Title
Development of an Integrative Framework to Assess the Environmental Impacts of Engineered Nanomaterials

Author
Romero Franco, Michelle

Publication Date
2018

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Development of an Integrative Framework to Assess the Environmental Impacts of Engineered Nanomaterials

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Environmental Health Sciences

by

Michelle Azucena Romero Franco

2018

ABSTRACT OF THE DISSERTATION

Development of an integrative process to assess the environmental impacts of engineered nanomaterials

by

Michelle Azucena Romero Franco

Doctor of Philosophy in Environmental Health Sciences

University of California, Los Angeles, 2018

Professor Hilary Godwin, Co-Chair

Professor Timothy Malloy, Co-Chair

The overarching goal of this work is to develop a framework and tools for assessing the environmental and human health impacts of Engineered Nanomaterials (ENMs). As a first step, the analysis focused on the identification of the types of decision makers that need to be able to assess the environmental impacts of ENMs and the contexts in which they are making decisions (decision scenarios). Next, a literature review was conducted to determine the utility of existing environmental impact assessment frameworks for these different decision scenarios, and to analyze which of these existing frameworks are most useful for assessing the potential impacts of

ENMs in each decision-making context and what gaps exist. This analysis revealed that there is a significant need for a practical tool that decision makers can use to assess whether they have sufficient data available to conduct an environmental impact assessment for a specific group of ENMs for a specific decision context. To address this gap, the core of this thesis presents the development of a decision-support tool that employs an evidential reasoning algorithm to assess data required for environmental impact assessment of ENMs. As a proof of concept, this approach was employed to evaluate whether sufficient data are available to assess the environmental impact of nano Copper and nano Copper Oxide, nano Zinc Oxide and nano Titanium Dioxide (nano Cu-CuO, nano ZnO and nanoTiO_2) in four different risk scenarios. This analysis revealed that sufficient data are available to assess the risk potential of TiO_2 in consumer products and occupational settings and that sufficient data are available to assess the risk potential of nano Cu-CuO with respect to aquatic environments. In all of the other combinations of materials and scenarios explored, the results show that additional data on the environmental fate and transport and regarding exposure potential are needed to be able to assess potential environmental impact. The final section of this thesis focuses on the implications that this and other work in the field of Environmental Health and Safety for Nanomaterials (Nano EH&S) have for our overall understanding of the risk of ENMs to human health. Specifically, key questions of concern to the public health community are proposed, and answers to these questions and remaining gaps are provided based on a comprehensive review of major accomplishments in the field of Nano EH&S over the last decade and a half. An overarching set of conclusions is provided in chapter five summarizing the scientific contributions of this work. The chapters of this work constitute important progress towards the development of frameworks and tools for characterizing the environmental and human health impacts of ENMs.

The dissertation of Michelle Azucena Romero Franco is approved.

Yoram Cohen

Michael Leo Brenna Jerrett

Timothy Malloy, Committee Co-Chair

Hilary Godwin, Committee Co-Chair

University of California, Los Angeles

2018

DEDICATION

A mis padres, hermano y mi familia, por tanto amor, apoyo y sobre todo la gran inspiración que son para mí, cada uno de ustedes a su manera. Sobre todo, a mis abuelos, quienes decidieron que la educación sería el regalo más preciado para todos nosotros. Gracias.

To my husband Brett for his infinite love and for his unconditional support. You make me feel blessed every day of my life. I love you.

To my extended family, who have made me feel at home and provided infinite hours of babysitting and all sorts of support. Thank you.

To Roquefort and Lady Bear, my two furry loving companions, thank you for the emotional support (that is a thing!).

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapter 1 Figures

Chapter 2 Figures

Chapter 2 Tables

Chapter 3 Figures

Chapter 3 Tables

Chapter 4 Figures

Chapter 4 Tables

Appendix A Tables

Appendix B Tables

Appendix C Figures

Appendix C Tables

GLOSSARY

Abbreviations	Name
AFM	Atomic-Force Microscopy
AML	NIST's Advanced Measurement Laboratory
AOPs	Adverse Outcome Pathways
ATS	Alternative Testing Strategies
BM	Benchmark
BMR	Benchmark Response
BN	Bayesian Network
BSA	Bovine Serum Albumin
BSF	Biological Simulation Fluid
CEA	U.S. EPA's Comprehensive Environmental Assessment
CEIN	UCLA Center for Environmental Implications of Nanotechnology
CEINT	Duke Center for Environmental Implications of Nanotechnology
CNs	Cellulose Nanomaterials
CNT	Carbon Nanotube
CPQRA	Chemical Process Quantitative Risk Assessment
CPTs	Conditional Probability Tables
CVD	Chemical Vapor Deposition
DEFRA	UK Department for Environment, Food and Rural Affairs
DEP	Diesel Exhaust Particulate

Abbreviations (cont.)	Name
DfE	Design for the Environment
DLS	Dynamic Light Scattering
DMP	Data Management Plan
DS	Dempster-Shaffer algorithm
DST	Decision Support Tool
DWCNT	Double-Walled Carbon Nanotube
EH&S/EHS	Environmental Health and Safety
EIA	Environmental Impact Assessment
ELSI	Ethical, Legal and Societal Issues
EMA	European Medicines Agency
ENHRES	Engineered nanoparticles: review of health and environmental safety
ENM	Engineered Nanomaterials
ENRHES RA	Engineered Nanoparticles - Review of Health and Environmental Safety: Human Health and Ecological Risk Assessment
ENTA	Europe Nanotechnology Trade Alliance
EPA/US EPA	U.S. Environmental Protection Agency
EPI	Exposure Potential Information
ERA	Ecological Risk Assessment
ESEM	Environmental Scanning Electron Microscope
EU-OSHA	European Agency for Safety and Health at Work
EXAFS	Extended X-Ray Absorption Fine-Structure Spectroscopy
FDA	U.S. Food and Drug Administration

Abbreviations (cont.)	Name
FINE	Forecasting of the Impacts of Nanomaterials in the Environment based on Bayesian Networks
FTIR	Fourier-Transform Infrared
GHS	Globally Harmonized System
HAR	High Aspect Ratio
HCS	High-Content Screening
HiPCO	High-Pressure Carbon Monoxide
HPI	Hazard Potential Information
HTS	High-Throughput Screening
IANano	Information Assessment Tool for Engineered Nanomaterials
IANH	International Alliance for NanoEHS Harmonization
IATA	Integrated Approaches on Testing and Assessment
IH	Industrial Hygiene
IHCP	Institute for Health and Consumer Protection
ILCs	Interlaboratory Comparisons
IRMM	Institute for Reference Materials and Measurements
ITS	Intelligent Testing Strategy
JRC	European Commission's Joint Research Center
JRC-IRMM	Institute for Reference Materials and Measurements of the European Commission's Joint Research Center
LCA	Life Cycle Analysis

Abbreviations (cont.)	Name
LC-APPI-MS	Liquid Chromatography-Atmospheric Pressure Photoionization-Mass Spectrometry
MCDA	Multi-Criteria Decision Analysis
MNPs	Magnetic Nanoparticles
MOA	(biological) Mode of Action
MOx	Metal Oxides
MWCNT	Multi-walled Carbon Nanotube
Nano LCRA	Life Cycle Risk Analysis for Nanomaterials
NCI	U.S. National Cancer Institute
NCL	Nanotechnology Characterization Laboratory
NEAT	Nanoparticle Emission Assessment Technique
NEPA	National Environmental Policy Act
NIBIB	U.S. National Institute of Biomedical Imaging and Bioengineering
NIEHS	U.S. National Institute of Environmental Health Sciences
NIH	U.S. National Institutes of Health
NIOSH	U.S. National Institute for Occupational Safety and Health
NISE	National Informal STEM Education Network
NIST	U.S. National Institute of Standards and Technology
NNCO	National Nanotechnology Coordination Office
NNI	U.S. National Nanotechnology Initiative

