQ_C5rExIINJEDhXKcpewynCA0GL9NArLk!/delta/base64xml/L0lDVE83b0pKN3VhQ1NZWmlncFJBL29Ob2dBRUlRaENFTVloQ0dJUUlTRkNJQWdBR0VRQkFBY0Z3VXNJQWdIQSEhLzRCMWljb25RVndHeE9VVG9LNzlZUTdEbUc0UkEvN19JXzNFTi84L2phdmF4LnNlcnZsZXQuaW5jbHVkZS5wYXRoX2luZm8vJTBncm91cFNlYXJjaC5qc3A!#7_I_3EN. 157696

Evonik (2008). AEROXIDE® TiO2 P 25. Retrieved July 07, 2009 from http://www.aerosil.com/product/aerosil/en/Pages/default.aspx#7_I_3EN. 157578

EWG (2008). Sunscreen investigation: Skin deep-cosmetic safety reviews. Retrieved June 02, 2009 from http://www.cosmeticsdatabase.com/special/sunscreens2008/. 196343

EWG (2009). 2009 sunscreen guide. Retrieved October 07, 2009 from http://www.ewg.org/whichsunscreensarebest/2009report. 196367

EWG (2009). EWG's 2009 sunscreen investigation - Section 5: Impact of nanoparticles. Retrieved April 29, 2010 from http://www.ewg.org/cosmetics/report/sunscreen09/investigation/impact-of-nanoparticles. 625314

Fabian E; Landsiedel R; Ma-Hock L; Wiench K;W Wohlleben W; van Ravenzwaay B (2008). Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. Arch Toxicol, 82: 151-157. 157576

Fairhurst D; Mitchnick M (1997). Particulate sun blocks: General principles. In Lowe NJ; Shaath NA; Pathak MA (Eds.), Sunscreens: Development, evaluation, and regulatory aspects (pp. 313-352). New York: Marcel Dekker. 196248

Fairley JA; Rasmussen JE (1983). Comparison of stratum corneum thickness in children and adults. J Am Acad Dermatol, 8: 652-654. http://www.ncbi.nlm.nih.gov/pubmed/6863621. 193370

Fang J; Shan X-q; Wen B; Lin J-M; Owens G (2009). Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. Environ Pollut, 157: 1101-1109. http://dx.doi.org/10.1016/j.envpol.2008.11.006 193371

Faux SP; Tran CL; Miller BG; Jones AD; Monteiller C; Donaldson K (2003). In vitro determinants of particulate toxicity: The dose-metric for poorly soluble dusts (Report No. 154). United Kingdom: Institute of Occupational Medicine. http://www.hse.gov.uk/research/rrpdf/rr154.pdf. 625074

Federici G; Shaw BJ; Handy RD (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol, 84: 415-430. 091222

Feng Z; Xia Y; Tian D; Wu K; Schmitt M; Kwok RK; Mumford JL (2001). DNA damage in buccal epithelial cells from individuals chronically exposed to arsenic via drinking water in Inner Mongolia, China. Anticancer Res, 21: 51-58. 193374

Fenoglio I; Greco G; Livraghi S; Fubini B (2009). Non-UV-induced radical reactions at the surface of TiO2 nanoparticles that may trigger toxic responses. Chemistry, 15: 4614-4621. http://dx.doi.org/10.1002/chem.200802542 180383

Fent K; Kunz PY; Gomez E (2008). UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. Chimia, 62: 368-375. http://dx.doi.org/10.2533/chimia.2008.368 157574

Ferguson MA; Hoffman MR; Hering JG (2005). TiO2-photocatalysed As(III) oxidation in aqueous suspensions: reaction kinetics and effects of adsorption. Environ Sci Technol, 39: 1880-1886. http://dx.doi.org/10.1021/es048795n 090572

Finnegan MP; Zhang H; Banfield JF (2007). Phase stability and transformation in titania nanoparticles in aqueous solutions dominated by surface energy. J Phys Chem C, 111: 1962-1968. http://dx.doi.org/10.1021/jp063822c 193379

Fond AM; Meyer GJ (2006). Biotoxicity of metal oxide nanoparticles. In C Kumar (Ed.), Nanomaterials: Toxicity, health and environmental issues (pp. 3-34). Weinheim, Germany: Wiley-VCH. 196337

Fostier AH; Pereira MDS; Rath S; Guimaraes JR (2008). Arsenic removal from water employing heterogeneous photocatalysis with TiO2 immobilized in PET bottles. Chemosphere, 72: 319-324. 193381

French RA; Jacobson AR; Kim B; Isley SL; Penn RL; Baveye PC (2009). Influence of ionic strength, pH, and cation valence on aggregation kinetics of titanium dioxide nanoparticles. Environ Sci Technol, 43: 1354-1359. http://dx.doi.org/10.1021/es802628n 193384

Fryzek JP; Chadda B; Marano D; White K; Schweitzer S; McLaughlin JK; Blot WJ (2003). A cohort mortality study among titanium dioxide manufacturing workers in the United States. J Occup Environ Med, 45: 400-409. http://dx.doi.org/10.1097/01.jom.0000058338.05741.45 157864

Gabrielson J; Kühn I; Colque-Navarro P; Hart M; Iversen A; McKenzie D; Möllby R (2003). Microplate-based microbial assay for risk assessment and (eco)toxic fingerprinting of chemicals [Errata]. Anal Chim Acta, 488: 133. 157862

Gabrielson J; Kühn I; Colque-Navarro P; Hart M; Iversen A; McKenzie D; Möllby R (2003). Microplate-based microbial assay for risk assessment and (eco)toxic fingerprinting of chemicals. Anal Chim Acta, 485: 121-130. 157863

Gallagher J; Heinrich U; George M; Hendee L; Phillips DH; Lewtas J (1994). Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. Carcinogenesis, 15: 1291-1299. 045102

Gambogi J (2008). Titanium mineral concentrates. In Mineral Commodity Summaries 2008 (pp. 178-179). Washington, DC: U.S. Geological Survey; U.S. Department of the Interior. http://minerals.usgs.gov/minerals/pubs/mcs/2008/mcs2008.pdf. 155622

Gamer AO; Leibold E; Van Ravenzwaay B (2006). The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin. Toxicol In Vitro, 20: 301-307. 090588

Gebhart J (1992). To the relevant diameter of aerosol particles in the 0.1 to 1 ?M transition range. J Aerosol Sci, 23: S305-S308. 157951

Geiser M; Rothen-Rutishauser B; Kapp N; Schurch S; Kreyling W; Schulz H; Semmler M; Im Hof V; Heyder J; Gehr P (2005). Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect, 113: 1555-1560. 087362

Geller AC; Colditz G; Oliveria S; Emmons K; Jorgensen C; Aweh GN; Frazier AL (2002). Use of sunscreen, sunburning rates, and tanning bed use among more than 10,000 U.S. children and adolescents. Pediatrics, 109: 1009-1014. 193390

Gerritzen MA; Lambooij E; Stegeman JA; Spruijt BM (2006). Slaughter of poultry during the epidemic of avian influenza in the Netherlands in 2003. Vet Rec, 159: 39-42. 097620

Golanski L; A. Guiot; Tardif F (2008). Experimental evaluation of individual protection devices against different types of nanoaerosols: graphite, TiO2 and Pt. Retrieved March 22, 2009 from http://www.nanosafe2008.org/home/liblocal/docs/Oral%20presentations/O6-1_Golanski.pdf. 196048

Gontier E; Habchi C; Pouthier T; Aguer P; Barberet P; Barbotteau Y; Incerti S; Ynsa MD; Surleve-Bazeille JE; Moretto P (2004). Nuclear microscopy and electron microscopy studies of percutaneous penetration of nanoparticles in mammalian skin [Abstract]. J Invest Dermatol, 123: A11. 193398

Gonzalez L; Lison D; Kirsch-Volders M (2008). Genotoxicity of engineered nanomaterials: A critical review. Nanotoxicology, 2: 252 - 273. 157569

Gopee NV; Cozart C; Siitonen PH; Smith CS; Walker NJ; Howard PC (2009, March). Lack of dermal penetration following topical application of uncoated nano-sized titanium dioxide to intact and dermabraded skin in mice. Poster presented at Society of Toxicology 48th Annual Meeting, Baltimore, Maryland. 193399

Gopee NV; Roberts DW; Webb P; Cozart CR; Siitonen PH; Latendresse JR; Warbitton AR; Yu WW; Colvin VL; Walker NJ; Howard PC (2009). Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. Toxicol Sci, 11: 37-48. http://dx.doi.org/10.1093/toxsci/kfp139 667592

Gottbrath S; Mueller-Goymann CC (2003). Penetration and visualization of titanium dioxide microparticles in human stratum corneum - Effect of different formulations on the penetration of titanium dioxide. SOFW J, 129: 11-17. 193401

Gottschalk F; Sonderer T; Scholz RW; Nowack B (2009). Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for different regions. Environ Sci Tech, 43: 9216-9222. http://dx.doi.org/10.1021/es9015553 633897

Gottschalk F; Sonderer T; Scholz RW; Nowack B (2010). Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. Environ Toxicol Chem, 29: 1036-1048. http://dx.doi.org/10.1002/etc.135 635674

Grassian VH; Adamcakoca-Dodd A; Pettibone JM; O'Shaughnessy PI; Thorne PS (2007). Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes. Nanotoxicology, 1: 211-226. 093170

Grassian VH; O'Shaughnessy PT; Adamcakova-Dodd A; Pettibone JM; Thorne PS (2007). Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ Health Perspect, 115: 397-402. 090606

Griffitt RJ; Luo J; Gao J; Bonzongo JC; Barber DS (2008). Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem, 27: 1972-1978. 157565

Guizard B; Tenegal F (2008). Liquid Recovery of TiO2 nanoparticles synthesized by laser pyrolysis. Retrieved March 30, 2009 from http://www.nanosafe2008.org/home/liblocal/docs/Oral%20presentations/O6-4_Guizard.pdf. 196049

Haigh JM; Smith EW (1994). The selection and use of natural and synthetic membranes for in-vitro diffusion experiments. Eur J Pharm Sci, 2: 311-330. http://dx.doi.org/10.1016/0928-0987(94)90032-9 625322

Han F; Kambala VSR; Srinivasan M; Rajarathnam D; Naidu R (2009). Tailored titanium dioxide photocatalysts for the degradation of organic dyes in wastewater treatment: A review. Appl Catal A-Gen, 359: 25-40. http://www.sciencedirect.com/science/article/B6TF5-4VTCM60-2/2/d667b7a884153da9fd5cc5b7b474f67f. 193407

Handy R; Henry T; Scown T; Johnston B; Tyler C (2008). Manufactured nanoparticles: Their uptake and effects on fish. A mechanistic analysis. Ecotoxicology, 17: 396-409. 157563

Handy RD; Owen R; Valsami-Jones E (2008). The ecotoxicology of nanoparticles and nanomaterials: Current status, knowledge gaps, challenges, and future needs. Ecotoxicology, 17: 315-325. 157562

Hansen SF; Michelson ES; Kamper A; Borling P; Stuer-Lauridsen F; Baun A (2008). Categorization framework to aid exposure assessment of nanomaterials in consumer products. Ecotoxicology, 17: 438-447. 157560

Hansen T; Clermont G; Alves A; Eloy R; Brochhausen C; Boutrand JP; Gatti AM; Kirkpatrick CJ (2006). Biological tolerance of different materials in bulk and nanoparticulate form in a rat model: sarcoma development by nanoparticles. J R Soc Interface, 3: 767-775. 090611

Harbour JR; Tromp J; Hair ML (1985). Photogeneration of hydrogen peroxide in aqueous TiO2 dispersions. Can J Chem, 63: 204-208. 090632

Harford AJ; Edwards JW; Priestly BG; Wright PFA (2007). Current OHS Best Practices for the Australian nanotechnology industry: A position paper by the NanoSafe Australia Network. Victoria, Australia: NanoSafe Australia Network. http://mams.rmit.edu.au/72nuxiavskpg.pdf. 196051

Haridasan PP; Pillai PMB; Tripathi RM; Puranik VD (2008). Thorium in ilmenite and its radiological implications in the production of titanium dioxide. Radiat Prot Dosimetry, 129: 381-385. http://dx.doi.org/10.1093/rpd/ncm446 155625

Hartmann NB; Von der Kammer F; Hofmann T; Baalousha M; Ottofuelling S; Baun A (2010). Algal testing of titanium dioxide nanoparticles—Testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology, 269: 190-197. http://dx.doi.org/10.1016/j.tox.2009.08.008 196322

Hassellöv M; Readman JW; Ranville JF; Tiede K (2008). Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Ecotoxicology, 17: 344-361. http://dx.doi.org/10.1007/s10646-008-0225-x 157559

Heinlaan M; Ivask A; Blinova I; Dubourguier HC; Kahru A (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. Chemosphere, 71: 1308-1316. http://dx.doi.org/10.1016/j.chemosphere.2007.11.047 193414

Heinrich U; Fuhst R; Rittinghausen S; Creutzenberg O; Bellmann B; Koch W; Levsen K (1995). Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol, 7: 533-556. 076637

Henderson RF; Driscoll KE; Harkema JR; Lindenschmidt RC; Chang I-Y; Maples KR; Barr EB (1995). A comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. Toxicol Sci, 24: 183-197. http://dx.doi.org/10.1093/toxsci/24.2.183 002744

Henry TB; Menn FM; Fleming JT; Wilgus J; Compton RN; Sayler GS (2007). Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. Environ Health Perspect, 115: 1059-65. 157684

Hewitt J (2002). A moment of clarity. Soap, Perfum Cosmet, 75, 47-50. 093307

Hewitt J (2009). Public Comment submitted by Julian P. Hewitt, Croda Europe Ltd; Docket ID: EPA-HQ-ORD-2009-0495-0002. Retrieved September 11, 2009 from http://www.regulations.gov/search/Regs/home.html#documentDetail?R=0900006480a1e72f. 625212

Hewitt JP (1995). Formulating with physical sunscreens: Control of emulsion pH. Drug Cosmet Ind, 157: 28-32. 157939

Hewitt JP (1996). The influence of emollients on dispersion of physical sunscreens. Drug Cosmet Ind, 159: 62-65. 157936

Hewitt JP (2000). Partners in protection. Soap, Perfum Cosmet, 73, 85-86. 157898

Hext PM; Tomenson JA; Thompson P (2005). Titanium dioxide: inhalation toxicology and epidemiology. Ann Occup Hyg, 49: 461-472. 090567

Hext PM; Warheit DB; Mangum J; Asgharian B; Wong B; Bermudez E; Everitt J (2002). Comparison of the pulmonary responses to inhaled pigmentary and ultrafine titanium dioxide particles in the rat, mouse and hamster. Ann Occup Hyg, 46: 191-196. 157878

Heyder J; Gebhart J; Scheuch G (1985). Interaction of diffusional and gravitational particle transport in aerosols. Aerosol Sci Technol, 4: 315-326. 006919

Hidaka H; Kobayshi H; Kuga M; Koike T (2005). Photoinduced characteristics of metal-oxide cosmetic pigments by agarose gel electrophoresis of DNA plasmids in vitro under UV-illumination. J Oleo Sci, 54: 487-494. 157804

Himics R; Pineiro R (2008). The Importance of Particle Size in Liquid Coatings - Coating problems and solutions associated with particle size reduction. Prod Finish, 63: 46-53. http://www.pfonline.com/articles/the-importance-of-particle-size-in-liquid-coatings. 155626

Holbrook RD; Murphy KE; Morrow JB; Cole KD (2008). Trophic transfer of nanoparticles in a simplified invertebrate food web. Nat Nanotechnol, 3: 352-355. http://dx.doi.org/10.1038/nnano.2008.110 192383

Holsapple MP; Lehman-McKeeman LD (2005). Forum series: research strategies for safety evaluation of nanomaterials. Toxicol Sci, 87: 315. 088087

Horie M; Nishio K; Fujita K; Endoh S; Miyauchi A; Saito Y; Iwahashi H; Yamamoto K; Murayama H; Nakano H; Nanashima N; Niki E; Yoshida Y (2009). Protein adsorption of ultrafine metal oxide and its influence on cytotoxicity toward cultured cells. Chem Res Toxicol, 22: 543-553. http://dx.doi.org/10.1021/tx800289z 193433

Hostynek JJ (2003). Factors determining percutaneous metal absorption. Food Chem Toxicol, 41: 327-345. http://dx.doi.org/10.1016/S0278-6915(02)00257-0 193435