Abbreviations (cont.)	Name
NPEC	Nanotechnology Public Engagement and Communications
NRC	U.S. National Research Council
NRST	Nanomaterial Risk Screening Tool
NSF	U.S. National Science Foundation
NTA	Nanoparticle Tracking Analysis
NTP	U.S. National Toxicology Program
OECD	Organization for Economic Co-operation and Development
OELs	Occupational Exposure Limits
OSHA	U.S. Occupational Safety and Health Administration
OSTP	U.S. Office of Science and Technology Policy
PEC	Predicted Environmental Concentrations
PMFA RA	Risk Quantification based on Probabilistic Mass Flow Modeling Analysis
PPE	Personal Protective Equipment
PPS	Primary Particle Size
QSAR	Quantitative Structure Activity Relationship
R&D	Research and Development
RA	Risk Assessment
RA	Risk Assessment
RCIP	Risk Classification based on an Industry Insurance Protocol
REACH	Registration, Evaluation, Authorization of Chemicals
REMS	Risk Evaluation and Mitigation Strategy

Abbreviations (cont.)	Name
ROS	Reactive Oxygen Species
SAD	Surface Area-Dose
SARs	Structure Activity Relationships
SAS	Synthetic Amorphous Silica
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SEM	Scanning Electron Microscopy
SERS	Surface Enhanced Raman Scattering
SNURs	Significant New Use Rules
SPM	Swiss Precautionary Matrix
STIS	Short Term Inhalation Studies
SWCNT	Single-walled Carbon Nanotube
TEM	Transmission Electron Microscopy
TSCA	Toxic Substances Control Act
TWA	Time Weighted Average
UFPs	Ultrafine Particles
WOE	Weight of Evidence
XAFS	X-Ray Absorption Fine Structure
XANES	X-Ray Absorption Near-Edge Spectroscopy

ACKNOWLEDGMENTS

I would like to thank my advisors, Hilary Godwin and Yoram Cohen, whose support, teaching and mentoring were invaluable to completing my academic degree. I could not express how grateful I am to have had Dr. Godwin play the utmost important roles not only as my academic adviser but as a mentor, life coach and occasional therapist for the last seven years. Dr. Godwin has definitely become a role model to me not only as a professional but as a woman in science. I would also like to acknowledge the moral and financial support and constructive criticism provided by Dr. Cohen. I am very proud to have learned so much from such a distinguished professor. And to the members of my committee, Professors Timothy Malloy and Michael Jerrett, thank you for taking the time to revise my work and for your input.

The achievement of my academic goals at UCLA would not have been possible without the help and support of my fellow doctoral students, professors, administrative staff and research colleagues at the Center for Environmental Implications of Nanotechnology (CEIN). For that reason, I extend my gratitude to my classmates and friends, Bryan Moy, Sharona Sokolow, Evelyn Alvarez, Katy McNamara, Tamanna Rahman and Tyler Watson, thank you for your feedback and discussions in our research seminars. A special thanks to the CEIN staff and the professors from the Environmental Health Sciences Department at the Fielding School of Public Health, especially Dr. Richard Jackson, who made me feel welcome from day one and helped plan my coursework.

As a graduate student, I received financial support (including tuition, fees and stipend) from CONACYT – UC MEXUS as well as from the University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). The research presented in this work was supported by funding from the National Science Foundation and the Environmental Protection Agency under Cooperative Agreement Numbers DBI-0830117 and DBI-12266377 to the UC-

CEIN. I would also like to acknowledge the financial support received from the UCLA-Fogarty Program, and specially Dr. John R. Froines, for all his career mentoring and advice. I am forever grateful for his inspirational classes and for taking the time to help me adjust to a new environment at UCLA.

Chapter 2 is a modified version of a previous publication, which appeared in the *Beilstein Journal of Nanotechnology* (Year 2017, Volume 8, Pages 989–1014). I would like to acknowledge the contributions of the co-authors in the paper: Dr. Hilary Godwin and Dr. Yoram Cohen, for their extensive contribution to the analysis of the manuscript and revisions, and Dr. Muhammad Bilal for his critique and editing. Dr. Cohen was the principal investigator for this study and played a primary role with writing and editing of the manuscript.

Chapter 3 was submitted as a manuscript to the Journal of Nanoparticle Research in May 2018. The original idea of the article was conceived as a result of continuous discussions with Dr. Yoram Cohen, Dr. Hilary Godwin and Dr. Muhammad Bilal. For this manuscript, Dr. Cohen and Dr. Godwin provided critical suggestions about the analysis and case studies, as well as the structure of the publication. Dr. Bilal contributed with his expertise to the calculations and presentation of results, including the algorithm implementation. Dr. Cohen was the principal investigator for this study and played a primary role in the writing and editing of the manuscript.

Chapter 4 is a version of a manuscript that is in preparation for submission to the American Journal of Public Health. For this manuscript, I recognize the instrumental role played by Dr. Hilary Godwin for her insights into the field of Nano Environmental Health and Safety. I would also like to extend a special acknowledgement to Dr. Yoram Cohen and Dr. Muhammad Bilal for their critique and editing of the contents. Dr. Godwin was the principal investigator for this study and contributed by revising and editing the manuscript.

VITA

Michelle Azucena Romero Franco

EDUCATION

2009	M.S., Environmental Health Sciences, Escuela de Salud Publica de México, Instituto Nacional de Salud Pública, Cuernavaca, México.
2004	B.E. Biochemical Engineering, Instituto Tecnologico de Zacatepec, Zacatepec, Mexico.

HONORS AND AWARDS

2013	Dean's Global Health Fellowship, UCLA Fielding School of Public Health
2011	National Council of Science and Technology, Mexico's Scholarship for Doctoral studies
2009	Irving J Selikoff – Mount Sinai scholarship
2009	Irving J Selikoff Scholarship, International Training Program in Environmental and Occupational Health, Mount Sinai School of Medicine, New York
2009	International Society for Environmental Epidemiology (ISEE) Travel award, Dublin Ireland
2007	National Council of Science and Technology, Mexico's Scholarship for Masters studies, School of Public Health of Mexico

PUBLICATIONS

- **Romero-Franco, M**., Godwin, H. A., Bilal, M., & Cohen, Y. (2017). Needs and challenges for assessing the environmental impacts of engineered nanomaterials (ENMs). *Beilstein Journal of Nanotechnology, 8*, 989-1014.
- Malloy, T. F., Zaunbrecher, V. M., Batteate, C., Blake, A., Carroll, W. F., Corbett, C. J., Foss Hansen, S., Lempert, R., Linkov, I., McFadden, R., Moran, K., Olivetti, E., Ostrom,

N., **Romero, M**., Schoenung, J.M., Seager, T.P., Sinsheimer, P., Thayer, K. (2016). Advancing alternative analysis: integration of decision science. *Environ Health Perspect, 125*, 066001-1- 066001-12.

- Riojas-Rodriguez, H., **Romero-Franco, M.** The deterioration of ecosystems and biodiversity: Consequences for human health in: *Environmental and Social Determinants of Health*. Washington, DC: PAHO, 2016. ISBN 978-92-75-13129-9.
- **Romero-Franco, M.**, Hernández-Ramírez, R. U., Calafat, A. M., Cebrián, M. E., Needham, L. L., Teitelbaum, S., Wolff, M.S., López-Carrillo, L. (2011). Personal care product use and urinary levels of phthalate metabolites in Mexican women. *Environment international*, 37(5), 867-871.

PRESENTATIONS

- June 2015 – Poster presentation "Development of a framework for environmental impact assessment of ENMS" Gordon Research Conference Environmental Nanotechnology. West Dover, Vermont, U.S.
- March 2014 – Oral presentation "Multidisciplinary research as an opportunity for gender equity: Nanotechnology as a case study". First symposium of Education, Outreach and Gender. Autonomous University of Morelos, Mexico.
- March 2014 – Seminar "Environmental health implications of nanotechnology". National Institute of Public Health. Cuernavaca, Morelos, Mexico.
- November 2013 – Video presentation at the Fielding School of Public Health Global Health Internships Showcase, Los Angeles, California.
- July 2010 – Poster presentation at The XII International Congress of Toxicology Barcelona, Spain
- August 2009 – Oral presentation "Personal care products' use and its association with urine concentrations of phthalate metabolites". ISEE 21st Annual Conference, Dublin, Ireland.

CHAPTER 1: INTRODUCTION AND OVERVIEW OF THE ORGANIZATION OF THE THESIS

THE ROLE OF SCIENTIFIC EVIDENCE IN ENVIRONMENTAL HEALTH DECISION-MAKING: ASSESSING ENVIRONMENTAL IMPACTS OF ENGINEERED NANOMATERIALS

Environmental health decision-making is a complex undertaking that involves navigating and balancing scientific evidence and policy development to protect the environment and human population from adverse effects. Holistic approaches are needed to provide an understanding and assessment of the scientific evidence that also consider uncertainties related to this evidence. For this purpose, decision support systems can be developed and adapted to specific decision contexts and agents of concern (e.g., chemicals, technologies, materials). In this work, an illustration of a decision support system (**Figure 1.4**) and its corresponding elements is presented for the case of Engineered Nanomaterials (ENMs) by first introducing an overview of the environmental health and safety implications of ENMs and a critical review of needs and challenges in assessing the environmental impacts of ENMs in different decision-contexts, followed by a scientific approach to assess the information availability to assess such impacts and a synthesis of the progress in the field with regard to public health implications. It is envisioned that the present decision-support system can be tailored to chemicals, technologies or other materials of concern, given the overlapping elements such as the critical evaluation of available data (e.g., a clear scientific rationale behind the required environmental impact assessment information) and transparency of the proposed methods.

HISTORICAL BACKGROUND ON ENVIRONMENTAL HEALTH AND SAFETY RESEARCH ON ENGINEERED NANOMATERIALS

One of the drivers to the development of studies on the Environmental Health and Safety of engineered nanomaterials (i.e., materials manufactured/synthesized with at least one dimension <100 nanometers) (Nano EH&S) was the work from the air quality literature, which demonstrated that ultrafine particles (UFPs) (i.e., particles that are <100 nm in size and inadvertently produced through combustion or other processes) pose significant threats to human health. In the late 1990's and early 2000's, a series of studies suggested that inhalation of particles that are less than 100 nm in size is particularly hazardous. Pioneering studies (Oberdörster et al. 1995, Donaldson et al. 1998) demonstrated that UFPs possess specific properties that differ from those of larger particles and, in some cases, elicit a greater toxicity response than larger analogs. Subsequent studies revealed that in vitro exposure to UFPs results in oxidative stress in cells lines (i.e., macrophages and epithelial cells) (Li et al. 2003), whereas epidemiological studies demonstrated that inhalation exposure to UFPs leads to a decrease of pulmonary function (peak expiratory flow), and increased exposure to UFPs also results in cough in non-smoking asthmatic individuals (Peters et al. 1997). Given the above-mentioned findings and the similar size range between engineered nanomaterials (ENMs) and ultrafine particles (i.e., less than 100 nm), scientists from the air quality field began to assess the potential of ENMs to elicit similar adverse biological properties (Li et al. 2016).

Another driver contributing to interest in Nano EH&S studies has been the exponential market growth in Nanotechnology and ENMs. As a result of their unique properties, ENMs have been incorporated into over 1000 products and are anticipated to reach a market of $1 trillion globally by 2020 (Roco 2011). The forecasted $1 trillion market of Nanotechnology and Nano-enabled products is also reflected in an increasing trend in the diversity in patents for commercial

applications. Between 2000 and 2008, the worldwide growth rate of the number of nanotechnology patent applications reached 34.5% (Dang et al. 2010). This growth has been fueled by the incorporation of ENMs into semiconductor devices, medical treatments, chemical and physical processes, among others. This growth in commercial applications of ENMs along with the quick rise in the number of novel ENMs being produced each year has fueled safety concerns.

Based on early studies and concerns, several federal agencies in the United States set aside funding to support targeted research on the environmental applications and impacts of ENMs during the early 2000's. One of the earliest funding efforts to establish research centers in this area came from the National Science Foundation (NSF), which granted ~$12 million to Rice University for the establishment of the *"Center for Biological and Environmental Nanotechnology"*(CBEN) in 2001, which was directed by Vicki Colvin and Richard Smalley. One of the objectives of this research center was to address concerns regarding interaction of ENMs and organic materials with biochemical and cellular processes (NSF 2006). Subsequent funding programs focused on Nano EH&S as well as greater coordination between different U.S. agencies. In 2003, CBEN nominated ENMs for testing by the National Toxicology Program (NTP) (Colvin 2003), which resulted in studies by the NTP evaluating nanoparticle translocation, characterizing the inhalation toxicology of high aspect ratio materials and determining the immune responses to ENMs. At that time, the U.S. Environmental Protection Agency (EPA) launched its first exploratory grants program on Nano EH&S (NRC 2003) and the Nanotechnology Environmental and Health Implications (NEHI) workgroup was established within the National Science and Technology Council (NSTC) to specifically address the needs in terms of Nano EH&S (NSET 2006). Another milestone at this time was the enactment of the 21st Century Nanotechnology Research and Development Act,

which aimed to ensure safe development of nanotechnology via the allocation of federal funding for Nano EH&S (U.S.C. 2003).

These research initiatives from the U.S. federal government agencies resulted in the publication of several seminal studies and reports in 2004-2007 that focused on developing Nano EH&S research milestones and guidelines. In 2004, in the U.S., Donaldson et al. (Donaldson et al. 2004) highlighted the need for adequate toxicity and risk assessment of ENMs, and used the term "nanotoxicology" for the first time in peer-reviewed literature (Santamaria 2012). This led to the development of "nanotoxicology" as an identifiable field (Oberdorster et al. 2005). In 2005 the EPA published a "Nanotechnology White Paper" (EPA 2005, Morris and Willis 2007), which identified and described the issues that EPA needed to address to ensure protection of human health and the environment. Likewise, in 2005, the U.S. National Institute for Occupational Safety and Health (NIOSH) published the report "Approaches to Safe Nanotechnology and Information Exchange with NIOSH" (NIOSH 2005) and the draft of the "Strategic Plan for NIOSH Nanotechnology Research: Filling the Knowledge Gaps" (NIOSH 2005), both of which were aimed at promoting recommendations for occupational safety and health practices for ENMs. Around the same time, the Royal Academy of Engineering in the UK published the report "Nanoscience and Nanotechnologies: Opportunities and Uncertainties", which outlined potential risks of ENMs (Royal 2004), and the Scientific Committee on Emerging and Newly Identified Health Risks of ENMs (SCENIHR) of the European Commission issued a report outlining critical knowledge gaps that required attention for risk assessment (SCENIHR 2005). In 2007, a collaborative project, "NanoRisk", was launched by DuPont aiming at analyzing exposure and hazard potential of nanomaterials (DUPONT 2007).

CHALLENGES OF ENVIRONMENTAL HEALTH AND SAFETY RESEARCH OF ENGINEERED NANOMATERIALS

Through the above-mentioned activities, a number of "challenges" related to the environmental health and safety implications of ENMs were identified by the scientific and regulatory communities. Based on a literature review of the Nano EH&S, these challenges have been summarized into the list below, which serves as a guideline to illustrate the progress achieved over the last two decades in this field (**Figure 1.1, Figure 1.2** and **Table A.1** in **Appendix A**):

Challenges Related to Characterizing the Physicochemical Properties of ENMs

1. Early syntheses of ENMs often resulted in high batch to batch variability in materials and hazard properties. This variability led to the need to develop standard reference materials and protocols for preparation and characterization of ENMs.
2. As a result of the rapid growth in discovery and increasingly large production of new ENMs and safety data gaps, research on Nano EH&S has struggled to keep up with the pace of new materials being discovered and placed in the market.
3. The increase in experimental research of ENM safety has resulted in the production of large datasets that has led to the need for new computational and statistical approaches to manage data sets of ENMs.