Hoyt M (2009). Analysis of nanoparticles in the environment. In Sellers K; Mackay C; Bergeson LL; Clough SR (Eds.), Nanotechnology and the Environment (pp. 99-122). Boca Raton: CRC Press. 633900

Hristovski K; Baumgardner A; Westerhoff P (2007). Selecting metal oxide nanomaterials for arsenic removal in fixed bed columns: From nanopowders to aggregated nanoparticle media. J Hazard Mater, 147: 265-274. http://www.sciencedirect.com/science/article/B6TGF-4MSHY3F-1/2/32e33288255e28bc90232dc38b91106c. 193436

Hsu LY; Chein HM (2007). Evaluation of nanoparticle emission for TiO2 nanopowder coating materials. J Nanopart Res, 9: 157-163. http://dx.doi.org/10.1007/s11051-006-9185-3 193437

Huang CP; Cha DK; Ismat SS (2005). 2005 Progress report: Short-term chronic toxicity of photocatalytic nanoparticles to bacteria, algae, and zooplankton. Retrieved May 15, 2009 from http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/7384/report/2005. 157801

Hund-Rinke K; Simon M (2006). Ecotoxic effect of photocatalytic active nanoparticles (TiO2) on algae and daphnids. Environ Sci Pollut Res Int, 13: 225-232. http://dx.doi.org/10.1065/espr2006.06.311 090607

Huntsman (2008). Tioxide R-HD2. Retrieved September 08, 2008 from https://www.huntsmanservice.com/Product_Finder/ui/PSDetailProductList.do?PCId=4893. 157555

IARC (2010). Titanium dioxide. In Carbon black, titanium dioxide and talc (pp. 193-276). Lyon, France: International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Monographs/vol93/index.php. 157762

Illum L (2000). Transport of drugs from the nasal cavity to the central nervous system. Eur J Pharm Sci, 11: 1-18. http://dx.doi.org/10.1016/S0928-0987(00)00087-7 157897

International Council on Nanotechnology (2010). NanoEHS virtual journal [database]. http://icon.rice.edu/virtualjournal.cfm 644440

ISLI Risk Science Institute Workshop Participants (2000). The relevance of the rat lung response to particle overload for human risk assessment: A workshop consensus report. Inhal Toxicol, 12: 1-17. 002892

ISO (1996). Water quality: Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxiciy test (Report No. ISO 6341). Geneva, Switzerland: International Organization for Standardization. http://www.iso.org/iso/catalogue_detail.htm?csnumber=21923. 667232

ISO (2004). Water Quality - Freshwater algal growth inhibition test with unicellular green algae (Report No. ISO 8692). Geneva, Switzerland: International Organization for Standardization. http://www.iso.org/iso/catalogue_detail.htm?csnumber=29924. 667212

Jeffries N (2007, February). SPF, efficacy and innovation. GCI Magazine. http://www.gcimagazine.com/marketstrends/segments/suncare/27627099.html. 157682

Jemec A; Drobne D; Remskar M; Sepcic K; Tisler T (2008). Effects of ingested nano-sized titanium dioxide on terrestrial isopods Porcellio scaber. Environ Toxicol Chem, 27: 1904-1914. 157552

Jeng HA; Swanson J (2006). Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Health A Tox Hazard Subst Environ Eng, 41: 2699-2711. 090085

Jiang J; Oberdrster G; Elder A; Gelein R; Mercer P; Biswas P (2008). Does nanoparticle activity depend upon size and crystal phase? Nanotoxicology, 2: 33-42. http://dx.doi.org/10.1080/17435390701882478 156609

Jiang JK; Oberdorster G; Biswas P (2009). Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J Nanopart Res, 11: 77-89. 193450

Jing C; Liu S; Patel M (2005). Arsenic leachability in water treatment adsorbents. Environ Sci Technol, 39: 5481-5487. http://dx.doi.org/10.1021/es050290p 193452

Jing C; Meng X; Calvache E; Jiang G (2009). Remediation of organic and inorganic arsenic contaminated groundwater using a nanocrystalline TiO2-based adsorbant. Environ Pollut, 157: 2514-2519. 193453

Jing C; Meng X; Liu S; Baidas S; Patraju R; Christodoulator C; Korfiatis GP (2005). Surface complexation of organic arsenic on nanocrystalline titanium oxide. J Colloid Interface Sci, 290: 14-21. http://dx.doi.org/10.1016/j.jcis.2005.04.019 193454

Johnson A; Bowes M; Crossley A; H Jarvie; Jürgens M; Lawlor A; Lofts S; Park B; Rowlands P; Singer A; Spurgeon D; Svendsen C; Thompson I; Williams R (2009, May). Should we be concerned about the discharge of sunscreen nano TiO2 to the environment? Poster presented at SETAC Europe 19th Annual Meeting, Göteborg, Sweden. 644432

Johnson R (2005). Relative size of several biological contaminants with the pore size of some common filters. Retrieved July 05, 2008 from http://www.cyber-nook.com/water/Solutions.html#pores. 157799

Kaegi R; Ulrich A; Sinnet B; Vonbank R; Wichser A; Zuleeg S; Simmler H; Brunner S; Vonmont H; Burkhardt M; Boller M (2008). Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. Environ Pollut, 156: 233-239. http://www.ncbi.nlm.nih.gov/pubmed/=18824285. 193457

Kalia YN; Nonato LB; Lund CH; Guy RH (1998). Development of skin barrier function in premature infants. J Invest Dermatol, 111: 320-326. http://dx.doi.org/10.1046/j.1523-1747.1998.00289.x 196039

Kandlikar M; Ramachandran G; Maynard A; Murdock B; Toscano WA (2007). Health risk assessment for nanoparticles: A case for using expert judgment. J Nanopart Res, 9: 137-156. 091626

Kapp N; Kreyling W; Schulz H; Im Hof V; Gehr P; Semmler M; Geiser M (2004). Electron energy loss spectroscopy for analysis of inhaled ultrafine particles in rat lungs. Microsc Res Tech, 63: 298-305. http://dx.doi.org/10.1002/jemt.20044 156624

Kasparian NA; McLoone JK; Meiser B (2009). Skin cancer-related prevention and screening behaviors: a review of the literature. J Behav Educ, 32: 406-428. 193465

Keeney S; McKenna H; Fleming P; McIlfatrick S (2009). Attitudes, knowledge and behaviours with regard to skin cancer: a literature review. Eur J Oncol Nurs, 13: 29-35. 193466

Keller CA; Frost A; Cagle PT; Abraham JL (1995). Pulmonary alveolar proteinosis in a painter with elevated pulmonary concentrations of titanium. Chest, 108: 277-280. http://dx.doi.org/10.1378/chest.108.1.277 157938

Kemira (2000). UV-Titan M160 product data sheet. Retrieved May 15, 2008 from http://www.kemira.com/NR/rdonlyres/A2D96838-D712-4276-AD28-6AF720835003/0/M160_e.pdf. 157896

Kertesz Z; Szikszai Z; Gontier E; Moretto P; Surleve-Bazeiille JE; Kiss B; Juhasz I; Hunyadi J; Kiss AZ (2005). Nuclear microprobe study of TiO2-penetration in the epidermis of human skin xenografts. Nucl Instrum Methods Phys Res B, 231: 280-285. http://dx.doi.org/10.1016/j.nimb.2005.01.071 180334

Khataee AR; Vatanpour V; Ghadim ARA (2009). Decolorization of CI Acid Blue 9 solution by UV/Nano-TiO2, Fenton, Fenton-like, electro-Fenton and electrocoagulation processes: A comparative study. J Hazard Mater, 161: 1225-1233. http://dx.doi.org/10.1016/j.jhazmat.2008.04.075 193468

Kim CS; Bao L; Okuyama K; Shimada M; Niinuma H (2006). Filtration efficiency of a fibrous filter for nanoparticles. J Nanopart Res, 8: 215-221. 193470

Kim J; Shan W; Davies SHR; Baumann MJ; Masten SJ; Tarabara VV (2009). Interactions of aqueous NOM with nanoscale TiO2: Implications for ceramic membrane filtration-ozonation hybrid process. Environ Sci Technol, 43: 5488–5494. http://dx.doi.org/10.1021/es900342q 635778

Kim M-S; Hong K-M; Chung JG (2003). Removal of Cu(II) from aqueous solutions by adsorption process with anatase-type titanium dioxide. Water Res, 37: 3524-3529. http://www.sciencedirect.com/science/article/B6V73-48XBK1V-F/2/9d950217ff1af7c663f9396bf59cfe49. 193472

Kim SJ; Lee HG; Kim SJ; Lee JK; Lee EG (2003). Photoredox properties of ultrafine rutile TiO2 acicular powder in aqueous 4-chlorophenol, Cu-EDTA and Pb-EDTA solutions. Appl Catal A-Gen, 242: 89-99. 157861

Kiser MA; Ryu H; Jang H; Hristovski K; Westerhoff P (2010). Biosorption of nanoparticles to heterotrophic wastewater biomass. Water Res, 44: 4105-4114. http://dx.doi.org/10.1016/j.watres.2010.05.036 634458

Kiser MA; Westerhoff P; Benn T; Wang Y; Perez-Rivera J; Hristovski K (2009). Titanium nanomaterial removal and release from wastewater treatment plants. Environ Sci Tech, 43: 6757-6763. http://dx.doi.org/10.1021/es901102n 225305

Kiss B; Bíró T; Czifra G; Tóth BI; Kertész Z; Szikszai Z; Kiss AZ; Juhász I; Zouboulis CC; Hunyadi J (2008). Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Exp Dermatol, 17: 659-67. http://dx.doi.org/10.1111/j.1600-0625.2007.00683.x 157547

Kobo Products Inc (2009). Attenuation grade TiO2 dispersions. Retrieved July 16, 2009 from http://www.koboproductsinc.com/Downloads/Kobo-TiO2Dispersions.pdf. 196045

Kolár M; Mest'ánková H; Jirkovský J; Heyrovský M; Subrt J (2006). Some aspects of physico-chemical properties of TiO2 nanocolloids with respect to their age, size, and structure. Langmuir, 22: 598-604. 193478

Kollias L (2009). Public Comment submitted by Louis Kollias, Director, Monitoring and Research, Metropolitan Water Reclamation District of Greater Chicago Docket ID: EPA-HQ-ORD-2009-0495-0010. Retrieved September 23, 2009 from http://www.regulations.gov/search/Regs/home.html#documentDetail?R=0900006480a28dc7. 624994

Komaguchi K; Nakano H; Araki A; Harima Y (2006). Photoinduced electron transfer from anatase to rutile in partially reduced TiO2 (P-25) nanoparticles: An ESR study. Chem Phys Lett, 428: 338-342. 193479

Kormann C; Bahnemann DW; Hoffman MR (1988). Preparation and characterization of quantum-size titanium dioxide. J Phys Chem B, 92: 5196-5201. http://dx.doi.org/10.1021/j100329a027 090582

Krishna V; Noguchi N; Koopman B; Moudgil B (2006). Enhancement of titanium dioxide photocatalysis by water-soluble fullerenes. J Colloid Interface Sci, 304: 166-171. 193482

KRONOS International (2006). The innovation of catalytic pollutant degradation: KRONOS vlp 7000. Retrieved July 28, 2009 from http://www.kronostio2.com/khome.nsf/KRONOS%20vlp%20-%20Cleaning%20with%20light.pdf. 196046

Ku SK; Lee HS (2006). An immunohistochemical study of the pancreatic endocrine cells of the nude mouse, Balb/c-nu/nu. European Journal of Histochemistry, 50: 61-68. http://www.ncbi.nlm.nih.gov/pubmed/16584986?dopt=Citation. 625354

Kuhn KP; Chaberny IF; Massholder K; Stickler M; Benz VW; Sonntag HG; Erdinger L (2003). Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light. Chemosphere, 53: 71-77. http://dx.doi.org/10.1016/S0045-6535(03)00362-X 090597

Kuwabara JS; Davis JA; Chang CCY (1986). Algal growth response to particle-bound orthophosphate and zinc. Limnol Oceanogr, 31: 503-511. http://www.aslo.org/lo/toc/vol_31/issue_3/0503.pdf. 625577

Lademann J; Richter H; Schaefer UF; Blume-Peytavi U; Teichmann A; Otberg N; Sterry W (2006). Hair follicles - a long-term reservoir for drug delivery. Skin Pharmacol Physiol, 19: 232-236. http://dx.doi.org/10.1159/000093119 157758

Lademann J; Richter H; Teichmann A; Otberg N; Blume-Peytavi U; Luengo J; Weiss B; Schaefer UF; Lehr CM; Wepf R; Sterry W (2007). Nanoparticles--an efficient carrier for drug delivery into the hair follicles. European Journal Pharm Biopharm, 66: 159-164. 157678

Lademann J; Weigmann H; Rickmeyer C; Barthelmes H; Schaefer H; Mueller G; Sterry W (1999). Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Physiol, 12: 247-256. http://dx.doi.org/10.1159/000066249 090591

Lademann J; Weigmann H; Schafer H; Muller G; Sterry W (2000). Investigation of the stability of coated titanium microparticles used in sunscreens. Skin Pharmacol Physiol, 13: 258-264. 157895

Lansdown AB; Taylor A (1997). Zinc and titanium oxides: promising UV-absorbers but what influence do they have on the intact skin? Int J Cosmet Sci, 19: 167-72. 157928

Larese FF; D'Agostin F; Crosera M; Adami G; Renzi N; Bovenzi M; Maina G (2009). Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology, 255: 33-37. http://www.ncbi.nlm.nih.gov/pubmed/=18973786. 193493

LeBlanc C (2009). Public Comment submitted by Carole LeBlanc, Special Expert, Chemical and Material Risk Management Directorate, Office of the Deputy Under Secretary of Defense, U. S. Department of Defense (DoD); Docket ID: EPA-HQ-ORD-2009-0495-0009. Retrieved September 17, 2009 from http://www.regulations.gov/search/Regs/home.html#documentDetail?R=0900006480a23355. 625209

Lecoanet HF; Bottero JY; Wiesner MR (2004). Laboratory assessment of the mobility of nanomaterials in porous media. Environ Sci Technol, 38: 5164-5169. 089258

Lee CK; Lin KS; Wu CF; Lyu MD; Lo CC (2008). Effects of synthesis temperature on the microstructures and basic dyes adsorption of titanate nanotubes. J Hazard Mater, 150: 494-503. 098739

Lee H; Choi W (2002). Photocatalytic oxidation of arsenite in TiO2 suspension: kinetics and mechanisms. Environ Sci Technol, 36: 3872-3878. 193498

Lee HA; Leavens TL; Mason SE; Monteiro-Riviere NA; Riviere JE (2009). Comparison of quantum dot biodistribution with a blood-flow-limited physiologically based pharmacokinetic model. Nano Lett, 9: 794-799. 157457

Lee KP; Trochimowicz HJ; Reinhardt CF (1985). Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol Appl Pharmacol, 79: 179-92. http://www.ncbi.nlm.nih.gov/pubmed/=4002222. 193501

Lee KP; Trochimowicz HJ; Reinhardt CF (1985). Transmigration of titanium dioxide (TiO2) particles in rats after inhalation exposure. Exp Mol Pathol, 42: 331-343. 067628

Lei Z; Mingyu S; Xiao W; Chao L; Chunxiang Q; Liang C; Hao H; Xiaoqing L; Fashui H (2008). Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biol Trace Elem Res, 121: 69-79. 157540

Lekki J; Stachura Z; Dabros W; Stachura J; Menzel F; Reinert T; Butz T; Pallon J; Gontier E; Ynsa MD; Moretto P; Kertesz Z; Szikszai Z; Kiss AZ (2007). On the follicular pathway of percutaneous uptake of nanoparticles: Ion microscopy and autoradiography studies. Nucl Instrum Methods Phys Res B, 260: 174-177. http://dx.doi.org/10.1016/j.nimb.2007.02.021 180280

Li Q; Easter NJ; Shang JK (2009). As(III) removal by palladium-modified nitrogen-doped titanium oxide nanoparticle photocatalyst. Environ Sci Technol, 43: 1534-1539. http://dx.doi.org/10.1021/es8025837 193506

Li Q; Mahendra S; Lyon DY; Brunet L; Liga MV; Li D; Alvarez PJ (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. Water Res, 42: 4591-4602. 157538

Li W; Liu Y; Want Q; Ichihara G; Kobayashi T; Fujitani T; Cui U; Hata N; Ichiharam S; Ding X (2008). Assessment of exposure and health status in workers handling titanium dioxide. Shanghai, China: Shanghai Institute of Planned Parenthood Research. http://www.nanosafe2008.org/home/liblocal/docs/Oral%20presentations/O1-1_Ichihara.pdf. 196055