Challenges Related to Assessing the Hazard Potential of ENMs

4. The hazard potential and toxicity of ENMs typically depend on environmental and biological conditions, which can also influence the fate and transport of ENMs. As a result, there can be substantial differences between the hazards of pristine and transformed ENMs studies need to consider these effects.

5. The hazard potential of ENMs is highly dependent on their properties (e.g., size, shape, composition, surface coatings, etc.), which means that even closely related materials may have vastly different hazard potentials and need to be independently assessed for safety.

6. The vast numbers of ENMs (and continuous production growth) make it unfeasible to conduct detailed/extensive in vivo assessments for all new ENMS. As a result, there is a need for validated hazard assessment strategies and protocols that incorporate both in vitro and in vivo test results.

Challenges Related to Assessing the Exposure Potential of ENMs

7. The environmental conditions across media induce significant changes in the physicochemical properties of ENMs, which in turns affect intermedia transport. Hence data and models are needed to quantify environmental releases and fate and transport of ENMs in the environment to assess environmental exposure, while considering the transformations of ENMs in the environment.

8. Naturally-occurring nano-sized particles can interfere with quantitative measurement of ENMs in real world settings. As a result, improved and more specific field monitoring and measurement data are needed.

9. Occupational exposure assessment requires methods that (i) address the potential of other particulate matter to interfere with ENMs and, (ii) translate exposure levels measured in animals to values relevant for humans.

Challenges Related to Assessing the Risk of ENMs

10. Given the above-mentioned challenges and significant differences between ENMs and chemicals, researchers have questioned the suitability of traditional environmental impact assessment approaches for ENMs. In cases where traditional approaches are not suitable, alternative approaches would need to be developed to assess ENM safety.
11. Tools are needed to assess whether available data are sufficient/adequate to conduct a risk/environmental impact assessment of ENMs under different scenarios and for different decision contexts, and, if not, identify what additional data are required.
12. Assessments are needed to determine whether/when/which traditional Life Cycle Analysis (LCA) tools are applicable to ENMs. In cases where the traditional LCA tools are not suitable as is, it is necessary to determine how such tools must be modified.
13. Epidemiological studies are needed to demonstrate whether effects of ENMs observed in animal models can be extrapolated to human populations.
14. The increasing numbers of ENMs applied to consumer products is associated with a need for practical risk reduction strategies for ENMs. These risk reduction strategies require Nano EH&S research and data that is adequate to inform regulatory decision-making.

Challenges Related to Ensuring the Safety Next Generation ENMs

15. The promising benefits of complex, next generation ENMs in commercial applications (e.g., therapeutics and diagnostics) necessitates improved methods for

screening these materials for safety and the development and dissemination of best practices for design of safer ENMs.

16. Safety assessment procedures developed for relatively simple ENMs need to be validated for and applied to more complex (3rd and 4th generation) ENMs.

Challenges Related to Communicating the Risks of ENMs

17. To reduce the possibility of rejection of this important new technology by the public, key stakeholders (e.g., NGOs, industry and policy makers) need to be engaged in prioritizing Nano EH&S research and education strategies.

18. NGOs, industry and policy makers need to be engaged in the development of risk communications strategies for Nano EH&S and communicating research results to different communities.

In the sections that follow, a critical analysis is provided with regard to the scientific milestones over the last two decades, to what extent advances in the field of Nano EHS have addressed the challenges listed above, and what gaps remain. Next, an explanation follows on how the work reported in this thesis addresses those remaining gaps. At the end of this chapter, the organization of the body of this thesis is provided.

KEY FINDINGS AND ADVANCES OVER THE LAST TWO DECADES IN NANO EH&S

Over the last two decades large amounts of additional funding have been allocated to address the above listed challenges (i.e., characterization, exposure, hazard, risk assessment and communication) in Nano EH&S. The U.S. National Nanotechnology Coordination Office

(NNCO) reported U.S. spending for Nano EH&S in 2004 to be $8.5 million (approximately 1% of the NNI's total budget). By 2006, this increased to $38.5 million (almost 4% of the total of the NNI's budget). Around the same time, the U.S. Environmental Defense Organization proposed that the annual Nano EH&S budget in the U.S. should be increased to $100 million to meet emerging research needs (Denison 2005). The amount allocated to Nano EH&S research increased to 5% of the NNI's budget in 2007 and surpass the $100 million mark in 2011 (National Nanotechnology 2014). As part of the above trend, the EPA and NSF launched a call for proposals in 2008 to create a collaborative center dedicated to Nano EH&S issues, which resulted in funding of two different centers. The first of these is the University of California Center for Environmental Implications of Nanotechnology (UC CEIN), which has the goal of developing environmental decision making tools that consider ENM physicochemical properties, environmental fate, transport, exposure, and hazard generation across a wide spectrum of nano/bio interfaces in cells, bacteria, organisms, communities and ecosystems. The second is the Duke Center for Environmental Implications of NanoTechnology (CEINT), led by Duke University, which has the goal of exploring the relationship between a vast array of nanomaterials — from natural, to manufactured, to those produced incidentally by human activities — and their potential environmental exposure, biological effects, and ecological impacts. These centers were continuously funded from 2008-2018 to study how nanomaterials behave in the environment and interact with living systems, with the goal of translating this knowledge into mitigation strategies useful in the development of nanotechnology (NSF 2008).

In Europe, funding for nanotechnology risk-related research (Nano EH&S) in the European Union's Framework Program 7 (FP7) was €261 million (USD $218 million) between 2006 and 2013, with a further €71 million (USD $59 million approximately) allocated in Horizon 2020 (the

successor to the FP7 program) (Maynard and Aitken 2016). In Asia, the budget allocated for Nano EH&S in Taiwan was about 10% of the total R&D expenditure in 2010, while Japan's budget averaged less than 2% for the same time period (Roco 2011). Following this second wave of Nano EH&S funding, the research output in the field of Nano EH&S skyrocketed. The increase in the number of publications consistent with raises in the U.S. Federal budget allocated for Nano EH&S research is shown in **Figure 1.3.** The above increase in the number of publications is also an indicator that significant progress was made towards addressing the Nano EH&S challenges described in the previous section. In the following sections, major highlights of the progress achieved for each challenge are further discussed.

Progress Related to Characterizing the Physicochemical Properties of ENMs

The highly variable physicochemical properties of ENMs that make them so desirable for many applications also makes them challenging for toxicological analysis. One of the most important characteristics of ENMs is that their properties differ substantially from those bulk materials of the same composition, and many have exceptional properties (e.g., conductivity, reactivity, and optical sensitivity). These properties can be modified systematically by varying parameters of the ENMs (e.g., size, surface charge, core composition, coatings, etc.) to achieve a specific desired function. However, some physicochemical properties, such as size, are also closely related to their hazard potential or bioactivity (Wiesner et al. 2009). Hence, researchers need to know *exactly what nanomaterials they have* if they wish to understand how toxicity of a particular class of ENMS and interactions with biological and/or environmental systems depends on their physicochemical properties (Nel et al. 2006). The broad diversity of ENM properties can lead to inconsistent results in toxicity testing/assays if proper controls are not put in place (**Challenge #1**). In the early days

of the Nano EH&S field, the lack of standard reference materials, standard preparation protocols and standardized analytical characterization protocols meant that researchers often had difficulties reproducing each other's results (Maynard 2006, Roebben et al. 2011, Roebben et al. 2013).

One of the most significant advances made towards reducing inconsistency of results from toxicity assays was the development and dissemination of standard reference nanomaterials by the National Institute of Standards and Technology (NIST). Since 2004, when the NIST's Advanced Measurement Laboratory (AML) was established (Rashba et al. 2004), NIST has actively worked on the development of measurement protocols of ENMs and the development of standard reference nanomaterials. These standard reference nanomaterials are selected because they are sufficiently homogeneous and stable with respect to one or more particular properties. NIST makes these materials widely available to be fit for its intended use in a measurement process (NIST 2017). These standard reference materials have proven to be essential tools in the quest for comparable and reliable measurement results (Roebben et al. 2011) and have largely helped address variability issues within the field. The NIST website (https://www.nist.gov/mml/nano-measurement-protocols) also includes measurement and protocols developed in collaboration with external partners (e.g., CEINT and UC CEIN). These include protocols for sample preparation, physicochemical measurements and biological/environmental measurements (NIST 2017). Some of the most influential advances include those for preparing dispersed solutions of ENMs (Ji et al. 2010), characterization of the aggregation state in various media using Dynamic Light Scattering (DLS) (Taurozzi et al. 2013) and assays to study nanomaterial induced genotoxicity (Nelson et al. 2015).

Several international groups/organizations have also piloted efforts in the development of standard reference nanomaterials including *representative* test materials (i.e., nanomaterial from a

single batch, which is sufficiently homogeneous and stable with respect to one or more specified properties and has been validated as fit for use in the development of test methods). For example, the European Commission's Joint Research Center (JRC) at the Institute for Reference Materials and Measurements (IRMM), in collaboration with the Institute for Health and Consumer Protection (IHCP) have developed several representative test nanomaterials (Roebben et al. 2013). These test materials are available to researchers through the European Nanomaterials Repository (Totaro et al. 2016)

The diverse structures and compositions of ENMs studied by researchers also necessitates the use of a wide array of analytical characterization techniques. Comprehensive characterization of ENMs properties is necessary not only to provide an understanding of pristine materials, but also to understand how these properties are altered in situ by environmental and biological conditions and how measured ENM properties correlate with biological and environmental responses (Holbrook et al. 2015). Two characterization techniques, scanning electron microscopy (SEM) and transmission electron microscopy (TEM), have been particularly crucial to the advancement of hazard assessment of ENMs. SEM and TEM are useful for tracking cellular and tissue uptake (Buzea et al. 2007). These methods are most useful for studying electron-dense (metallic) nanoparticles and are not very useful for studying soft materials (e.g., dendrimers and liposomes) (Nel et al. 2009). Other techniques that have been adopted for characterization of ENMs (NRC 2012) include:

- Small-angle x-ray scattering, which is used to define nanoparticle cores;
- X-ray photoelectron spectroscopy and Fourier-transform infrared (FTIR) spectroscopy, which is used to define ENM surface chemistry;

- Atomic-Force Microscopy (AFM), which provides information about the overall dimensions of cores and shells of ENMs;
- Thermal gravimetric analysis, which provides the ratio of ligand mass to core mass in ENMs; and
- Nuclear magnetic resonance spectrometry, which can be used to detect small-molecule impurities in ENM preparations.

Given that the application of comprehensive measurements is not always feasible, a number of groups have proposed minimal characterization studies that should be used for all Nano EH&S studies (Boverhof and David 2010, Pettitt and Lead 2013). Typically, the following properties are included in this minimum characterization set (Boverhof and David 2010, Pettitt and Lead 2013):

- Size: particle size distribution (PSD), mean/median, variability;
- Core properties: chemical composition, crystal structure (crystallinity), chemical interactions, morphology and shape;
- Surface properties: specific surface area, charge, oxidation state, coordination chemistry, surface capping/coating agents;
- Aggregation state/agglomeration: aggregate size and morphology, kinetics and mechanisms of aggregation/disaggregation; and
- Solubility (for metal based ENMs): dissolution rate.

Other considerations that should be considered when assessing toxicity of ENMs include the stability of the ENM (e.g., how the ENM changes over time), the media and (where possible) relevant dose metrics, mass and number concentration. Rigorous characterization of ENMs is critical to safety assessment of ENMs, and the inclusion of such information in toxicity studies has been proposed as metric of the quality of ENM toxicity studies (Card and Magnuson 2010).

The exponential growth of the field of Nanotechnology has also created challenges in characterizing exposure potential and hazard for a large number of new materials in a small amount of time (**Challenge #2**). Advances that have allowed researchers to gather a large amount of data in a short amount of time include the development of:

- Alternative Testing Strategies (ATS), which allows a large number of materials to be tested rapidly and can be used to reduce animal experimentation and the time/cost for testing;
- In silico methods, which use computational approaches to predict toxicological outcomes; and
- Read-across approaches, in which known information from well characterized chemicals is extrapolated to novel ENMs (Oomen et al. 2015).

Examples of ATS include high-throughput (HTS) and high-content (HCS) screening methods. These methods can be used to assess toxicological injury pathways, signaling pathways, membrane damage, organelle damage, apoptosis and necrosis pathways, DNA damage, and mutagenicity (Meng et al. 2009). HCS methods focus exclusively on cell-based assays that use microscopic image analysis to assess certain cellular responses (e.g., reactive oxygen species production and membrane changes) (Huo et al. 2015)), while HTS methods include a wide variety of assays to study multiple biological effects (e.g., in vivo zebrafish models (Lin et al. 2013), and cell-free biochemical assays (Rogers et al. 2008)).

In silico toxicology approaches involve the integration of modern computing and information technology with molecular biology (Raunio 2011)) and have been key in the development of models such as quantitative structure activity relationships (QSARs), which are valuable tools for filling data gaps (Roco 2011). High-throughput screening and in silico methods are closely related

as high-throughput data can form the basis for developing and validating in silico toxicity models (e.g., QSARs). Both ATS and in silico methods can be used not only to address data gaps but also to generate data-driven hypotheses to establish and/or validate possible toxicity mechanisms. Data mining techniques (e.g., the process of examining large databases to generate new information) have been shown to be useful during exploratory analysis of HTS data. Data mining can be used to extract information for hypothesis formulation of possible toxicity mechanisms and relationship among different cell responses, and relevance of environmental conditions and ENMs properties (Cohen et al. 2012). One example is the use of heatmaps, which provide ordered representations of data and can facilitate identification of similarity patterns via row and/or column clustering(Cohen et al. 2012). Read-across approaches have also been proposed to predict toxic endpoint information for a given ENM by using data from another ENM or bench chemical that has similar physicochemical properties or a similar chemical composition (Arts et al. 2015, Gajewicz et al. 2015).

The large amounts of data generated from these approaches have created an additional challenge, which is the need for computational and statistical approaches for handling ENMs data sets and providing Nano EH&S assessments (**Challenge #3**). A major advance has been the development of professionally curated data repositories and nanoinformatics tools, which have helped facilitate studies that link the physicochemical properties of ENMs to their toxicological behavior (Guzan et al. 2013, Liu and Cohen 2015, Powers et al. 2015). Examples of initiatives for data curation include, the Nanomaterial Registry (Guzan et al. 2013), ISATAB Nano (Thomas et al. 2013), caNanoLab and eNano Mapper. The Nanomaterial Registry was established to provide reported information on nanomaterials, which have been put through a robust curation process (available at the website www.nanomaterialregistry.org) (Guzan et al. 2013). This registry was

developed by RTI International and is funded by the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the National Institute of Environmental Health Sciences (NIEHS) and the National Cancer Institute (NCI). The Registry captures details about biological and environmental studies on ENMs and provides links, when possible, to the detailed endpoint data associated with the studies. ISATAB Nano comprises four spreadsheet-based file formats for representing and integrating various types of nanomaterial data. These spreadsheet formats are broken down into the following elements: (1) investigation files (reference information about each investigation, study, assay, protocol, study file and assay file); (2) study files (names and attributes of protocols used for preparing samples for analysis); (3) assay files (values of measured endpoint variables and references to external data files for each analyzed sample) and (4) material files (description of the material sample and its structural parts and chemical components). One advantage of such standardized file formats is that they provide a framework for recording and integrating ENM descriptions, assay data (metadata and endpoint measurements) and protocol information (Thomas et al. 2013). In this regard, another data-sharing portal is caNanoLab, which is designed to facilitate information sharing in the biomedical nanotechnology research community to expedite and validate the use of nanotechnology in biomedicine (available at https://cananolab.nci.nih.gov/caNanoLab/) (Panneerselvam and Choi 2014). Likewise, within the NanoSafety Cluster (a cluster of projects funded by the European Commission), eNanoMapper was developed as a computational infrastructure for toxicological data management of ENMs. eNanoMapper works towards supporting the collaborative safety assessment for ENMs by creating a modular and extensible infrastructure for data sharing, data analysis, and building computational toxicology models for ENMs (Jeliazkova et al. 2015).