Li XZ; Liu H; Cheng LF; Tong HJ (2003). Photocatalytic oxidation using a new catalyst-TiO2 microsphere-for water and wastewater treatment. Environ Sci Technol, 37: 3989-3994. http://dx.doi.org/10.1021/es0262941 090581

Liao C-M; Chiang Y-H; Chio C-P (2009). Assessing the airborne titanium dioxide nanoparticle-related exposure hazard at workplace. J Hazard Mater, 162: 57-65. http://dx.doi.org/10.1016/j.jhazmat.2008.05.020 157456

Limbach LK; Bereiter R; Muller E; Krebs R; Galli R; Stark WJ (2008). Removal of oxide nanoparticles in a model wastewater treatment plant: Influence of agglomeration and surfactants on clearing efficiency. Environ Sci Technol, 42: 5828-5833. 155628

Lin HF; Valsaraj KT (2003). A titania thin film annular photocatalytic reactor for the degradation of polycyclic aromatic hydrocarbons in dilute water streams. J Hazard Mater, 99: 203-19. http://www.ncbi.nlm.nih.gov/pubmed/=12719152. 193511

Lindberg JE; Quinn MM (2007). A survey of environmental, health and safety risk management information needs and practices among nanotechnology firms in the Massachusetts region (Report No. PEN Brief No. 1). Washington, DC: Woodrow Wilson International Center for Scholars: Project on Emerging Nanotechnologies. 155629

Linglan M; Chao L; Chunxiang Q; Sitao Y; Jie L; Fengqing G; Fashui H (2008). Rubisco activase mRNA expression in spinach: Modulation by nanoanatase treatment. Biol Trace Elem Res, 122: 168-178. 157534

Lison D; Thomassen LC; Rabolli V; Gonzalez L; Napierska D; Seo JW; Kirsch-Volders M; Hoet P; Kirschhock CE; Martens JA (2008). Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. Toxicol Sci, 104: 155-162. 157530

Liu H; Ma L; Zhao J; Liu J; Yan J; Ruan J; Hong F (2009). Biochemical toxicity of nano-anatase TiO2 particles in mice. Biol Trace Elem Res, 129: 170-180. (Available online December 2008). http://dx.doi.org/10.1007/s12011-008-8285-6 193516

Lodén M; Åkerström U; Schwan E (2006). Patent No. EP 1 688 129 A1. Sweden: European Patent Office. https://publications.european-patent-office.org/PublicationServer/getpdf.jsp?cc=EP&pn=1688129&ki=A1. 157757

Löhr AJ; De Kort T; Van Straalen NM; Van Gestel CAM (2007). Unraveling the causes of the toxicity of extremely acid waters of volcanic origin. Environ Int, 33: 743-749. http://dx.doi.org/10.1016/j.envint.2007.02.008 193518

Lohrke H; Hesse B; Goerttler K (1984). Spontaneous tumors and lifespan of female NMRI mice of the outbred stock Sut:NMRT during a lifetime study. J Cancer Res Clin Oncol, 108: 192-196. http://dx.doi.org/10.1007/BF00402466 157978

Lomer MC, Thompson RP, Commisso J, Keen CL, Powell JJ. (2000). Determination of titanium dioxide in foods using inductively coupled plasma optical. Analyst, 125: 2339-2343. http://dx.doi.org/10.1039/b006285p 635672

Lomer MCE; Hutchinson C; Volkert S; Greenfield SM; Catterall A; Thompson RPH; Powell JJ (2004). Dietary sources of inorganic microparticles and their intake in healthy subjects and patients with Crohn's disease. Br J Nutr, 92: 947-955. http://dx.doi.org/10.1079/BJN20041276 157382

Long SR (1989). Rhizobium-legume nodulation: Life together in the underground. Cell, 56: 203-214. http://dx.doi.org/10.1016/0092-8674(89)90893-3 644893

Long TC; Saleh N; Tilton RD; Lowry GV; Veronesi B (2006). Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol, 40: 4346-4352. 089584

Lovern SB; Klaper R (2006). Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environ Toxicol Chem, 25: 1132-1137. 088040

Lovern SB; Strickler JR; Klaper R (2007). Behavioral and physiological changes in Daphnia magna when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC7OHx). Environ Sci Technol, 41: 4465-4470. 091069

Lu CS; Chen CC; Mai FD; Li HK (2009). Identification of the degradation pathways of alkanolamines with TiO2 photocatalysis. J Hazard Mater, 165: 306-316. http://www.sciencedirect.com/science/article/B6TGF-4TP49S3-3/2/f21af6c7afd7ea838aaa4cfcc025b87b. 193528

Lu N; Zhu Z; Zhao X; Tao R; Yang X; Gao Z (2008). Nano titanium dioxide photocatalytic protein tyrosine nitration: a potential hazard of TiO2 on skin. Biochem Biophys Res Commun, 370: 675-680. 157526

Luoma SN (2008). Silver nanotechnologies and the environment: Old problems or new challenges. Washington, DC: Project on Emerging Nanotechnologies. 157525

Ma-Hock L; Burkhardt S; Strauss V; Gamer AO; Wiench K; van Ravenzwaay B; Landsiedel R (2009). Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. Inhal Toxicol, 21: 102-118. (Available online September 2008). http://dx.doi.org/10.1080/08958370802361057 193534

Määttä K; Arstila AU (1975). Pulmonary deposits of titanium dioxide in cytologic and lung biopsy specimens. Light and electron microscopic x-ray analysis. Lab Invest, 33: 342-6. 157979

Mahe B; Vogt A; Liard C; Duffy D; Abadie V; Bonduelle O; Boissonnas A; Sterry W; Verrier B; Blume-Peytavi U; Combadiere B (2009). Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice. J Invest Dermatol, 129: 1156-1164. http://dx.doi.org/10.1038/jid.2008.356 225307

Maier M; Hannebauer B; Holldorff H; Albers P (2006). Does lung surfactant promote disaggregation of nanostructured titanium dioxide? J Occup Environ Med, 48: 1314-1320. http://dx.doi.org/10.1097/01.jom.0000215405.72714.b200043764-200612000-00010 090451

Makri A; Goveia M; Balbus J; Parkin R (2004). Children's susceptibility to chemicals: A review by developmental stage. J Toxicol Environ Health B Crit Rev, 7: 417-35. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15586877. 193537

Maness PC; Smolinski S; Blake DM; Huang Z; Wolfrum EJ; Jacoby WA (1999). Bactericidal activity of photocatalytic TiO2 reaction: Toward an understanding of its killing mechanism. Appl Environ Microbiol, 65: 4094-8. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10473421. 193538

Marquis BJ; Love SA; Braun KL; Haynes CL (2009). Analytical methods to assess nanoparticle toxicity. Analyst, 134: 425-439. http://dx.doi.org/10.1039/B818082b 193539

Mavon A; Miquel C; Lejeune O; Payre B; Moretto P (2007). In vitro percutaneous absorption and in vivo stratum corneum distribution of an organic and a mineral sunscreen. Skin Pharmacol Physiol, 20: 10-20. 090587

Maynard AD (2008). Living with nanoparticles. Nano Today, 3: 64. 157522

Maynard AD; Aitken RJ (2007). Assessing exposure to airborne nanomaterials: Current abilities and future requirements. Nanotoxicology, 1: 26-41. 090674

McKeytta JJ (1984). Encyclopedia of chemical processing and design. Boca Raton, FL: CRC Press. 196036

MedlinePlus (2009). MedlinePlus Medical Encyclopedia: LDH isoenzymes. Retrieved November 17, 2010 from http://www.nlm.nih.gov/medlineplus/ency/article/003499.htm. 193814

Mehrvar M; Anderson WA; Moo-Young M (2002). Comparison of the photoactivities of two commercial titanium dioxide powders in the degradation of 1,4-dioxane. Intl J Photoenergy, 4: 141-146. 193541

Menzel F; Reinert T; Vogt J; Butz T (2004). Investigations of percutaneous uptake of ultrafine TiO2 particles at the high energy ion nanoprobe LIPSION. Nucl Instrum Methods Phys Res B, 219-220: 82-86. http://dx.doi.org/10.1016/j.nimb.2004.01.032 180361

Meridian Institute (2006). Overview and comparison of conventional and nano-based water treatment technologies Supplement to nanotechnology, water, and development. Washington, DC: Meridian Institute. http://sites.merid.org/nano/watertechpaper/?item=%2fnano%2fwatertechpaper%2f&user=extranet%5cAnonymous&site=website. 090595

Millennium Inorganic Chemicals (2007). Titanium dioxide manufacturing processes. Retrieved February 16, 2009 from http://www.millenniumchem.com/Products+and+Services/Products+by+Type/Titanium+Dioxide+-+Paint+and+Coatings/r_TiO2+Fundamentals/Titanium+Dioxide+-+Paint+and+Coatings+TiO2+Fundamentals_EN.htm. 195899

Miller AL; Hoover MD; Mitchell DM; Stapleton BP (2007). The nanoparticle information library (NIL): A prototype for linking and sharing emerging data. J Occup Environ Hyg, 4: D131-134. http://dx.doi.org/10.1080/15459620701683947 092297

Miller TJ; Knapton A; Adeyemo OO; Noory LS; Weaver JL; Hanig JP; Honchel R; Zhang J; Espandiari P; Benedick MF; Umbreit TH; Tomazic-Jezic VJ; Sadrieh N (2007). Toxicology of titanium dioxide (TiO2) nanoparticles: In vitro and in vivo evaluation of macrophage uptake of TiO2. FASEB J, 21: A812. http://www.fasebj.org. 157668

Mineral Information Institute (2009). Titanium dioxide manufacturing processes. Retrieved February 16, 2009 from http://www.mii.org/Minerals/phototitan.html. 195905

Minerva M (2009). Public Comment submitted by Michael Minerva, Director, Global Environmental Technical Services, Schering-Plough; Docket ID: EPA-HQ-ORD-2009-0495-0005. Retrieved September 17, 2009 from http://www.regulations.gov/search/Regs/home.html#documentDetail?R=0900006480a22910. 625210

Mingyu S; Fashui H; Chao L; Xiao W; Xiaoqing L; Liang C; Fengqing G; Fan Y; Zhongrui L (2007). Effects of nano-anatase TiO2 on absorption, distribution of light, and photoreduction activities of chloroplast membrane of spinach. Biol Trace Elem Res, 118: 120-130. http://dx.doi.org/10.1007/s12011-007-0006-z 157667

Mingyu S; Xiao W; Chao L; Chunxiang Q; Xiaoqing L; Liang C; Hao H; Fashui H (2007). Promotion of energy transfer and oxygen evolution in spinach photosystem II by nano-anatase TiO2. Biol Trace Elem Res, 119: 183-92. http://dx.doi.org/10.1007/s12011-007-0065-1 157666

Mitchnick M; O'Lenick AJ Jr (1996). Patent No. 5565591 (class: 556/10). United States: United States Patent Office. http://www.freepatentsonline.com/5565591.html. 157935

Mitoraj D; Janczyk A; Strus M; Kisch H; Stochel G; Heczko PB; Macyk W (2007). Visible light inactivation of bacteria and fungi by modified titanium dioxide. Photochem Photobiol Sci, 6: 642-648. http://dx.doi.org/10.1039/b617043a 157665

Mohr U; Ernst H; Roller M; Pott F (2006). Pulmonary tumor types induced in Wistar rats of the so-called "19-dust study". Exp Toxicol Pathol, 58: 13-20. 097493

Monteiro-Riviere NA (1991). Comparative anatomy, physiology, and biochemistry of mammalian skin. In DW Hobson (Ed.), Dermal and ocular toxicology: Fundamentals and methods (pp. 2-72). Washington, DC: CRC Press. 157957

Monteiro-Riviere NA (2004). Evaluation of Nanoparticle interactions with skin. Talk presented at U.S. EPA 2004 Nanotechnology Science to Achieve Results (STAR) Progres Review Workshop, Philadelphia, PA. 157834

Monteiro-Riviere NA; Bristol DG; Manning TO; Rogers RA; Riviere JE (1990). Interspecies and interregional analysis of the comparative histological thickness and laser Doppler blood flow measurements at five cutaneous sites in nine species. J Invest Dermatol, 95: 582-586. 625073

Moore LE; Smith AH; Hopenhayn-Rich C; Biggs ML; Kalman DA; Smith MT (1997). Micronuclei in exfoliated bladder cells among individuals chronically exposed to arsenic in drinking water. Cancer Epidemiol Biomarkers Prev, 6: 31-36. 193553

Moore MN (2006). Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int, 32: 967-976. http://www.sciencedirect.com/science/article/B6V7X-4KFV3DR-1/1/9fa06c0513b92d164f2855ccadf46296. 089839

Moran CA; Mullick FG; Ishak KG; Johnson FB; Hummer WB (1991). Identification of titanium in human tissues: Probable role in pathologic processes. Hum Pathol, 22: 450-454. 157956

Morgan K (2005). Development of a preliminary framework for informing the risk analysis and risk management of nanoparticles. Risk Anal, 25: 1621-1635. 088831

Mortensen LJ; Oberdorster G; Pentland AP; DeLouise LA (2008). In vivo skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR. Nano Lett, 8: 2779-2787. 155612

Moyal D (2008). How to measure UVA protection afforded by sunscreen products. Expert Review of Dermatology, 3: 307-313. http://dx.doi.org/10.1586/17469872.3.3.307 193559

Mueller NC; Nowack B (2008). Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol, 42: 4447-4453. http://dx.doi.org/10.1021/es7029637 157519

Muhle H; Bellmann B; Creutzenberg O; Dasenbrock C; Ernst H; Kilpper R; MacKenzie JC; Morrow P; Mohr U; Takenaka S; Mermelstein R (1991). Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam Appl Toxicol, 17: 280-299. http://toxsci.oxfordjournals.org/cgi/content/abstract/17/2/280. 063996

Muhle H; Mangelsdorf I (2003). Inhalation toxicity of mineral particles: Critical appraisal of endpoints and study design. Toxicol Lett, 140-141: 223-228. http://dx.doi.org/10.1016/S0378-4274(02)00514-3 157859

Muhlfeld C; Geiser M; Kapp N; Gehr P; Rothen-Rutishauser B (2007). Re-evaluation of pulmonary titanium dioxide nanoparticle distribution using the "relative deposition index": Evidence for clearance through microvasculature. Part Fibre Toxicol, 4: 7. 091106

Mulenweg H (2004). Gas-phase reactions open up new roads to nanoproducts. Degussa Science Newsletter, 8, 12-16. 090592

Murdock RC; Braydich-Stolle L; Schrand AM; Schlager JJ; Hussain SM (2008). Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci, 101: 239-253. http://dx.doi.org/10.1093/toxsci/kfm240 193563

Murr LE; Esquivel EV; Bang JJ (2004). Characterization of nanostructure phenomena in airborne particulate aggregates and their potential for respiratory health effects. Journal of Materials Science: Materials in Medicine, 15: 237-247. http://dx.doi.org/10.1023/B:JMSM.0000015483.06136.68 196310

Myllynen P; Loughran M; Howard C; Sormunen R; Walsh A; Vahakangas K (2008). Kinetics of gold nanoparticles in the human placenta. Reprod Toxicol, 26: 130-137. 187028

Nagaveni K; Sivalingam G; Hegde MS; Madras G (2004). Photocatalytic degradation of organic compounds over combustion-synthesized nano-TiO2. Environ Sci Technol, 38: 1600-1604. 090578

Nakagawa Y; Wakuri S; Sakamoto K; Tanaka N (1997). The photogenotoxicity of titanium dioxide particles. Mutat Res Genet Toxicol Environ Mutagen, 394: 125-132. http://dx.doi.org/10.1016/S1383-5718(97)00126-5 157927

NanoDerm (2007). Quality of skin as a barrier to ultra-fine particles (Final Report) (Report No. QLK4-CT-2002-02678). Germany: Nanoderm. http://www.uni-leipzig.de/~nanoderm/Downloads/Nanoderm_Final_Report.pdf. 157660

Nanosafe (2008). Dissemination report: Are conventional protective devices such as fibrous filter media, respirator cartridges, protective clothing and gloves also efficient for nanoaerosols? (Report No. DR-325/326-200801-1). France: Sixth Framework Programme. http://www.nanosafe.org/scripts/home/publigen/content/templates/show.asp?P=63&L=EN&ITEMID=13. 196066

Nanosafe (2008). Dissemination report: Is it possible to easily measure the engineered nanoparticles at workplaces? Retrieved June 02, 2009 from http://www.nanosafe.org/scripts/home/publigen/content/templates/show.asp?P=63&L=EN&ITEMID=13. 594868

National Nanotechnology Initiative (2006). What is nanotechnology? Retrieved September 25, 2007 from http://www.nano.gov/html/facts/whatIsNano.html. 091186

National Psoriasis Foundation (2006). About psoriasis. Frequently asked questions. Retrieved August 22, 2006 from http://www.psoriasis.org/about/faq/. 157748

Navarro E; Baun A; Behra R; Hartmann NB; Filser J; Miao AJ; Quigg A; Santschi PH; Sigg L (2008). Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology, 17: 372-386. 157517

Neal AL (2008). What can be inferred from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology, 17: 362-371. http://dx.doi.org/10.1007/s10646-008-0217-x 196069

Nemmar A; Melghit K; Ali BH (2008). The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO2 nanorods in rats. Exp Biol Med, 233: 610-619. http://dx.doi.org/10.3181/0706-RM-165 157514

Newman KA (2006, December). Sun protection report. GCI Magazine. http://www.gcimagazine.com/marketstrends/segments/suncare/4829426.html?page=1. 157745

Nichols G; Byard S; Bloxham MJ; Botterill J; Dawson NJ; Dennis A; Diart V; North NC; Sherwood JD (2002). A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization. J Pharm Sci, 91: 2103-2109. 202114

Nielsen HD; Berry LS; Stone V; Burridge TR; Fernandes TF (2008). Interactions between carbon black nanoparticles and brown algae Fucus serratus: Inhibition of fertilization and zygotic development. Nanotoxicology, 2: 88-97. http://dx.doi.org/10.1080/17435390802109185 644828

NIOSH (2005). NIOSH current intelligence bulletin: Evaluation of health hazard and recommendations for occupational exposure to titanium dioxide. Retrieved April 24, 2007 from http://www.cdc.gov/niosh/review/public/tio2/pdfs/TIO2Draft.pdf. 196072

NIOSH (2009). Approaches to safe nanotechnology: Managing the health and safety concerns associated with engineered nanomaterials. Retrieved June 02, 2009 from http://cdc.gov/niosh/docs/2009-125/. 196073

Nohynek GJ; Lademann J; Ribaud C; Roberts MS (2007). Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. Crit Rev Toxicol, 37: 251-277. http://dx.doi.org/10.1080/10408440601177780 090619

Nowack B; Bucheli TD (2007). Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut, 150: 5-22. http://dx.doi.org/10.1016/j.envpol.2007.06.006 092294

NRC (1999). Toxicity of military smokes and obscurants. Washington, DC: National Research Council. http://books.nap.edu/catalog.php?record_id=9621. 091188

NSF International (2009). NSF certified products: Public water supply system components. Retrieved June 02, 2009 from http://www.nsf.org/Certified/PwsComponents/Listings.asp?Company=4J270&Stasndard=061. 196092

Nurkiewicz TR; Porter DW; Hubbs AF; Cumpston JL; Chen BT; Frazer DG; Castranova V (2008). Nanoparticle inhalation augments particle-dependent systemic microvascular dysfunction. Part Fibre Toxicol, 5: 1. http://dx.doi.org/10.1186/1743-8977-5-1 156816

Nurkiewicz TR; Porter DW; Hubbs AF; Stone S; Chen BT; Frazer DG; Boegehold MA; Castranova V (2009). Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling. Toxicol Sci, 110: 191-203. http://dx.doi.org/10.1093/toxsci/kfp051 191961

Oberdörster G; Maynard A; Donaldson K; Castranova V; Fitzpatrick J; Ausman K; Carter J; Karn B; Kreyling W; Lai D; Olin S; Monteiro-Riviere N; Warheit D; Yang H (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Part Fibre Toxicol, 2: 8. http://www.particleandfibretoxicology.com/content/2/1/8. 090087

Oberdörster E; Zhu S; Blickley TM; McClellan-Green P; Haasch ML (2006). Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C60) on aquatic organisms. Carbon N Y, 44: 1112-1120. 088054

Oberdörster G (2000). Toxicology of ultrafine particles: in vivo studies. Philos Transact A Math Phys Eng Sci, 358: 2719-2740. http://dx.doi.org/10.1098/rsta.2000.0680 036303

Oberdörster G; Ferin J; Gelein R; Soderholm SC; Finkelstein J (1992). Role of the alveolar macrophage in lung injury: Studies with ultrafine particles. Environ Health Perspect, 97: 193-199. 045110

Oberdörster G; Ferin J; Lehnert BE (1994). Correlation between particle size, in vivo particle persistence, and lung injury. Environ Health Perspect, 102: 173-179. http://www.ncbi.nlm.nih.gov/pubmed/=7882925. 046203

Oberdörster G; Oberdörster E; Oberdörster J (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect, 113: 823-839. http://www.ncbi.nlm.nih.gov/pubmed/=16002369. 087559

Oberdörster G; Sharp Z; Atudorei V; Elder A; Gelein R; Kreyling W; Cox C (2004). Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol, 16: 437-445. http://dx.doi.org/10.1080/08958370490439597 055639

OECD (1992). Draft revised guideline 203: Fish, acute toxicity test. In OECD Guideling for testing of chemicals OECD Publishing. Retrieved November 17, 2010, from: http://www.oecd.org/dataoecd/45/30/41888165.pdf. 667208

OECD (2004). Test No. 202: Daphnia sp. acute immobilisation test. In OECD Guidelines for the Testing of Chemicals / Section 2: Effects on Biotic Systems OECD Publishing. Retrieved November 17, 2010, from: http://www.oecdbookshop.org/oecd/display.asp?K=5LMQCR2K7S28&DS=Test-No.-202-Daphnia-sp.-Acute-Immobilisation-Test. 667207

OECD (2006). Test No. 201: Alga, growth inhibition test. In OECD Guidelines for the Testing of Chemicals / Section 2: Effects on Biotic Systems OECD Publishing. Retrieved November 17, 2010, from: http://www.oecdbookshop.org/oecd/display.asp?K=5LMQCR2K7S5H&DS=Test-No.-201-Alga-Growth-Inhibition-Test. 199838

OECD (2008). Series on the safety of manufactured nanomaterials,Number 6: List of manufactured nanomaterials and list of endpoints for phase one of the OECD testing programme (Report No. ENV/JM/MONO(2008)13/REV). Paris, France: Organisation for Economic Co-operation and Development. http://www.olis.oecd.org/olis/2008doc.nsf/LinkTo/NT000034C6/$FILE/JT03248749.PDF. 157512

OECD (2008). Test No. 211: Daphnia magna reproduction Test. In OECD Guidelines for the testing of chemicals / Section 2: Effects on Biotic Systems OECD Publishing. Retrieved November 17, 2010, from: http://www.oecdbookshop.org/oecd/display.asp?K=5LMQCR2K7R8S&DS=Test-No.-211-Daphnia-magna-Reproduction-Test. 667210

OECD (2009). OECD database on research into safety of manufactured nanomaterials [database]. http://webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx? 644433

OECD (2010). Report of the questionnaire on regulatory regimes for manufactured nanomaterials (Report No. ENV/JM/MONO(2010)12). Paris, France: Inter-Organization Programme for the Sound Management of Chemicals. 644192

Osier M; Baggs RB; Oberdörster G (1997). Intratracheal instillation versus intratracheal inhalation: Influence of cytokines on inflammatory response. Environ Health Perspect, 105: 1265-1271. http://www.ncbi.nlm.nih.gov/pubmed/=9400736. 086056

Osterwalder N; Capello C; Hungerbühler K; Stark WJ (2006). Energy consumption during nanoparticle production: how economic is dry synthesis? J Nanopart Res, 8: 1-9. 157743

Ostraat ML (2009). Industry-led initiative for occupational health and safety. In M Hull; S Friedrichs (Eds.), Risk governance of nanotechnology: Environmental, health and safety concerns (pp. 181-246). Norwich, NY: William Andrew Publishing. 196077

Ostraat ML; Swain KA; Krajewski JJ (2008). SiO2 aerosol nanoparticle reactor for occupational health and safety studies. J Occup Environ Hyg, 5: 390-398. http://dx.doi.org/10.1080/15459620802071646 193591

Ostraat ML; Swain KA; Krajewski JJ; Small RJ (2006, November). Aerosol Nanoparticle Behavior and Barrier Efficiency of Filter Media to Engineered Aerosol Nanoparticles. Abstract from 2006 Annual Meeting of the Nanoparticle Occupational Safety and Health Consortium, San Francisco, CA. http://aiche.confex.com/aiche/2006/techprogram/P68014.htm. 667690

Ostrowski A; Martin T; Conti J; Hurt I; Harthorn B (2009). Nanotoxicology: Characterizing the scientific literature, 2000-2007. J Nanopart Res, 11: 251-257. http://dx.doi.org/10.1007/s11051-008-9579-5. 193592

Oxonica (2005). Technical notes: Example formulation details and protocol tips to obtain optimal dispersion of Optisol (TM) UV Absorber. Retrieved April 04, 2007 from http://www.oxonica.com/media/media_promoliterature.php?start=6. 157793

Packaged Facts (2001). The U.S. market for suncare and lipcare products. Retrieved March 31, 2001 from http://www.mindbranch.com/listing/product/R567-393.html. 196053

Paquin PR; Santore RC; Farley KJ; Kavvadas C; Wu KB; Mooney K; Winfield RP; Di Toro DM (eds) (2003). Metals in aquatic systems: a review of exposure, bioaccumulation, and toxicity models. Pensacola, FL: SETAC Press. 196867

Park GB; Knowland JS; Flutter BR (2006). Patent No. 20060134026, class: 424/59. United States: U.S. Patent and Trademark Agency. http://www.freepatentsonline.com/y2006/0134026.html. 193593

Pena M; Meng X; Korfiatis GP; Jing C (2006). Adsorption mechanism of arsenic on nanocrystalline titanium dioxide. Environ Sci Technol, 40: 1257-1262. http://www.ncbi.nlm.nih.gov/pubmed/=16572784. 090573

Pflucker F; Hohenberg H; Holzle E; Will T; Pfeiffer S; Wepf R; Diembeck W; Wenck H; Gers-Barlag H (1999). The outermost stratum corneum layer is an effective barrier against dermal uptake of topically applied micronized titanium dioxide. Int J Cosmet Sci, 21: 399-411. http://dx.doi.org/10.1046/j.1467-2494.1999.211924.x 644132

Pflücker F; Wendel V; Hohenberg H; Gärtner E; Will T; Pfeiffer S; Wepf R; Gers-Barlag H (2001). The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. Skin Pharmacol Physiol, 14: 92-97. 157887

Pichat P (2003). Photocatalytic degradation of pollutants in water and air: basic concepts and application. In MA Tarr (Ed.), Chemical Degradation Methods for Wastes and Pollutants: Environmental and Industrial Applications (pp. 77-120). New York, NY: Marcel-Dekker, Inc. 196037

Pinheiro T; Pallon J; Alves LC; Verissimo A; Filipe P; Silva JN; Silva R (2007). The influence of corneocyte structure on the interpretation of permeation profiles of nanoparticles across skin. Nucl Instrum Methods Phys Res B, 260: 119-123. http://dx.doi.org/10.1016/j.nimb.2007.02.014 180160

Porter D; Sriram K; Wolfarth M; Jefferson A; Schwegler-Berry D; Andrew ME; Castranova V (2008). A biocompatible medium for nanoparticle dispersion. Nanotoxicology, 2: 144-154. http://dx.doi.org/10.1080/17435390802318349 157508

Pott F; Roller M (2005). Carcinogenicity study with nineteen granular dusts in rats. Eur J Oncol, 10: 249-281. 157790

Pott F; Ziem U; Reiffer FJ; Huth F; Ernst H; Mohr U (1987). Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. Exp Toxicol Pathol, 32: 129-152. 029823

Powers KW; Brown SC; Krishna VB; Wasdo SC; Moudgil BM; Roberts SM (2006). Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. Toxicol Sci, 90: 296-303. 088783

Powers KW; Palazuelos M; Moudgil BM; Roberts SM (2007). Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology, 1: 42-51. http://dx.doi.org/10.1080/17435390701314902 090679

Project on Emerging Nanotechnologies (2007). Kids tear free SPF 30 Company: Banana Boat®. Retrieved December 24, 2008 from http://www.nanotechproject.org/inventories/consumer/browse/products/5419/. 157648

Project on Emerging Nanotechnologies (2010). The Project on Emerging Nanotechnologies database on environment, health, and safety research [database]. http://www.nanotechproject.org/inventories/ehs/ 644439

Purifics Solutions (2008). Briefing: Photo-Cat water purification for potable and reuse. Retrieved June 02, 2009 from http://www.purifics.com/solutions/index.html. 196040

Ramanakumar AV; Parent MÉ; Latreille B; Siemiatycki J (2008). Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: results from two case-control studies in Montreal. Int J Cancer, 122: 183-189. http://dx.doi.org/10.1002/ijc.23021 157507

Reeves JF; Davies SJ; Dodd NJF; Jha AN (2008). Hydroxyl radicals (OH) are associated with titanium dioxide (TiO2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. Mutat Res, 640: 113-122. http://dx.doi.org/10.1016/j.mrfmmm.2007.12.010 157506

Rehn B; Seiler F; Rehn S; Bruch J; Maier M (2003). Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicol Appl Pharmacol, 189: 84-95. 090613

Reisch MS (2005). New-wave sunscreens: active ingredient makers are frustrated by the long list of sunscreens and UV-A testing protocols that are still awaiting FDA decisions. Chem Eng News, 83: 18-22. http://pubs.acs.org/cen/coverstory/83/8315sunscreens.html. 155634

Reliene R; Schiestl RH (2003). Mouse models for induced genetic instability at endogenous loci. Oncogene, 22: 7000-7010. 157857

Renwick LC; Brown D; Clouter A; Donaldson K (2004). Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup Environ Med, 61: 442-447. http://www.ncbi.nlm.nih.gov/pubmed/=15090666. 056067

Richardson SD; Thruston AD; Collette TW; Patterson KS; Lykins BW; Ireland JC (1996). Identification of TiO2/UV disinfection byproducts in drinking water. Environ Sci Technol, 30: 3327-3334. http://dx.doi.org/10.1021/es960142m 193612

Ridley MK; Hackley VA; Machesky ML (2006). Characterization and surface-reactivity of nanocrystalline anatase in aqueous solutions. Langmuir, 22: 10,972-10,982. 090599

Rincon AG; Pulgarin C (2003). Photocatalytical inactivation of E. coli: effect of (continuous-intermittent) light intensity and of (suspended-fixed) TiO2 concentration. Appl Catal B-Environ, 44: 263-284. http://dx.doi.org/10.1016/S0926-3373(03)00076-6 157856

Rittinghausen S; Kaspareit J; Mohr U (1997). Incidence and spectrum of spontaneous neoplasms in Han:NMRI mice of both sexes. Exp Toxicol Pathol, 49: 347-349. 157924

Riviere JE; Monteiro-Riviere NA (1991). The isolated perfused porcine skin flap as an in vitro model for percutaneous absorption and cutaneous toxicology. Crit Rev Toxicol, 21: 329-344. http://dx.doi.org/10.3109/10408449109019570 625197

Robichaud CO; Uyar AE; Darby MR; Zucker LG; Wiesner MR (2009). Estimates of upper bounds and trends in nano-TiO2 production as a basis for exposure assessment. Environ Sci Technol, 43: 4227-4233. http://dx.doi.org/10.1021/es8032549 193617

Robinson JK; Rigel DS; Amonette RA (2000). Summertime sun protection used by adults for their children. J Am Acad Dermatol, 42: 746-753. 193618

Rodil R; Moeder M (2008). Development of a simultaneous pressurised-liquid extraction and clean-up procedure for the determination of UV filters in sediments. Anal Chim Acta, 612: 152-159. http://dx.doi.org/10.1016/j.aca.2008.02.030 157503

Rouse JG; Yang J; Ryman-Rasmussen JP; Barron AR; Monteiro-Riviere NA (2007). Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. Nano Lett, 7: 155-160. http://dx.doi.org/10.1021/nl062464m 157644

Ryu J; Choi W (2004). Effects of TiO2 surface modifications on photocatalytic oxidation of arsenite: the role of superoxides. Environ Sci Technol, 38: 2928-2933. 193622

Ryu J; Choi W (2006). Photocatalytic oxidation of arsenite on TiO2: understanding the controversial oxidation mechanism involving superoxides and the effect of alternative electron acceptors. Environ Sci Technol, 40: 7034-7039. 090579