Efforts to host the large amounts of Nano EH&S data, data repositories and data management plans have been facilitated by broadening data requirements by U.S. agencies regarding data collection and distribution that are not nano-specific but are relevant to nano research. For instance, in the United States, the U.S. Office of Science and Technology Policy (OSTP) coordinates with U.S. Federal agencies to develop policies to promote public access to the results of federally funded research, including digital data (NSF 2015). As part of their grants program, U.S. Federal funding agencies have established requirements for data repositories and data sharing. In the United States, NSF requires applicants for funding to prepare a Data Management Plan (DMP) (NSF 2015). NSF requires that (to the extent feasible), consistent with applicable law and policy, and agency mission, digitally-formatted scientific data resulting from unclassified research supported wholly or in part by NSF funding should be stored and publicly accessible to search, retrieve, and analyze (NSF 2015). Other U.S. research institutions and funding agencies have also developed open data policies. For example, the U.S. National Institutes of Health (NIH) developed a data-sharing policy as early as 2003 to encourage NIH-funded researchers to share scientific data sets (OECD 2015). The policy requires of applicants requesting USD $500,000 or more of funding to include a data-sharing plan in the grant application procedure, or to justify why data sharing is not possible (OECD 2015).

Despite all of these advances, expert systems specific to Nano EH&S are still needed for researchers to manage the information and knowledge linked to data, and to aid in the process of defining the minimal information required to describe ENMs. Currently, heterogeneous formatting of data from different sources continues to challenge integration for nano-databases. Furthermore, data sharing within the Nano EH&S field is still hampered as a result of concerns related to intellectual property and “authorship” of data. Another limitation at present is that curation of data

into data repositories is primarily conducted manually by extraction from the literature. Significant challenges include the time required, as well as uncertainty about what criteria to use when selecting data for analysis (Harper et al. 2013). To address some of the challenges involved in information management, researchers at the UC CEIN have developed a new and improved web-based data/information management system (NanoDatabank: http://nanoinfo.org/nanodatabank/) for Nano EH&S research. This data management system has provisions for convenient data storage, classification, retrieval and sharing (with individual users and/or user groups). This system allows researchers to access data from various studies; these data are classified via a dynamic system that automatically generates Meta Data. At the date at which this was written (July 2018), NanoDatabank contained over 1000 uploaded investigations from UC CEIN and it is expected to be deployed for use of external investigators as well.

Progress Related to Assessing the Hazard Potential of ENMs

Because ENMs are in the same size range as biological receptors, they can have unique biological properties. Hence, the study of ENM interactions with proteins, membranes, cells, DNA and organelles and their nanoparticle/biological interfaces is critical to ascertain their potential hazardous effects.

The interactions of ENMs with proteins and other biological entities can also lead to the formation of protein coronas, particle wrapping, intracellular uptake and biocatalytic processes which can in turn lead to changes in hazard potential (**Challenge #4**). The effects produced by interaction with biomolecules include ENM phase transformations, free energy releases, restructuring and dissolution (Nel et al. 2009).

Given the need to study these interactions to develop safer-by-design ENMs, many technologies are emerging or are being adapted for studying nano–bio interfaces (Nel et al. 2009). For example, Surface Enhanced Raman Scattering (SERS), which is a common technique used for bioimaging of cells and intact animals and has proven very valuable for studying nano-bio interactions (Salata 2004, Nel et al. 2009, Cardinal et al. 2017). SERS is sensitive enough to detect single molecules and can be used as a molecular imaging technique in living organisms (Nel et al. 2009, Cardinal et al. 2017). Other imaging techniques, such as transmission electron microscopy (TEM) cryomicroscopy can be used to image unstained biomolecules and intercellular structures at the sub-nanometer level. TEM, combined with data processing, enables the molecular topographies of single biomolecules to be visualized in conformational states that are not accessible through X-ray diffraction (Nel et al. 2009).

Some properties of ENMs that are related to a high potential for eliciting hazard and such properties can also interfere with standard toxicological assays (**Challenge #5**). For example, in ENMs such as Carbon Nanotubes (CNTs) aspect ratio, impurities, and coatings (Fubini et al. 2011) are important determinants of hazard potential (e.g., CNTs evoke pulmonary inflammatory response similar to asbestos due to their high aspect ratio). For other materials, such as Quantum Dots, the hazard potential has been correlated with their diameter and surface properties including shell, ligand and surface modifications in a recent analysis of more than 300 publications (1,741 toxicity data samples) (Oh et al. 2016). For nano silica, the hazard potential has been correlated with oxidation state/hydration state of surface (Napierska et al. 2010, Fruijtier-Pölloth 2012). In the case of synthetic amorphous silica (SAS), biological activity has been related to the particle shape and surface characteristics interfacing with biological media (Fruijtier-Pölloth 2012).

Major advances have been achieved in understanding the mechanisms of toxicity of ENMs by studying how toxicity varies with particle size distribution, surface area, particle shape, hydrophobicity, chemical composition, redox potential and band gap (Gao and Lowry 2017), thus leading to the development of approaches to predict hazard based on ENMs properties. For metal oxide ENMs (MOx), two properties that have been identified as relevant to their hazard potential are redox potential and band gap energy (Zhang et al. 2012). A study conducted with 24 metal oxide (MOx) nanoparticles showed that it is possible to use conduction band energy levels to delineate their potential toxicity on cellular and animal levels. For MOx ENMs, an overlap of conduction band energy (Ec) levels with cellular redox potential strongly correlates with the ability of ENMs to induce oxygen radicals, oxidative stress, and inflammation (Zhang et al. 2012). In silico approaches, such as nano-SAR (structure activity relationships) leveraging those data have revealed that conduction band energy and ionic index (often correlated with the hydration enthalpy) can be used as suitable descriptors of toxicity mechanisms for MOx ENMs and metal ions (Liu et al. 2013).

Despite the progress made in hazard assessment involving the influence of ENM properties and biological interactions, an area that still requires further research is understanding how transformations of ENMs in the environment can affect hazard outcomes. A recent review (Holden et al. 2016) suggests that pristine ENMs, including those with surface functionalization, capping agents, or adsorbed species or coatings, are more frequently assessed, with only a minor fraction of transformed versions of ENMs being studied. However, studies on textiles, paints, and nanocomposites suggest that released particles significantly transform and age in the environment and exhibit different environmental behavior and effects compared to pristine ENMs (Holden et al. 2016). Hence, based on expert elicitation Holden et al. identify the following strategies to

improve toxicology assays, a) choose test end points, duration, and study conditions (e.g., use of ENM test concentrations that align with realistic exposure scenarios from modeling or monitoring studies), and b) consider environmental realism in ENM hazard assessments (e.g., using receptors and exposure conditions that reflect real life scenarios) (Holden et al. 2016). In their study, Holden et al. recommend improving the coordination among ENM quantitative analysts, exposure modelers, and ecotoxicologists, and stakeholders across government, industry, and academia (Holden et al. 2016).