Ryu J; Choi W (2008). Substrate-specific photocatalytic activities of TiO2 and multiactivity test for water treatment application. Environ Sci Technol, 42: 294-300. http://pubs3.acs.org/acs/journals/doilookup?in_doi=10.1021/es071470x. 157501

Sadauskas E; Wallin H; Stoltenberg M; Vogel U; Doering P; Larsen A; Danscher G (2007). Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol, 4: 10. http://dx.doi.org/10.1186/1743-8977-4-10 091407

Sadrieh N; Wokovich AM; Gopee NV; Siitonen PH; Cozart CR; Howard PC; Doub WH; Bushes LF (2008). Analysis of dermal penetration of titanium dioxide (TiO2) from sunscreen formulations containing micro- and nano-scale particles of TiO2. Abstract from Society of Toxicology 47th Annual Meeting, Seattle, WA. 157500

Sadrieh N; Wokovich AM; Gopee NV; Zheng J; Haines D; Parmiter D; Siitonen PH; Cozart CR; Patri AK; McNeil SE; Howard PC; Doub WH; Buhse LF (2010). Lack of significant dermal penetration of titanium dioxide (TiO2) from sunscreen formulations containing nano- and sub-micron-size TiO2 particles. Toxicol Sci, 115: 156-166. http://dx.doi.org/10.1093/toxsci/kfq041 594511

Sager TM; Castranova V (2009). Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: Comparison to ultrafine titanium dioxide. Part Fibre Toxicol, 6: 15. 193625

Sager TM; Kommineni C; Castranova V (2008). Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: Role of particle surface area. Part Fibre Toxicol, 5: 17. http://dx.doi.org/10.1186/1743-8977-5-17 157499

Sager TM; Porter DW; Robinson VA; Lindsley WG; Schwegler-Berry DE; Castranova V (2007). Improved method to disperse nanoparticles for in vitro and in vivo investigation of toxicity. Nanotoxicology, 1: 118-129. http://dx.doi.org/10.1080/17435390701381596 091214

Sager TM; Robinson VA; Porter DW; Schwegler-Berry D; Lindsley WG; Castranova V (2007). An improved method to prepare suspensions of nanoparticles for treatment of lung cells in culture or in vivo exposure by pharyngeal aspiration or intratracheal instillation. Abstract from Society of Toxicology 46th Annual Meeting, Charlotte, NC. 090633

Santmyire BR; Feldman SR; Fleischer AB Jr (2001). Lifestyle high-risk behaviors and demographics may predict the level of participation in sun-protection behaviors and skin cancer primary prevention in the United States. Results of the 1998 National Health Interview Survey. Cancer, 92: 1315-1324. 193629

Sayes CM; Wahi R; Kurian PA; Liu Y; West JL; Ausman KD; Warheit DB; Colvin VL (2006). Correlating nanoscale titania structure with toxicity: A cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelian cells. Toxicol Sci, 92: 174-185. http://dx.doi.org/10.1093/toxsci/kfj197 090569

SCCNFP (2000). Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning titanium dioxide Colipa n S75 (Report No. SCCNFP/0005/98). Brussels, Belgium: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out135_en.pdf. 092740

Schlossman D; Shao Y; Detrieu P (2006). Perspectives on supplying attenuation grades of titanium dioxide and zinc oxide for sunscreen applications. Retrieved October 28, 2007 from http://www.fda.gov/nanotechnology/meetings/kobo_files/textmostly/index.htm. 093309

Schmidt J; Vogelsberger W (2006). Dissolution kinetics of titanium dioxide nanoparticles: The observation of an unusual kinetic size effect. J Phys Chem B, 110: 3955-3963. http://dx.doi.org/10.1021/jp055361 193634

Schulz J; Hohenberg H; Pflücker F; Gärtner E; Will T; Pfeiffer S; Wepf R; Wendel V; Gers-Barlag H; Wittern KP (2002). Distribution of sunscreens on skin. Adv Drug Deliv Rev, 54: S157-S163. 157872

Schwerin MR; Walsh DL; Coleman Richardson D; Kisielewski RW; Kotz RM; Routson LB; David Lytle C (2002). Biaxial flex-fatigue and viral penetration of natural rubber latex gloves before and after artificial aging. J Biomed Mater Res, 63: 739-745. 193636

Scientific Committee on Computer Products (2007). Safety of nanomaterials in cosmetic products (Report No. SCCP/1147/07). Brussels: European Commission, Health and Consumer Protection Directorate-General. 196826

Scientific Committee on Computer Products (2007). Preliminary opinion on safety of nanomaterials in cosmetic products. Brussels, Belgium: European Commission, Health and Consumer Protection Directorate-General. 157639

Seeger EM; Baun A; Kastner M; Trapp S (2009). Insignificant acute toxicity of TiO2 nanoparticles to willow trees. Journal of Soils and Sediments, 9: 46-53. http://dx.doi.org/10.1007/s11368-008-0034-0 644124

Seok S-I; Ahn B-Y; Kim H (2006). Patent No. 7611688 B2. United States: United States Patent Office. http://www.freepatentsonline.com/7611688.html. 091198

Serpone N; Salinaro A; Horikoshi S; Hidaka H (2006). Beneficial effects of photo-inactive titanium dioxide specimens on plasmid DNA, human cells and yeast cells exposed to UVA/UVB simulated sunlight. J Photochem Photobiol A, 179: 200-212. http://dx.doi.org/10.1016/j.jphotochem.2005.08.017 157736

Shao Y; Schlossman D (1999). Effect of particle size on performance of physical sunscreen formulas. Talk presented at PCIA Conference, Shanghai, China. http://www.koboproductsinc.com/Downloads/PCIA99-Sunscreen.pdf. 093301

Shao Y; Schlossman D (2004). Discovering an optimum small micropigment for high UV shielding and low skin whitening. Talk presented at 23d IFSCC Congress, Orlando, FL. http://www.koboproductsinc.com/Downloads/IFSCC2004.pdf. 157825

Sharma VK; Sohn M (2009). Aquatic arsenic: Toxicity, speciation, transformations, and remediation. Environ Int, 35: 743-759. http://www.sciencedirect.com/science/article/B6V7X-4VNCC30-2/2/f35c556755d8d67ddc278d738be8dcc2. 193641

Shatkin JA (2008). Informing environmental decision making by combining life cycle assessment and risk analysis. J Ind Ecol, 12: 278-281. http://dx.doi.org/10.1111/j.1530-9290.2008.00031.x 180065

Shvedova AA; Kisin E; Murray AR; Johnson VJ; Gorelik O; Arepalli S; Hubbs AF; Mercer RR; Keohavong P; Sussman N; Jin J; Yin J; Stone S; Chen BT; Deye G; Maynard A; Castranova V; Baron PA; Kagan VE (2008). Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: Inflammation, fibrosis, oxidative stress, and mutagenesis. Am J Physiol Lung Cell Mol Physiol, 295: L552-L565. 157491

Siemiatycki J (1991). Risk factors for cancer in the workplace. Boca Raton, FL: CRC Press. 157954

Simon GA; Maibach HI (1998). Relevance of hairless mouse as an experimental model of percutaneous penetration in man. Skin Pharmacol Appl Skin Physiol, 11: 80-6. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9603658. 193647

Simonet BM; Valcárcel M (2009). Monitoring nanoparticles in the environment. Anal Bioanal Chem, 393: 17-21. http://dx.doi.org/10.1007/s00216-008-2484-z 193648

Singh R; Lillard JW Jr (2009). Nanoparticle-based targeted drug delivery. Exp Mol Pathol, 86: 215-223. http://www.sciencedirect.com/science/article/B6WFB-4VB01W6-1/2/15abcbb36ae3fedb3ee77c8b553aad3e. 193650

Skin Cancer Foundation (2008). iVillage Survey Results from May 2007. Retrieved June 02, 2009 from http://www.skincancer.org/ivillage-survey-results.html. 594955

Sonavane G; Tomoda K; Makino K (2008). Biodistribution of colloidal gold nanoparticles after intravenous administration: Effect of particle size. Colloids Surf B Biointerfaces, 66: 274-280. http://www.sciencedirect.com/science/article/B6TFS-4T0FF49-1/2/8faec63ca55e0b4080399510ccc34c8c. 193652

Srinivas CR; Lal S; Thirumoorthy M; Sundaram SV; Karthick PS (2006). Sunscreen application: Not less, not more. Indian J Dermatol Venereol Leprol, 72: 306-307. 157734

Steinberg DC (2007). Global regulations of sunscreens. IFSCC, 10: 3-13. 193656

Stone V; Nowack B; Baun A; van den Brink N; Kammer F; Dusinska M; Handy R; Hankin S; Hassellöv M; Joner E; Fernandes TF (2010). Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation. Sci Total Environ, 408: 1745-1754. http://dx.doi.org/10.1016/j.scitotenv.2009.10.035 633898

Sugibayashi K; Todo H; Kimura E (2008). Safety evaluation of titanium dioxide nanoparticles by their absorption and elimination profiles. J Toxicol Sci, 33: 293-298. 157489

Sun H; Zhang X; Niu Q; Chen Y; Crittenden JC (2007). Enhanced accumulation of arsenate in carp in the presence of titanium dioxide nanoparticles. Water Air Soil Pollut, 178: 245-254. 193662

Sunscreens: Some are short on protection. (2007). Consum Rep, 72, 6. 155618

Takeda K; Suzuki KI; Ishihara A; Kubo-Irie M; Fujimoto R; Tabata M; Oshio S; Nihei Y; Ihara T; Sugamata M (2009). Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. Eisei Kagaku, 55: 95-102. 193667

Tan MH; Commens CA; Burnett L; Snitch PJ (1996). A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. Australas J Dermatol, 37: 185-187. http://dx.doi.org/10.1111/j.1440-0960.1996.tb01050.x 157933

Taylor MR (2008). Assuring the Safety of Nanomaterials in Food Packaging. The Regulatory Process and Key Issues. Washington, DC: Woodrow Wilson International Center for Scholars. http://www.nanotechproject.org/process/assets/files/6704/taylor_gma_pen_packaging1.pdf. 157487

TGA (2006). A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens. Australia: Australian Government, Department of Health and Ageing, Therapeutic Goods Administration. http://www.tga.gov.au/npmeds/sunscreen-zotd.pdf. 089202

The Project on Emerging Nanotechnologies (2009). Inventory of nanotechnology-based consumer products. Retrieved March 01, 2009 from http://www.nanotechproject.org/inventories/consumer/. 196052

Thomas K; Sayre P (2005). Research strategies for safety evaluation of nanomaterials, Part I: Evaluating the human health implications of exposure to nanoscale materials. Toxicol Sci, 87: 316-321. 088085

Tian D; Ma H; Feng Z; Xia Y; Le XC; Ni Z; Allen J; Collins B; Schreinemachers D; Mumford JL (2001). Analyses of micronuclei in exfoliated epithelial cells from individuals chronically exposed to arsenic via drinking water in inner Mongolia, China. J Toxicol Environ Health A Curr Iss, 64: 473-484. 193679

Tiede K; Boxall ABA; Tear SP; Lewis J; David H; Hassellöv M (2008). Detection and characterization of engineered nanoparticles in food and the environment. Food Addit Contam, 25: 795-821. http://dx.doi.org/10.1080/02652030802007553 196278

Tiede K; Hassellov M; Breitbarth E; Chaudhry Q; Boxall AB (2009). Considerations for environmental fate and ecotoxicity testing to support environmental risk assessments for engineered nanoparticles. J Chromatogr A, 1216: 503-509. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18805541. 193680

Tiede K; Tear SP; David H; Boxall AB (2009). Imaging of engineered nanoparticles and their aggregates under fully liquid conditions in environmental matrices. Water Res, 43: 3335-3343. http://dx.doi.org/10.1016/j.watres.2009.04.045 633895

Tin Tin Win S; Mitsushima D; Yamamoto S; Fukushima A; Funabashi T; Kobayashi T; Fujimaki H (2008). Changes in neurotransmitter levels and proinflammatory cytokine mRNA expressions in the mice olfactory bulb following nanoparticle exposure. Toxicol Appl Pharmacol, 226: 192-198. http://dx.doi.org/10.1016/j.taap.2007.09.009 157486

Tok AIY; Boey FYC; Su LT; Ng SH (2009). Flame synthesis of nanoparticles. Retrieved June 02, 2009 from http://www.mse.ntu.edu.sg/research/?op=raree/flame.html. 196054

Trouiller B; Solaimani P; Westbrook A; Reliene R; Schiestl RH (2008, October). TiO2 nanoparticles induce genetic instability and oxidative DNA damage in vivo in mice. Poster presented at Environmental Mutagen Society (EMS) 39th Annual Meeting, Rio Grande, Puerto Rico. 157484

TRS Environmental (2009). TSI-AeroTrak model 9000 nanoparticle aerosol monitor. Retrieved June 02, 2009 from http://www.trs-environmental.com/Model/TSI_AEROTRAK_9000.aspx. 196057

Tsuang YH; Sun JS; Huang YC; Lu CH; Chang WH; Wang CC (2008). Studies of photokilling of bacteria using titanium dioxide nanoparticles. Artif Organs, 32: 167-174. 157483

U.S. Food and Drug Administration (2009). Sunburn protection ractor (SPF). Retrieved October 07, 2009 from http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm106351.htm. 196372

U.S.EPA (2009). Nanomaterial research strategy (final report) (Report No. EPA/620/K-09/011). Washington, DC: Office of Research and Development, U.S. Environmental Protection Agency. http://www.epa.gov/nanoscience/files/nanotech_research_strategy_final.pdf. 625484

U.S. EPA (1991). Acute freshwater crustacean bioassay: 48 hour acute toxicity test using Daphnia magna or Daphnia pulex. In Compendium of ERT toxicity testing procedures (pp. 17-20). (Report No. 40/P-91/009). Washington, DC: U.S. Environmental Proctection Agency; Office of Solid Waste and Emergency Response. http://nepis.epa.gov/Exe/ZyNET.exe/10001YL9.txt?ZyActionD=ZyDocument&Client=EPA&Index=1991%20Thru%201994|Hardcopy%20Publications&Docs=&Query=540P91009%20&Time=&EndTime=&SearchMethod=2&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A\ZYFILES\INDEX%20DATA\91THRU94\TXT\00000000\10001YL9.txt&User=ANONYMOUS&Password=anonymous&SortMethod=-|h&MaximumDocuments=15&FuzzyDegree=0&ImageQuality=r85g16/r85g16/x150y150g16/i500&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x. 667211

U.S. EPA (1992). Guidelines for exposure assessment. Washington, DC: U.S. Environmental Protection Agency . 041875

U.S. EPA (1994). A plain english guide to the EPA Part 503 Biosolids Rule (Report No. EPA/832/R-93/003). Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/OW-OWM.html/mtb/biosolids/503pe/503pe_toc.pdf. 090659

U.S. EPA (1997). Exposure factors handbook (final report) (Report No. EPA/600/P-95/002F a-c). Washington, DC: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464. 594981

U.S. EPA (2002). Community water system survey 2000 Volume I: overview. Washington, DC: Office of Water, U.S. Environmental Protection Agency. http://www.epa.gov/ogwdw/cwssvr.html. 091192

U.S. EPA (2002). Filter backwash recycling rule: Technical guidance manual (Report No. EPA 816-R-02-014). Washington, DC: U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water. http://www.epa.gov/safewater/mdbp/pdf/filterbackwash/fbrr_techguidance.pdf. 644800

U.S. EPA (2004). Air quality criteria for particulate matter (Report No. EPA/600/P-99/002aF-bF). Research Triangle Park, NC: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=87903. 056905

U.S. EPA (2006). 2006 Edition of the drinking water standards and health advisories (Report No. EPA 822-R-06-013). Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/waterscience/criteria/drinking/dwstandards.pdf. 091193

U.S. EPA (2006). Biosolids: Frequently asked questions. Retrieved September 27, 2007 from http://www.epa.gov/owm/mtb/biosolids/genqa.htm. 090658

U.S. EPA (2006). FACTOIDS: Drinking water and ground water statistics for 2005 (Report No. EPA 816-I-03-001). Washington, D.C.: U.S. Environmental Protection Agency. http://www.epa.gov/safewater/databases/pdfs/statistics_data_factoids_2005.pdf. 091194

U.S. EPA (2006). U.S. Environmental Protection Agency peer review handbook (Report No. EPA/100/B-06/002). Washington, DC: U.S. Environmental Protection Agency, Science Policy Council. http://www.epa.gov/peerreview/pdfs/peer_review_handbook_2006.pdf. 194566