Assessing hazard of ENMs also requires standard protocols and materials to avoid difficulties in comparing studies done with similar ENMs and inconsistencies in outcomes of in vivo and in vitro studies (**Challenge #6**). Discordance between findings from in vivo and in vitro studies can be partially attributed to batch to batch variability, method and duration of dosing and dose level and method of ENM manipulation prior to testing (Bonner et al. 2013). In this regard, important steps have been made towards standardization of toxicity testing. Relevant examples include efforts by the U.S. National Institute of Environmental Health Sciences (NIEHS) Nano Go Consortium (Xia et al. 2013), the European group QualityNano (Hole et al. 2013) and international groups, such as the International Alliance for NanoEHS Harmonization (IANH) (Roebben et al. 2011) and the OECD (Petersen et al. 2015).

U.S. – Nano GO Consortium

Nano GO Consortium is an interlaboratory, multi-investigator project involved in (NIEHS)-funded consortium studies that conducted a series of in vivo and in vitro interlaboratory experiments to determine if it could generate consistent data sets using a well-characterized and commonly sourced panel of ENMs. This consortium conducted in vitro experiments on bioactivity

evaluations on zinc oxide (ZnO), three forms of titanium dioxide (TiO_2), and three forms of multi-walled carbon nanotubes (MWCNTs) using mammalian cell lines, lung epithelial cells and macrophages (Xia et al. 2013). Their results suggest that conducting studies with multiple relevant cell types to avoid false-negative outcomes is critical for accurate evaluation of nanomaterial bioactivity (Xia et al. 2013). The second set of experiments conducted by members of the consortium included in vivo models to evaluate lung responses in mice and rats to nano TiO_2 and multi-walled carbon nanotubes (MWCNTs) exposure. The results from the Nano GO consortium revealed similar patterns of pathology in rats and mice produced by the nanomaterials. Although interlaboratory variability was observed for the degree of neutrophilia caused by the three types of TiO_2 nanoparticles, similar findings for relative potency for the three types of MWCNTs were found across all laboratories. As a result, this initiative has generated greater confidence in the utility of interlaboratory comparisons (Bonner et al. 2013).

European research consortium Quality Nano

Another group working towards validation of hazard assays is the Quality Nano initiative, which is an EU FP7-funded research group that integrates researchers from 28 European analytical and experimental facilities that study nanotechnology, medicine and natural sciences (Hole et al. 2013). The goal of Quality Nano is to develop and implement best practice and quality in all aspects of nanosafety assessment. The results of an interlaboratory comparison (ILC) exercise measuring modal particle size, via nanoparticle tracking analysis (NTA), showed that even a well-defined protocol (much more complete and detailed than usually described in published experimental studies) can still lead to variability of outcomes (Hole et al. 2013).

International consortiums

The International Alliance for Nano EHS (IANH), established in 2007, is one of the pioneer groups working towards the development of standard (reference) materials, methods, and procedures relevant to Nano EH&S (Roebben et al. 2011). IANH was an interdisciplinary group of scientific experts from Europe, Japan and the United States. This group conducted a series of interlaboratory comparisons (ILCs) among ten different laboratories using reference materials provided by the U.S. National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements of the European Commission's Joint Research Center (JRC-IRMM) and following ISO standards to assess the size and surface charge of suspended nanoparticles. Results showed that, when detailed shipping, measurement, and reporting protocols are followed, measurement of the hydrodynamic particle diameter of nanoparticles in predispersed monomodal suspensions using the dynamic light scattering method results in reproducible (Roebben et al. 2011). On the other hand, measurements of more polydisperse suspensions of nanoparticle aggregates or agglomerates were not reproducible between laboratories (Roebben et al. 2011).

The OECD has also led efforts towards the standardization and validation of toxicity tests. One of the main objectives of the OECD has been to develop aquatic toxicity tests for use with ENMs. To develop such standardized tests, an expert workshop was convened to develop guidelines using OECD aquatic and sediment tests. As a result of this workshop, specific requirements for testing were identified, including ones related to preparation of dispersions, dose metrics, the importance and challenges associated with maintaining and monitoring exposure levels, and the need for reliable methods to quantify ENMs in complex media (Petersen et al. 2015).

Progress Related to Assessing the Exposure Potential of ENMs

Exposure assessment of ENMs poses a unique challenge to Nano EH&S, given the changes that occur in ENM properties that result from contact of ENMs with biological and environmental media. An important point to be considered is that not all nano-enabled products will result in exposures, and not all exposures will lead to new risks. For instance, the use of modern nano-semiconductors is unlikely to lead directly to ENM exposure. Materials and products of most concern are those with the potential to release nanoscale materials into the environment that may lead to biologically relevant exposure (e.g., aerosols, powders, and suspensions of engineered nanometer-diameter particles (nanoparticles) and micrometer-scale agglomerates or aggregates of these particles).

The exposure potential of ENMs depends on properties such as reactivity, toughness and solubility in water, which are highly dependent on ENM size, shape and structure. Once released, ENM properties such as solubility in water, colloidal stability and reactivity have been shown to influence how ENMs interact with the environment into which they are released (Garner and Keller 2014). These same properties also influence the fate and transport of ENMs in the environment and consequently the exposure potential of ENMs.

Data and models have been developed to estimate environmental releases of ENMs and understand how ENMs physicochemical properties influence their behavior, which have in turn improved our understanding of the fate and transport and exposure potential of ENMs (**Challenge #7**). Major advances in this field include the development of several probabilistic and mechanistic models to estimate environmental concentrations of ENMs in different environmental compartments. Relevant probabilistic models include material flow analysis (Gottschalk et al. 2009, Gottschalk et al. 2010, Sun et al. 2014, Gottschalk et al. 2015), mechanistic multimedia fate

and transport models (Liu and Cohen 2014, Meesters et al. 2014) and applied Bayesian Network tools to model environmental multimedia distribution of ENMs using probabilistic (Money et al. 2014) and mechanistic approaches (Bilal et al. 2017). These environmental fate and transport models have also been reviewed in terms of their applicability in Nano EH&S regulatory settings (Nowack 2017). Even though model validation has been limited by the availability of relevant analytical measurements, material flow analysis and mechanistic models can be useful in regulatory frameworks. Such models, for instance are likely to be useful to the European Chemicals Agency (ECHA), where predicted environmental concentrations (PECs) are accepted for the registration of conventional chemicals (Nowack 2017). To further improve this fate and transport models, the parameterization of release models (i.e., input data for fate and transport models) should rely more on measured data, and less on qualitative assessments. To reduce uncertainties derived from missing or conflicting data in such models, additional efforts should aim at reducing uncertainty in ENM production and emission data, as well as market penetration (Gottschalk et al. 2013). Finally, human exposure models have also been developed to estimate oral uptake of nanoparticles in consumer products (Fröhlich and Roblegg 2012) and inhalation exposure to nanoparticles from consumer spray products (Nazarenko et al. 2011) and powders (Nazarenko et al. 2012).

To address the complexities of exposure assessment of ENMs, improved technologies (e.g., analytical methods) have been developed to track the presence, fate and transport of ENMs in the environment (**Challenge #8**). A first step towards improving exposure assessment of ENMs has been the development of standardized methods for analysis and characterization of pristine ENMs (Nowack et al. 2015). Examples of advances in this field include the application of 1) liquid chromatography-atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS), 2) x-

ray absorption fine structure spectroscopy (XAFS) and 3) environmental scanning electron microscope (ESEM). LC-APPI-MS can be used to determine aqueous concentrations of ENMs with positive electron affinity at relatively low levels. Spectroscopic techniques, such as x-ray absorption fine structure (XAFS), including x-ray absorption near-edge spectroscopy (XANES) and extended x-ray absorption fine-structure spectroscopy (EXAFS), have been used in conjunction with electron microscopy to determine the chemical state and local atomic structure of inorganic ENMs and assess their chemical transformations (Domingos et al. 2009, Nowack et al. 2015). Another important method is the environmental scanning electron microscope (ESEM), which allows a gaseous environment in the specimen chamber, and hence can be useful for detecting ENMs in the environment (Farre et al. 2009, Nel et al. 2011). However, most of these the analytical tools are not yet capable of distinguishing the naturally occurring nano-sized materials/particles from engineered nanomaterials at the low ENM concentrations expected in complex environmental media (Nowack et al. 2015). Additional research is needed to further improve analytical technologies to track, sense, detect and image ENMs in environmental, biomedical and biological systems (Nel et al. 2011, Cohen et al. 2012, Nowack et al. 2015).