U.S. EPA (2007). Arsenic in drinking water: Basic information. Retrieved September 25, 2007 from http://www.epa.gov/safewater/arsenic/basicinformation.html. 091224

U.S. EPA (2007). Exposure and Fate Assessment Screening Tool Version 2.0 (E-FAST V2.0). Retrieved June 02, 2009 from http://www.epa.gov/opptintr/exposure/pubs/efast.htm. 196060

U.S. EPA (2007). Nanotechnology white paper (Report No. EPA 100/B-07/001). Washington, DC: Science Policy Council, Nanotechnology Workgroup, U.S. Environmental Protection Agency. http://www.epa.gov/osa/pdfs/nanotech/epa-nanotechnology-whitepaper-0207.pdf. 090564

U.S. EPA (2008). Child-specific exposure factors handbook (Report No. EPA/600/R-06/096F). Washington, D.C.: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243. 196062

U.S. EPA (2008). Sampling and analysis of nanomaterials in the environment: A state-of-the-science review (Report No. EPA/600/R-08/098). Washington, DC: U.S. Environmental Protection Agency. http://www.ntis.gov/search/product.aspx?ABBR=PB2009113239. 157480

U.S. EPA (2008). Technical overview of ecological risk assessment analysis phase: Ecological effects characterization. Retrieved July 13, 2008 from http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm. 157481

U.S. EPA (2009). Exposure assessment models. Retrieved June 02, 2009 from http://www.epa.gov/ceampubl/. 196064

U.S. EPA (2009). Integrated science assessment for particulate matter: Annex B Dosimetry: Table B-2 ultrafine disposition in animals. Washington, D.C.: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546. 196063

U.S. EPA (2009). Models knowledge base. Retrieved June 02, 2009 from http://cfpub.epa.gov/crem/knowledge_base/knowbase.cfm. 196065

U.S. EPA (2010). Drinking water treatment wastes. Retrieved August 24, 2010 from http://www.epa.gov/rpdweb00/tenorm/drinking-water.html#disposal. 635678

U.S. EPA (2010). Workshop Summary for the EPA Board of Scientific Counselors (Report No. EPA/600/R-10/042). Research Triangle Park, NC: National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. 625483

U.S. FDA (1999). Final rule for sunscreen drug products for over-the-counter human use. Retrieved June 02, 2009 from http://www.access.gpo.gov/nara/cfr/waisidx_08/21cfr352_08.html. 196374

U.S. FDA (2006). Nanoscale Materials: Nomination and Review of Toxicological Literature. Washington, DC: Chemical Selection Working Group, U.S. Food & Drug Administration. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Nanoscale_materials.pdf. 157728

U.S. Pharmacopeia (2006). Titanium dioxide . In U.S. pharmacopeia official monographs (p. 3364). Rockville, MD: U.S. Pharmacopeia. 155639

Uchino T; Tokunaga H; Ando M; Utsumi H (2002). Quantitative determination of OH radical generation and its cytotoxicity induced by TiO2-UVA treatment. Toxicol In Vitro, 16: 629-635. 090568

Umicore (2008). Nano-sized oxide powders for UV applications. Abstract from Innovation for Sustainable Production (i-SUP2008), Bruges, Belgium. 193688

Umweltbundesamt (2009). Anmeldung und Zulassung neuer Stoffe. Retrieved June 02, 2009 from http://www.umweltbundesamt-umwelt-deutschland.de/umweltdaten/public/theme.do?nodeIdent=2289. 196071

UNEP (2007). Chapter 7: Emerging challenges-nanotechnology and the environment. In GEO Year Book 2007 (pp. 61-70). Nairobi, Kenya: United Nations Environment Programme Division of Early Warning and Assessment. http://www.unep.org/yearbook/2007/ 196074

Uniqema (2004). Solaveil CT-200 (Report No. PC/E/03-03/GLOB/14.5/CT200). London: Uniqema. 155637

USGS (2009). Mineral commodity summaries 2009: Titanium and titanium dioxide. Washington, D.C.: U.S. Geological Survey. http://minerals.usgs.gov/minerals/pubs/commodity/titanium/mcs-2009-titan.pdf. 157454

Vanoverbeke J (2008). Modeling individual and population dynamics in a consumer-resource system: Behavior under food limitation and crowding and the effect on population cycling in Daphnia. Ecol Modell, 216: 385-401. http://dx.doi.org/10.1016/j.ecolmodel.2008.05.009 157477

van den Brink W (2008). Monitoring of airborne nano particles at industrial workplaces by means of a portable device will enable strategies to reduce exposure levels and improve the health and safety of workers. Retrieved June 02, 2009 from http://www.nanosafe.org/scripts/home/publigen/content/templates/show.asp?P=96&L=EN#6. 196075

van Ravenzwaay B; Landsiedel R; Fabian E; Burkhardt S; Strauss V; Ma-Hock L (2009). Comparing fate and effects of three particles of different surface properties: Nano-TiO2, pigmentary TiO2 and quartz. Toxicol Lett, 186: 152-159. http://www.sciencedirect.com/science/article/B6TCR-4V3HF9R-4/2/222953228b852fa49130adfc6334da99. 193689

Velzeboer I; Hendriks AJ; Ragas AM; van de Meent D (2008). Aquatic ecotoxicity tests of some nanomaterials. Environ Toxicol Chem, 27: 1942-1947. 157476

Verhulst D; Sabacky BJ; Spitler TM; Prochazka J (2003). Process for the production of nano-sized TiO2 and other ceramic oxides by spray hydrolysis. Reno, Nevada: Altair Nanomaterials Inc. 157854

Vevers WF; Jha AN (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO2) nanoparticles on fish cells in vitro. Ecotoxicology, 17: 410-420. 157475

Vormberg R (2004). Highly dispersed titanium dioxide: From a pigment to a marvel of versatility [brochure]. Dusseldorf, Germany: Degussa Corporate Communications. 157822

Wahi RK; Liu Y; Faulner JC; Colvin VL (2006). Solvothermal synthesis and characterization of anatase TiO2 nanocrystals with ultrahigh surface area. J Colloid Interface Sci, 302: 530-536. 090580

Wakefield G; Lipscomb S; Holland E; Knowland J (2004). The effects of manganese doping on UVA absorption and free radical generation of micronised titanium dioxide and its consequences for the photostability of UVA absorbing organic sunscreen components. Photochem Photobiol Sci, 3: 648-652. http://www.rsc.org/delivery/_ArticleLinking/DisplayArticleForFree.cfm?doi=b403697b&JournalCode=PP. 193693

Wang H; Wick RL; Xing B (2009). Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditis elegans. Environ Pollut, 157: 1171-1177. http://www.sciencedirect.com/science/article/B6VB5-4V3S568-1/2/97684af3036f267787e67152a75879a3. 193696

Wang J; Chen C; Liu Y; Jiao F; Li W; Lao F; Li Y; Li B; Ge C; Zhou G; Gao Y; Zhao Y; Chai Z (2008). Potential neurological lesion after nasal instillation of TiO2 nanoparticles in the anatase and rutile crystal phases. Toxicol Lett, 183: 72-80 . http://dx.doi.org/10.1016/j.toxlet.2008.10.001 157474

Wang J; Jiang Z; Zhang L; Kang P; Xie Y; Lv Y; Xu R; Zhang X (2009). Sonocatalytic degradation of some dyestuffs and comparison of catalytic activities of nano-sized TiO(2), nano-sized ZnO and composite TiO(2)/ZnO powders under ultrasonic irradiation. Ultrason Sonochem, 16: 225-231. http://dx.doi.org/10.1016/j.ultsonch.2008.08.005 157453

Wang J; Liu Y; Jiao F; Lao F; Li W; Gu Y; Li Y; Ge C; Zhou G; Li B; Zhao Y; Chai Z; Chen C (2008). Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO2 nanoparticles. Toxicology, 254: 82-90. http://dx.doi.org/10.1016/j.tox.2008.09.014 157473

Wang JX; Chen CY; Sun J; Yu HW; Li YF; Li B; Xing L; Huang YY; He W; Gao YX; Chai ZF; Zhao YL (2005). Translocation of inhaled TiO2 nanoparticles along olfactory nervous system to brain studied by synchrotron radiation X-ray fluorescence. HEP and NP, 29 Suppl: 76-79. 193703

Wang JX; Li YF; Zhou GQ; Li B; Jiao F; Chen CY; Gao YX; Zhao YL; Chai ZF (2007). [Influence of intranasal instilled titanium dioxide nanoparticles on monoaminergic neurotransmitters of female mice at different exposure time]. Zhonghua Yu Fang Yi Xue Za Zhi, 41: 91-95. 157616

Wang JX; Zhou GQ; Chen CY; Yu HW; Wang TC; Ma YM; Jia G; Gao YX; Li B; Sun J; Li YF; Jiao F; Zhao YL; Chai ZF (2007). Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett, 168: 176-185. 090290

Wang S; Zhang X; Zhang J; Chang A (2008). Research on the treatment for desulphurization wastewater by nano-titanium dioxide. Paper presented at 2nd International Conference on Bioinformatics and Biomedical Engineering (ICBBE), Shanghai, China. 193705

Warheit DB (2008). How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? Toxicol Sci, 101: 183-185. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18300382. 193706

Warheit DB (2008). Information on nano-TiO2 in DuPont reports from June 5, 2008 phone conversation. Retrieved November 18, 2010 from http://cfpub.epa.gov/ncea/hero/index.cfm?action=reference.edit&reference_id=157470. 157470

Warheit DB; Borm PJA; Hennes C; Lademann J (2007). Testing strategies to establish the safety of nanomaterials: Conclusions of an European Centre for Ecotoxicology and Toxicology of Chemicals workshop. Inhal Toxicol, 19: 631-643. 091305

Warheit DB; Hoke RA; Finlay C; Donner EM; Reed KL; Sayes CM (2007). Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicol Lett, 171: 99-110. http://www.sciencedirect.com/science/article/B6TCR-4NKJ0FS-2/2/e07ab27a1d6eae912b786e960541ade6. 091075

Warheit DB; Webb TR; Reed KL; Frerichs S; Sayes CM (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: Differential responses related to surface properties. Toxicology, 230: 90-104. http://dx.doi.org/10.1016/j.tox.2006.11.002 090594

Warheit DB; Webb TR; Sayes CM; Colvin VL; Reed KL (2006). Pulmonary instillation studies with nanoscale TiO2 rods and dots in rats: Toxicity is not dependent upon particle size and surface area. Toxicol Sci, 91: 227-236. 088436

Watlington K (2005). Emerging nanotechnologies for site remediation and wastewater treatment. Washington, D.C.: U.S. Environmental Protection Agency, Technology Innovation and Field Services Division. http://www.clu-in.org/download/studentpapers/K_Watlington_Nanotech.pdf. 196080

Weaver J; Umbreit TH; Miller TJ; Zhang J; Stratmeyer ME; Tomazic-Jezic FJ (2007). Toxicology of titanium dioxide (TiO2) nanoparticles: Immunological effects in subcutaneously and intravenously injected mice. Abstract from Society of Toxicology 46th Annual Meeting, Charlotte, NC. 193713

Weinstein JM; Yarnold P; Hornung R (2001). Parental knowledge and practice of primary skin cancer prevention: Gaps and solutions. Pediatr Dermatol, 18: 473-477. 191128

Weinstock MA; Rossi JS; Redding CA; Maddock JE; Cottrill SD (2000). Sun protection behaviors and stages of change for the primary prevention of skin cancers among beachgoers in southeastern New England. Ann Behav Med, 22: 286-293. 193716

Wiench K; Landsiedel R; Zok S; Hisgen V; Radkel K; Van Ravenzwaay B (2007). Aquatic fate and toxicity of nanoparticles: Agglomeration, sedimentation and effects of daphnia magna [Abstract no 1384]. Retrieved October 05, 2009 from www.sustainability.basf.com/basfcorp/img/sustainability/dialog/Wiench_SOT_OekoNano_Poster.pdf. . 090635

Wigginton NS; Haus KL; Hochella MF (2007). Aquatic environmental nanoparticles. J Environ Monit, 9: 1306-1316. 157415

Woodrow Wilson International Center for Scholars (2006). Innovative® Skincare SPF 20 Sunscreen Powder. Retrieved June 02, 2009 from http://www.nanotechproject.org/inventories/consumer/browse/products/5248/. 196083

Wu J; Liu W; Xue C; Zhou S; Lan F; Bi L; Xu H; Yang X; Zeng FD (2009). Toxicity and penetration of TiO2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicol Lett, 191: 1-8. http://dx.doi.org/10.1016/j.toxlet.2009.05.020 193721

Xia T; Kovochich M; Brant J; Hotze M; Sempf J; Oberley T; Sioutas C; Yeh JI; Wiesner MR; Nel AE (2006). Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett, 6: 1794-1807. 089620

Xu A; Chai Y; Nohmi T; Hei TK (2009). Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells. Part Fibre Toxicol, 6: 3. http://dx.doi.org/10.1186/1743-8977-6-3 157452

Xu T; Cai Y; O'Shea KE (2007). Adsorption and photocatalyzed oxidation of methylated arsenic species in TiO2 suspensions. Environ Sci Technol, 41: 5471-5477. http://dx.doi.org/10.1021/es0628349 193725

Xu X-R; Li S-X; Li X-Y; Gu J-D; Chen F; Li X-Z; Li H-B (2009). Degradation of n-butyl benzyl phthalate using TiO2/UV. J Hazard Mater, 164: 527-532. http://www.sciencedirect.com/science/article/B6TGF-4T7XGW2-6/2/f20c93956e21e788fa424d9d3e6e21f3. 193726

Xu Z; Jing C; Li F; Meng X (2008). Mechanisms of photocatalytical degradation of monomethylarsonic and dimethylarsinic acids using nanocrystalline titanium dioxide. Environ Sci Technol, 42: 2349-2354. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18504964. 193727

Yamadori I; Ohsumi S; Taguchi K (1986). Titanium dioxide deposition and adenocarcinoma of the lung. Acta Pathol Jpn, 36: 783-790. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3739712. 193728

Yamamoto A; Honma R; Sumita M; Hanawa T (2004). Cytotoxicity evaluation of ceramic particles of different sizes and shapes. J Biomed Mater Res A, 68: 244-256. 157820

Yang F; Hong F; You W; Liu C; Gao F; Wu C; Yang P (2006). Influences of nano-anatase TiO2 on the nitrogen metabolism of growing spinach. Biol Trace Elem Res, 110: 179-190. 157723

Yang K; Lin D; Xing B (2009). Interactions of humic acid with nanosized inorganic oxides. Langmuir, 25: 3571-3576. http://dx.doi.org/10.1021/la803701b 190513

Zhang LW; Monteiro-Riviere NA (2008). Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. Skin Pharmacol Physiol, 21: 166-80. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18523414. 193735

Zhang X; Sun H; Zhang Z; Niu Q; Chen Y; Crittenden JC (2007). Enhanced bioaccumulation of cadmium in carp in the presence of titanium nanoparticles. Chemosphere, 67: 160-166. http://dx.doi.org/10.1016/j.chemosphere.2006.09.003 090114

Zhang XZ; Sun HW; Zhang ZY (2006). [Bioaccumulation of titanium dioxide nanoparticles in carp]. Huan Jing Ke Xue, 27: 1631-1635. 157722

Zhang Y; Chen Y; Westerhoff P; Hristovski K; Crittenden JC (2008). Stability of commercial metal oxide nanoparticles in water. Water Res, 42: 2204-2212. 157462

Zheng L; Hong F; Lu S; Liu C (2005). Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. Biol Trace Elem Res, 104: 83-92. 157784

Zhu S; Oberdorster E; Haasch ML (2006). Toxicity of an engineered nanoparticle (fullerene, C60) in two aquatic species, Daphnia and fathead minnow. Mar Environ Res, 62 Suppl: S5-S9. http://dx.doi.org/10.1016/j.marenvres.2006.04.059 157721

Zhu X; Zhu L; Duan Z; Qi R; Li Y; Lang Y (2008). Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to Zebrafish (Danio rerio) early developmental stage. J Environ Sci Health A Tox Hazard Subst Environ Eng, 43: 278-284. http://www.informaworld.com/10.1080/10934520701792779. 193742

Zimmerer RE; Lawson KD; Calvert CJ (1986). The effects of wearing diapers on skin. Pediatr Dermatol, 3: 95-101. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3952034. 193744

Annex A. Nano-TiO_2 in Sunscreen: Background Information

Nano-TiO_2 has been used in topical sunscreen products since approximately 1990 (EWG, 2008, 196343). 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 approximately $38 million, and has maintained approximately a 20% share of the sunscreen ingredient market as a whole (Dransfield, 2005, 157809). Dransfield (2005, 157809) projected that the market for inorganic active ingredients in sunscreens would grow to approximately $75 million by 2010 which would account for one-third of the total market for sunscreen active ingredients. Dransfield (2005, 157809) 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 Ti sunscreens and 30% of zinc sunscreens in Australia were formulated with nanoparticles (TGA, 2006, 089202).

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, 196053). 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 to $575 million (Davis, 1994, 157946). 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, 157682). 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, 157745). As a "mature" market in the U.S., sun protection products are expected to have a growth rate of only approximately 2% per year (Jeffries, 2007, 157682). Between 2005 and 2010, however, growth in the sun-care market was expected to be much faster abroad than in the U.S. (Jeffries, 2007, 157682). If the growth in cosmeceuticals has dampened demand for conventional sunscreen, this growth has led to even greater demand for sunscreen active ingredients, including micronized TiO_2 (Davis, 1994, 157946).

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

UV radiation is classified by wavelength into three types: UV-A (320-400 nm), UV-B (290-320 nm), and UV-C (200-290 nm). The shorter the wavelength, the more energy the UV radiation transmits. Consequently, the shorter wavelength rays can cause more damage to skin than the longer wavelength rays. Approximately 10% of the solar radiation that reaches Earth's surface is UV, and approximately 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

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

can be dangerous, it is blocked by ozone in the atmosphere and does not reach Earth's surface (Jeffries, 2007, 157682; Shao and Schlossman, 1999, 093301).

The traditional SPF rating system measures protection against UV-B radiation only. The U.S. 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 (72 FR 49070). This system was expected to go into effect in November 2008 or later but has not been finalized as of April 2010. 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, 193559).

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 unique 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, 093301) found that a nano-TiO_2 dispersion with a primary particle size of approximately 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 indicated 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, 157809) presented data indicating that TiO_2 particles (not specifying whether they were primary or secondary particles) at 100 nm diameter provide the best UV-A protection but also significant visible light attenuation (i.e., leaving a white hue on skin if such particles are used in sunscreens), and particles in the range of 40 to 60 nm provided the best UV-B protection. In addition, 20-nm nano-TiO_2 did not provide sufficient protection against UV-A or UV-B. 200-nm TiO_2 particles provide poor UV-A and UV-B protection and high attenuation of visible light (Dransfield, 2005, 157809). According to Hewitt (2002, 093307), theoretical calculations suggest that the optimal mean TiO_2 primary particle size for good UV-B and UV-A protection is approximately 50 nm. Chaudhuri and Majewski (1998, 093308) noted that nano-TiO_2 with a primary crystal size of 10-20 nm and an effective particle size of approximately 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, 093301). Aggregation will also make a formulation more opaque (Chaudhuri and Majewski, 1998, 093308). 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, 093309) 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, 093309) 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, 093308) 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, 155626) 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, 093308) 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, 093307) 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, 155639), declares that TiO_2 "contains not less than 99.0% and not more than 100.5% of TiO_2." 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, 093308; Maynard, 2008, 157522). Although anatase is less photostable, it appears to be in common use. Barker and Branch (2008, 180141) 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, 092740). Other TiO_2 or nano-TiO_2 surface coatings mentioned in the literature include inorganic oxides (Bird, 2002, 093306), simethicone (Chaudhuri and Majewski, 1998, 093308), methicone, lecithin (Schlossman et al., 2006, 093309), stearic acid, glycerol, silica, aluminum stearate, dimethicone (SCCNFP, 2000, 092740), metal soap, isopropyl titanium triisostearate (ITT), triethoxy caprylylsilane, and C9-15 fluoroalcohol phosphate (Schlossman et al., 2006, 093309). 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-1.

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. 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, 155634; Wakefield et al., 2004, 193693). Doped TiO_2 is colored instead of white, which can have desirable cosmetic effects in products such as skin lighteners (Park et al., 2006, 193593).

Recent research by Barker and Branch (2008, 180141) 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% rutile, and is doped with manganese rather than surface-coated. It is not known 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, 193297; Maynard, 2008, 157522).

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 properties such as 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."

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, 093306). Surface coatings that make TiO_2 dispersible in nonaqueous 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, 093301). For methicone and C9-15 fluoroalcohol phosphate, silicone might be the preferred medium (Shao and Schlossman, 1999, 093301). Bird (2002, 093306) 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, 093308) describe one product, an "amphiphilic" powder (Eusolex® T-2000) containing approximately 80% 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_{pzc}, which is the pH 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_{pzc} 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, 157939). 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, 093308). Bird (2002, 093306) 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, 157939).

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, 093301). 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 made by 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, 157825). Mitchnick and O'Lenick (1996, 157935) 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 emulsions 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, 157898). 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, 157809).

The emulsifiers used to keep the two phases from separating are typically partially hydrophilic and partially hydrophobic. 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, 157793).

A.1.6. Other Ingredients – Active and Inactive

Nano-TiO_2 can be combined with other physical UV blockers, such as 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, 157939).

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, 093306; Chaudhuri and Majewski, 1998, 093308).

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, 157936).

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, 157898).

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

Table A-1 was compiled from information contained in the Environmental Working Group's cosmetic database "Skin Deep" (EWG, 2008, 196343) 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. 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	TiSilc Sheer, SPF 45	N/A
ProCyte	TiSilc Sheer, SPF 45 (tinted)	3.5%
ProCyte	TiSilc Sunblock, SPF 60+	8%
ProCyte	TiSilc 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: Used with permission from the Environmental Working Group, for their Skin Deep Database (EWG, 2008, 196343).

REFERENCES

Barker PJ; Branch A (2008). The interaction of modern sunscreen formulations with surface coatings. Progr Org Coating, 62: 313-320. http://dx.doi.org/10.1016/j.porgcoat.2008.01.008 180141

Bird S (2002). Sense and stability. Soap, Perfum Cosmet, 75, 42-44. 093306

Brausch JM; Smith PN (2009). Pesticide resistance from historical agricultural chemical exposure in Thamnocephalus platyurus (Crustacea: Anostraca). Environ Pollut, 157: 481-487. 193297

Chaudhuri RK; Majewski G (1998). Amphiphilic microfine titanium dioxide: Its properties and application in sunscreen formulations. Drug Cosmet Ind, 162: 24-31. 093308

Davis DA (1994). Sunscreen oddities. Drug Cosmet Ind, 155: 20-24. 157946

Dransfield G (2005). Manufacture of novel, transparent TiO2 based sunscreens. Retrieved May 09, 2008 from http://www.wun.ac.uk/nanomanufacturing/archive/05_06_series/documents/dransfield.pdf. 157809

EWG (2008). Sunscreen investigation: Skin deep-cosmetic safety reviews. Retrieved June 02, 2009 from http://www.cosmeticsdatabase.com/special/sunscreens2008/. 196343

Hewitt J (2002). A moment of clarity. Soap, Perfum Cosmet, 75, 47-50. 093307

Hewitt JP (1995). Formulating with physical sunscreens: Control of emulsion pH. Drug Cosmet Ind, 157: 28-32. 157939

Hewitt JP (1996). The influence of emollients on dispersion of physical sunscreens. Drug Cosmet Ind, 159: 62-65. 157936

Hewitt JP (2000). Partners in protection. Soap, Perfum Cosmet, 73, 85-86. 157898

Himics R; Pineiro R (2008). The Importance of Particle Size in Liquid Coatings - Coating problems and solutions associated with particle size reduction. Prod Finish, 63: 46-53. http://www.pfonline.com/articles/the-importance-of-particle-size-in-liquid-coatings. 155626

Jeffries N (2007, February). SPF, efficacy and innovation. GCI Magazine. http://www.gcimagazine.com/marketstrends/segments/suncare/27627099.html. 157682

Maynard AD (2008). Living with nanoparticles. Nano Today, 3: 64. 157522

Mitchnick M; O'Lenick AJ Jr (1996). Patent No. 5565591 (class: 556/10). United States: United States Patent Office. http://www.freepatentsonline.com/5565591.html. 157935

Moyal D (2008). How to measure UVA protection afforded by sunscreen products. Expert Review of Dermatology, 3: 307-313. http://dx.doi.org/10.1586/17469872.3.3.307 193559

Newman KA (2006, December). Sun protection report. GCI Magazine. http://www.gcimagazine.com/marketstrends/segments/suncare/4829426.html?page=1. 157745

Oxonica (2005). Technical notes: Example formulation details and protocol tips to obtain optimal dispersion of Optisol (TM) UV Absorber. Retrieved April 04, 2007 from http://www.oxonica.com/media/media_promoliterature.php?start=6. 157793

Packaged Facts (2001). The U.S. market for suncare and lipcare products. Retrieved March 31, 2001 from http://www.mindbranch.com/listing/product/R567-393.html. 196053

Park GB; Knowland JS; Flutter BR (2006). Patent No. 20060134026, class: 424/59. United States: U.S. Patent and Trademark Agency. http://www.freepatentsonline.com/y2006/0134026.html. 193593

Reisch MS (2005). New-wave sunscreens: active ingredient makers are frustrated by the long list of sunscreens and UV-A testing protocols that are still awaiting FDA decisions. Chem Eng News, 83: 18-22. http://pubs.acs.org/cen/coverstory/83/8315sunscreens.html. 155634

SCCNFP (2000). Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning titanium dioxide Colipa n S75 (Report No. SCCNFP/0005/98). Brussels, Belgium: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out135_en.pdf. 092740

Schlossman D; Shao Y; Detrieu P (2006). Perspectives on supplying attenuation grades of titanium dioxide and zinc oxide for sunscreen applications. Retrieved October 28, 2007 from http://www.fda.gov/nanotechnology/meetings/kobo_files/textmostly/index.htm. 093309

Shao Y; Schlossman D (1999). Effect of particle size on performance of physical sunscreen formulas. Talk presented at PCIA Conference, Shanghai, China. http://www.koboproductsinc.com/Downloads/PCIA99-Sunscreen.pdf. 093301

Shao Y; Schlossman D (2004). Discovering an optimum small micropigment for high UV shielding and low skin whitening. Talk presented at 23d IFSCC Congress, Orlando, FL. http://www.koboproductsinc.com/Downloads/IFSCC2004.pdf. 157825

TGA (2006). A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens. Australia: Australian Government, Department of Health and Ageing, Therapeutic Goods Administration. http://www.tga.gov.au/npmeds/sunscreen-zotd.pdf. 089202

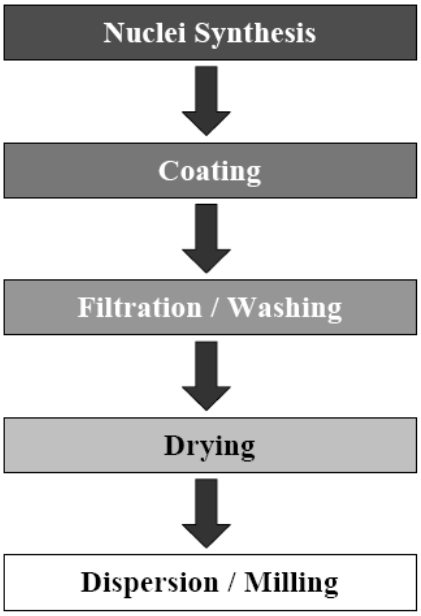
U.S. Pharmacopeia (2006). Titanium dioxide . In U.S. pharmacopeia official monographs (p. 3364). Rockville, MD: U.S. Pharmacopeia. 155639

Wakefield G; Lipscomb S; Holland E; Knowland J (2004). The effects of manganese doping on UVA absorption and free radical generation of micronised titanium dioxide and its consequences for the photostability of UVA absorbing organic sunscreen components. Photochem Photobiol Sci, 3: 648-652. http://www.rsc.org/delivery/_ArticleLinking/DisplayArticleForFree.cfm?doi=b403697b&JournalCode=PP. 193693

Annex B. Nano-TiO_2 in Sunscreen: Manufacturing Processes

B.1. Overview of Nano-TiO_2 Manufacturing Process

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



Source: Dransfield (2005, 157809)

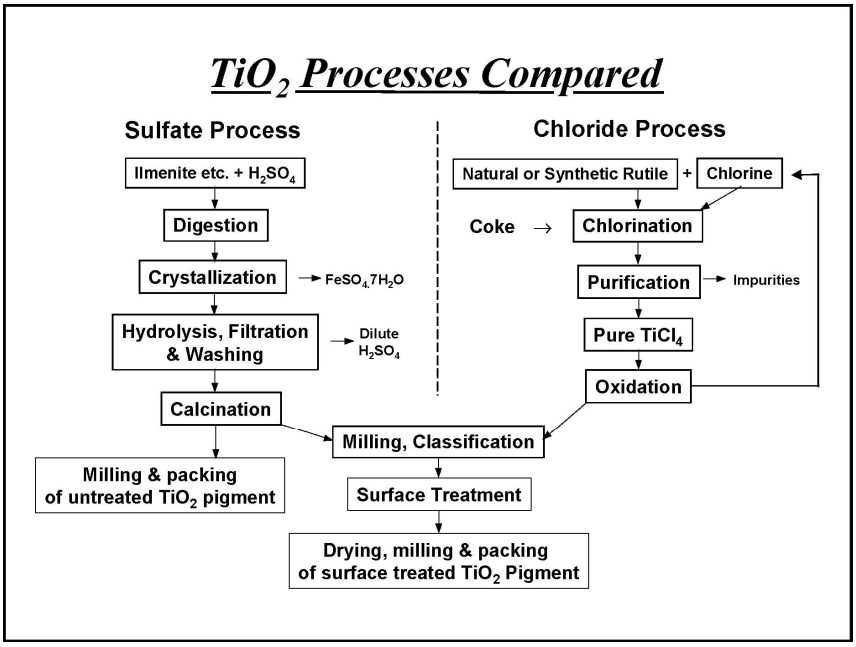
Figure B-1. Generic manufacturing process for nano-TiO_2 for sunscreens.

B.1.1. Titanium Dioxide Nuclei Synthesis

Commercial-scale TiO2 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 TiO2. 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-TiO2 synthesis by Chen and Mao (2007, 193313). The sulfate process and

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

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: Millennium Inorganic Chemicals (2007, 195899).

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

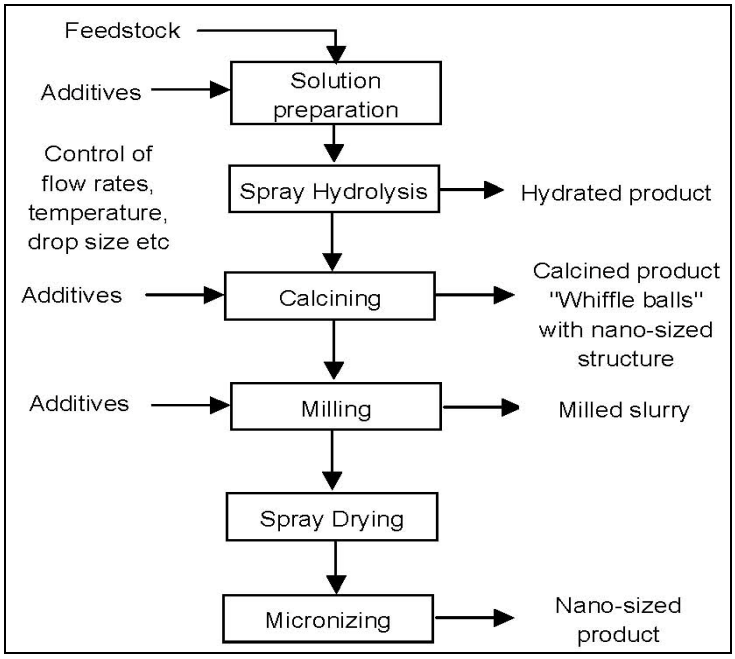
The sulfate process, a wet process for creating pigmentary TiO_2, dates from around 1930, and it was the dominant method used to produce TiO_2 until the chloride process was developed in the 1950s (Hext et al., 2005, 090567). The chloride process now accounts for approximately 60% of worldwide TiO_2 pigment production (Hext et al., 2005, 090567). 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, 157743). 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, 093309), 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, 157854). 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

[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" (Scientific Committee, 2007, 157639).

Verhulst et al. (2003, 157854), is the spray hydrolysis step, which eliminates the aqueous filtration step.



Source: Reprinted with permission for Verhulst et al. (2003, 157854)

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

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, 157809). 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, 195899).

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, 195899).

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, 195899).

The following equations represent the chemical processes involved in the sulfate process (Dransfield, 2005, 157809):

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

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

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 900ºC. 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, 195899).

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, 195899).

The following equations represent the chemical processes involved in the chloride process (Dransfield, 2005, 157809):

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

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

Specific Steps in the Altair Process – Spray Hydrolysis

The patented Altair process (Verhulst et al., 2003, 157854) 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, 157854).

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, 157854) 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, 157854).

Milling and Micronizing for Sulfate, Chloride, and Altair Processes

Milling breaks apart the hollow crystalline lattice [1] structure produced in the calcination step, but has to be mild enough not to break the individual crystallites (Verhulst et al., 2003, 157854). 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, 157854). After spray drying, the milled particles ("loosely agglomerated balls") can be "further micronized to produce a dispersed powder." While both micronizing and milling decrease the agglomerates, they are different processes.

In micronizing, agglomerates collide with each other in a circulating stream of air or steam, and the collision breaks down the agglomerated particles. In milling, an external grinding agent is used to decrease the size of agglomerates. For instance, agglomerates in a liquid medium are fed into a mill containing small ceramic beads, and the impact from the beads on the agglomerates during mixing break the agglomerated particles.

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, 157699; Schlossman et al., 2006, 093309; Wakefield et al., 2004, 193693). 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, 157935) 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 40-100ºC 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, 193593) 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 300ºC. 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 [2] or plasma routes (no additional detail provided).

[1] Lattice is the geometrical arrangement of atoms in a crystal.

[2] 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 B1 (SCCNFP, 2000, 092740), and an additional list is on the internet (EWG, 2009, 625314). 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 U.S.

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%
Tioveil dispersions	Uniqema	rutile	10-28 nm	alumina 10.5-12.5% or 5-15% and silica 3.5-5.5%; alumina 5-15% and aluminum stearate 5-15%

Source: Used with permission of the European Union, SSCNFP (Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers) (SCCNFP, 2000, 092740).

Three manufacturers of 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 [1] and specialty chemicals for cosmetics and personal care products. Uniqema was acquired by Croda in 2006 (Croda, 2006, 193851).

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

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

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, 196045). 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, 196045; Shao and Schlossman, 1999, 093301). Polyhydroxystearic acid is used to disperse the product in the solvent/carrier, C12-15 alkyl benzoate, which is an ester (Kobo Products Inc, 2009, 196045; Shao and Schlossman, 1999, 093301). 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, 196045; Shao and Schlossman, 1999, 093301).

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, 196045; Shao and Schlossman, 1999, 093301). Doping with manganese gives the sunscreen the advantages of increased UV-A absorption, reduced free radical generation, and increased free radical scavenging behavior (Reisch, 2005, 155634; Umicore, 2008, 193688).

Uniqema/Croda[1] manufactures several TiO_2 sunscreens, including a line of Solaveil™ Clarus using nano-TiO_2 (Chandler, 2006, 193834). 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, 193875). 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, 193878). The TiO_2 particle size distribution is very narrow, with the vast majority of particles falling in the nano-range (Croda, 2008, 193878). Uniqema (2004, 155637) recommended 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 water phase in water-in-oil (w/o) emulsion, or added separately to a w/o emulsion after emulsification (Uniqema, 2004, 155637).

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-2 shows a sample formulation using Croda Solaveil CT-10W and Solaveil CT-200 (Croda, 2009, 193880). Table B-3 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, 196045).

[1] Croda acquired Uniqema in 2006 (Croda, 2006, 193851). In this Appendix, information sources are cited as it was presented at the time of publication.

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) TiO_2 (and) isodeceth-6 (and) oleth-10 (and) aluminum stearate (and) alumina (and) simethicone]	5.00
Part B	
Solaveil CT-200 [TiO_2 (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, 193880).

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) TiO_2 (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 * Mix each Part separately. Make sure Net-WO is dispersed in Part 1. Heat both Parts to 40ºC and add Part 2 to Part 1 while stirring with homogenizer at 3,000 rotations per minute (rpm). Increase the rotation to 5,000 rpm and continue to emulsify for 5 minutes. Cool down to room temperature with sweeping mixer.	

Source: Kobo Products Inc. (2009, 196045).

REFERENCES

Chandler M (2006). Innovative UV Protection. Creating your advantage in sunscreen products. Talk presented at Croda Educational eSeminar, Edison, NJ. http://www.cosmeticsandtoiletries.com/networking/eventcoverage/3729901.html. 193834

Chen X; Mao SS (2007). Titanium dioxide nanomaterials: Synthesis, properties, modifications, and applications. Chem Rev, 107: 2891-2959. 193313

Croda (2006). Croda invests in growth: Acquires Uniqema [brochure]. Cosmetics & Toiletries. 193851

Croda (2007). Product Trade Name: SOLAVEIL CT-100 [brochure]. 193875

Croda (2008). Chemical Composition of Solaveil CT-200 [brochure]. 193878

Croda (2009). Weightless Morning Dew with Sun Protection (SC-383-1) [brochure]. 193880

Dransfield G (2005). Manufacture of novel, transparent TiO2 based sunscreens. Retrieved May 09, 2008 from http://www.wun.ac.uk/nanomanufacturing/archive/05_06_series/documents/dransfield.pdf. 157809

DuPont (2007). Nanomaterial Risk Assessment Worksheet DuPont(TM) Light Stabilizer. Retrieved June 18, 2008 from http://www.edf.org/documents/6913_TiO2_Worksheet.pdf. 157699

EWG (2009). EWG's 2009 sunscreen investigation - Section 5: Impact of nanoparticles. Retrieved April 29, 2010 from http://www.ewg.org/cosmetics/report/sunscreen09/investigation/impact-of-nanoparticles. 625314

Hext PM; Tomenson JA; Thompson P (2005). Titanium dioxide: inhalation toxicology and epidemiology. Ann Occup Hyg, 49: 461-472. 090567

Kobo Products Inc (2009). Attenuation grade TiO2 dispersions. Retrieved July 16, 2009 from http://www.koboproductsinc.com/Downloads/Kobo-TiO2Dispersions.pdf. 196045

Millennium Inorganic Chemicals (2007). Titanium dioxide manufacturing processes. Retrieved February 16, 2009 from http://www.millenniumchem.com/Products+and+Services/Products+by+Type/Titanium+Dioxide+-+Paint+and+Coatings/r_TiO2+Fundamentals/Titanium+Dioxide+-+Paint+and+Coatings+TiO2+Fundamentals_EN.htm. 195899

Mitchnick M; O'Lenick AJ Jr (1996). Patent No. 5565591 (class: 556/10). United States: United States Patent Office. http://www.freepatentsonline.com/5565591.html. 157935

Osterwalder N; Capello C; Hungerbühler K; Stark WJ (2006). Energy consumption during nanoparticle production: how economic is dry synthesis? J Nanopart Res, 8: 1-9. 157743

Park GB; Knowland JS; Flutter BR (2006). Patent No. 20060134026, class: 424/59. United States: U.S. Patent and Trademark Agency. http://www.freepatentsonline.com/y2006/0134026.html. 193593

Reisch MS (2005). New-wave sunscreens: active ingredient makers are frustrated by the long list of sunscreens and UV-A testing protocols that are still awaiting FDA decisions. Chem Eng News, 83: 18-22. http://pubs.acs.org/cen/coverstory/83/8315sunscreens.html. 155634

SCCNFP (2000). Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning titanium dioxide Colipa n S75 (Report No. SCCNFP/0005/98). Brussels, Belgium: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out135_en.pdf. 092740

Schlossman D; Shao Y; Detrieu P (2006). Perspectives on supplying attenuation grades of titanium dioxide and zinc oxide for sunscreen applications. Retrieved October 28, 2007 from http://www.fda.gov/nanotechnology/meetings/kobo_files/textmostly/index.htm. 093309

Scientific Committee on Computer Products (2007). Preliminary opinion on safety of nanomaterials in cosmetic products. Brussels, Belgium: European Commission, Health and Consumer Protection Directorate-General. 157639

Shao Y; Schlossman D (1999). Effect of particle size on performance of physical sunscreen formulas. Talk presented at PCIA Conference, Shanghai, China. http://www.koboproductsinc.com/Downloads/PCIA99-Sunscreen.pdf. 093301

Umicore (2008). Nano-sized oxide powders for UV applications. Abstract from Innovation for Sustainable Production (i-SUP2008), Bruges, Belgium. 193688

Uniqema (2004). Solaveil CT-200 (Report No. PC/E/03-03/GLOB/14.5/CT200). London: Uniqema. 155637

Verhulst D; Sabacky BJ; Spitler TM; Prochazka J (2003). Process for the production of nano-sized TiO2 and other ceramic oxides by spray hydrolysis. Reno, Nevada: Altair Nanomaterials Inc. 157854

Wakefield G; Lipscomb S; Holland E; Knowland J (2004). The effects of manganese doping on UVA absorption and free radical generation of micronised titanium dioxide and its consequences for the photostability of UVA absorbing organic sunscreen components. Photochem Photobiol Sci, 3: 648-652. http://www.rsc.org/delivery/_ArticleLinking/DisplayArticleForFree.cfm?doi=b403697b&JournalCode=PP. 193693

Annex C. Nano-TiO_2 Exposure Control in the Workplace and Laboratory

C.1. Workplace Exposure Controls

This section provides examples of strategies that are currently in place or recommended to decrease exposures to nanomaterials in the workplace (Nanosafe, 2008, 196066; NIOSH, 2009, 196073) and to ensure the effectiveness of personal protective equipment (PPE) against nano-TiO_2 (Golanski et al., 2008, 196048; Guizard and Tenegal, 2008, 196049) (Nanosafe, 2008, 196066). Other approaches to reduce worker exposure have been developed and are undergoing further refinement; the examples provided here are intended to be illustrative rather than exhaustive. 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, 2008, 196066) provided several tiers of approaches to decrease nanomaterial exposure in the workplace. During production, the first and preferred approach is to avoid potential exposure to 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, 2008, 196066). 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, 2008, 196066).

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, 2008, 196066). 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.

NIOSH has a nanotechnology program to increase safety and decrease potential exposures to nanomaterials in the workplace (NIOSH, 2009, 196073). In a NIOSH document for safe nanotechnology (NIOSH, 2009, 196073), occupational health surveillance and guidelines for working with engineered nanomaterials are discussed, among other topics. Some of these programs could also educate and encourage the general public to reduce environmental releases from the products into the environment. Some companies that manufacture nano-TiO_2 have engineering safeguards and additional programs in place to reduce or eliminate occupational and environmental exposures (e.g., BASF, 2008, 193811; DuPont, 2007, 157699). Various production methods to decrease worker exposure are also being investigated [for nano-TiO_2, see Guizard and Tenegal (2008, 196049)].

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

With a goal toward managing nanotechnology safely and effectively within the industrial setting, the 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, 196077). The longest exposure time within which the N100 filter performed at or exceeded the efficiency specified by the filter ranking (>99.97% filtration efficiency) was 210 minutes (Ostraat, 2009, 196077). No PPE specifically designed for nanomaterials exists or is under development (Klaessig, personal communication, 2008, 196042). For filter efficacy against nano-TiO_2 aerosol penetration tested by NanoSafe, see Section C.1.1.

C.1.1. Personal Protective Equipment

In this 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, 193470; Ostraat, 2009, 196077). Other physicochemical properties of nanoparticles that affect filtration efficiency include size, chemical composition, and shape. The size of the particle that most effectively penetrates 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 (McKeytta, 1984, 196036).

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, 196048). 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 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, 196048). The penetration efficacy of platinum (Pt) particles through HEPA filters was only slightly lower than that of nano-TiO_2 particles. Golanski et al. (2008, 196048) 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 efficiently than other nanomaterials, namely graphite and Pt. The exposure duration of the Golanski et al. (2008, 196048) 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, 196077).

The efficacy of protective clothing in preventing nano-TiO_2 penetration by diffusion was higher for nonwoven fabric than woven cotton and polyester fabric (Golanski et al., 2008, 196048). Air-tight, nonwoven, polyethylene Tyvek (115 µm thick) was more effective 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, 196048), 10-nm nano-Pt (Golanski et al., 2008, 196048), and 40- and 80-nm graphite (Nanosafe, 2008, 196066).

Nitrile, latex, and Neoprene gloves were reported to be effective 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, 196048) or 20- to 100-nm graphite (Nanosafe, 2008, 196066). However, continuous flex of gloves could lead to cracks and holes in the gloves (Schwerin et al., 2002, 193636), so changing gloves throughout the day is recommended (Harford et al., 2007, 196051).

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 of whether or not the nanomaterials they worked with or manufactured pose safety risks (Gerritzen et al., 2006, 097620).

Subsequently, the same research team published additional findings based on a larger sample size of 82 versus the original 64. 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, 155619).

A survey of 43 New England nanotechnology firms found that larger companies (with 500 or more employees) were more likely to recognize potential 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, 155629).

REFERENCES

BASF (2008). Guide to safe manufacture and for activities involving nanoparticles at workplaces in BASF AG. Germany: BASF. http://basf.com/group/corporate/en/function/conversions:/publish/content/sustainability/dialogue/in-dialogue-with-politics/nanotechnology/images/BASF_Guide_to_safe_manufacture_and_for_activities_involving_nanoparticles.pdf. 193811

Conti JA; Killpack K; Gerritzen G; Huang L; Mircheva M; Delmas M; Harthorn BH; Appelbaum RP; Holden PA (2008). Health and Safety Practices in the Nanomaterials Workplace: Results from an International Survey. Environ Sci Technol, 42: 3155-3162. 155619

DuPont (2007). Nanomaterial Risk Assessment Worksheet DuPont(TM) Light Stabilizer. Retrieved June 18, 2008 from http://www.edf.org/documents/6913_TiO2_Worksheet.pdf. 157699

Gerritzen MA; Lambooij E; Stegeman JA; Spruijt BM (2006). Slaughter of poultry during the epidemic of avian influenza in the Netherlands in 2003. Vet Rec, 159: 39-42. 097620

Golanski L; A. Guiot; Tardif F (2008). Experimental evaluation of individual protection devices against different types of nanoaerosols: graphite, TiO2 and Pt. Retrieved March 22, 2009 from http://www.nanosafe2008.org/home/liblocal/docs/Oral%20presentations/O6-1_Golanski.pdf. 196048

Guizard B; Tenegal F (2008). Liquid Recovery of TiO2 nanoparticles synthesized by laser pyrolysis. Retrieved March 30, 2009 from http://www.nanosafe2008.org/home/liblocal/docs/Oral%20presentations/O6-4_Guizard.pdf. 196049

Harford AJ; Edwards JW; Priestly BG; Wright PFA (2007). Current OHS Best Practices for the Australian nanotechnology industry: A position paper by the NanoSafe Australia Network. Victoria, Australia: NanoSafe Australia Network. http://mams.rmit.edu.au/72nuxiavskpg.pdf. 196051

Kim CS; Bao L; Okuyama K; Shimada M; Niinuma H (2006). Filtration efficiency of a fibrous filter for nanoparticles. J Nanopart Res, 8: 215-221. 193470

Lindberg JE; Quinn MM (2007). A survey of environmental, health and safety risk management information needs and practices among nanotechnology firms in the Massachusetts region (Report No. PEN Brief No. 1). Washington, DC: Woodrow Wilson International Center for Scholars: Project on Emerging Nanotechnologies. 155629

McKeytta JJ (1984). Encyclopedia of chemical processing and design. Boca Raton, FL: CRC Press. 196036

Nanosafe (2008). Dissemination report: Are conventional protective devices such as fibrous filter media, respirator cartridges, protective clothing and gloves also efficient for nanoaerosols? (Report No. DR-325/326-200801-1). France: Sixth Framework Programme. http://www.nanosafe.org/scripts/home/publigen/content/templates/show.asp?P=63&L=EN&ITEMID=13. 196066

NIOSH (2009). Approaches to safe nanotechnology: Managing the health and safety concerns associated with engineered nanomaterials. Retrieved June 02, 2009 from http://cdc.gov/niosh/docs/2009-125/. 196073

Ostraat ML (2009). Industry-led initiative for occupational health and safety. In M Hull; S Friedrichs (Eds.), Risk governance of nanotechnology: Environmental, health and safety concerns (pp. 181-246). Norwich, NY: William Andrew Publishing. 196077

Schwerin MR; Walsh DL; Coleman Richardson D; Kisielewski RW; Kotz RM; Routson LB; David Lytle C (2002). Biaxial flex-fatigue and viral penetration of natural rubber latex gloves before and after artificial aging. J Biomed Mater Res, 63: 739-745. 193636