Another important advance in exposure assessment of ENMs has been the development of rigorous methodologies for assessing the physical state of ENMs in complex media (dosimetry) (Liu et al. 2015). The goal of these studies is to address the difficulty to measure amounts of specific ENMs beyond the traditional mass-dose, particle number, and surface area-dose (SAD) considerations (Nel et al. 2011). Moreover, life cycle considerations have also been included in exposure assessment approaches. For instance, a recently published life cycle methodology simulates consumer use and disposal conditions of nano-enabled products and nanoparticle releases for *in vitro* and *in vivo* toxicological studies (Pal et al. 2015). The published methodology,

provides a standardized protocol to assess the release and toxicological implications of Nano particles released across the life cycle, which consists of the following elements:

1. Real-time monitoring and sampling of size-fractionated nanoparticles;
2. Efficient extraction of particles collected on substrates using aqueous or ethanol extraction protocols to ensure minimal physicochemical alterations;
3. Optimized particle dispersion preparation and characterization;
4. Use of dosimetric techniques for in vitro and in vivo toxicological studies (Pal et al. 2015).

To address the challenges involved in estimating workplace exposure (**Challenge #9**), monitoring approaches and techniques have increased over the last decade allowing scientists and decision makers to improve the identification of sources of exposure, quantification and the implementation of effective management measures. Since 2006, when the U.S. National Institute for Occupational Safety and Health (NIOSH) formally established a nanotechnology unit. One of NIOSH's goals has been to develop strategies to semi-quantitatively evaluate airborne ENM concentrations in the workplace. To achieve this goal, the project NEAT (Nanoparticle Emission Assessment Technique) (Methner et al. 2010) implemented approaches using portable direct-reading instrumentation supplemented by a pair of filter-based air samples (source-specific and personal breathing zone) in a series of field studies in research and development laboratories, pilot plants, and manufacturing facilities. The NIOSH team has used this approach to study a variety of ENMs, including CNTs (single-walled and multi-walled), Carbon Nanofibers (CNFs), fullerenes, Carbon Nano Pearls, MOx, electro spun nylon, and QDs. Of particular importance is that, by incorporating filter-based samples, the team was able to identify particle sources and address differentiation between incidental and process related ENMs (Methner et al. 2010). The results provided by NEAT were useful in evaluating ENM emissions and identifying readily available

engineering controls that can be applied to minimize nanomaterial emissions in the 12 facilities were NEAT's experiments were conducted (Methner et al. 2010). Additional studies include work performed by Harold et al. (Howard 2013) in facilities manufacturing or processing CNTs and Kuempel et al. in occupational settings involving nano TiO_2 (Kuempel et al. 2012). These studies highlighted pulmonary exposure as the main concern for workers, which can be mitigated using engineering controls and Personal Protective Equipment (PPE). Another recent analysis of ENM occupational exposure (studies published between January 2000 and January 2015) ranked ENMs in terms of the quality of evidence behind the exposure assessments (Debia et al. 2016). According to the analysis of these occupational exposure studies, high-quality evidence is available (as of 2016) to support controls of worker exposure to MWCNTs, SWCNTs, CNFs, nano Al_2O_3, nano TiO_2, and nano Ag worker exposure. Oppositely, there was insufficient high-quality studies to conduct an analysis on workers exposure to nano SiO_2, non-classified CNTs, nanoclays, nano Fe, fullerene C_{60}, DWCNTs (double-walled CNTs), and nano ZnO (Debia et al. 2016). In the study conducted by Debia et al., no information was identified about occupational exposure to CeO_2. Additional observations made in the above mentioned study included that exposure to ENMs is heavily related to handling tasks, and that the ENM forms that workers are exposed to include micro-sized agglomerated ENMs, and that engineering controls play a critical role in reducing workers' exposure. One limitation is that the information included in the analysis of occupational exposure conducted by Debia et al. included developed countries only, which highlights the need for ENM occupational exposure studies in low income countries (Debia et al. 2016).

Progress Related to Assessing the Risk of ENMs (Mitigation/Risk Management, Safe Deployment of ENMs)

One of the central questions in the field of Nano EH&S has been whether conventional risk assessment tools are suitable for ENMs, or whether they need to be modified (**Challenge #10**). Risk assessment of ENMs is complicated by the physicochemical properties of ENMs, which make the direct application of traditional chemical risk assessment to ENMs challenging. As a result, a number of alternative frameworks have been proposed ENM risk analysis. This topic remains a subject of high interest among scientists, organizations, governments and policy-makers.

Over the last decade, extensive work has been conducted not only to analyze the suitability of traditional risk assessment methodologies for ENMs (SCENIHR 2005, SCENIHR 2007, SCENIHR 2009), but also to assess the suitability of novel and modified risk assessment approaches for ENMs (Hristozov et al. 2012, Hristozov et al. 2016, Oomen et al. 2017, Romero-Franco et al. 2017). Since 2005, starting with the SCENIHR report (SCENIHR 2005), a wave of studies, meetings and reports have focused on developing new approaches to assess environmental and human health risks of ENMs and to adapt traditional chemical approaches to accommodate the complex physicochemical properties of ENMs and their transformations in the environment. In 2007, the OECD, in collaboration with DuPont, launched a collaborative project "NanoRisk" aiming at analyzing exposure and hazard potential of ENMs (DUPONT 2007). Another effort was led by the International Life Sciences Institute Research Foundation, which convened an expert working group to develop a screening strategy for the hazard identification of ENMs (Roco et al. 2011). The report issued by expert working group, as reported by Roco et al. 2011, included the elements of a screening strategy (i.e., physicochemical characteristics, cellular and non-cellular in vitro assays, and in vivo assays) applicable to an early stage in the development of a risk

assessment process for nanomaterials (Roco et al. 2011). Additional approaches that have been proposed as suitable for conducting risk analysis of ENMs (Erbis et al. 2016) including:

- Monte Carlo simulation methods, which provide a representation of uncertainty, producing distributions for performance measures (Sun et al. 2014),
- Decision tree analysis, in which graphical representation of all decisions and their possible outcomes, and associated uncertainties are incorporated into a risk model (Godwin et al. 2015),
- Multi-criteria decision analysis, in which a trade-off or compromise solutions are assessed to balance several often-competing criteria (Tervonen et al. 2009),
- Bayesian methods, in which a risk or outcome probability estimation is obtained based on observed prior distribution or historical information (Money et al. 2012), and
- Control banding, which is used to determines the safety control measures based on the hazard and exposure risk in the workplace (Paik et al. 2008).

Over the last decade, the application of statistical/probabilistic approaches to deal with uncertainty and or incomplete datasets (e.g., Bayesian Networks, Montecarlo) (Money et al. 2012, Gottschalk et al. 2013) has increased significantly, thus improving the researchers' ability to manage data gaps (Erbis et al. 2016).

Overall, several frameworks and approaches have been identified as partially applicable to risk assessment of ENMs under specific conditions/scenarios (see Romero-Franco et al. 2017) and **Chapter 2)**. However, some gaps still remain, in which a lack of consensus regarding how to standardize knowledge/information and a need for detailed information elements for improving risk assessment in practice (e.g., certain risk assessment frameworks discuss elements theoretically

rather than demonstrating the application in case studies) (Oomen et al. 2017). Furthermore, various studies have shown that further research is needed to address how full risk assessments of ENMs could be conducted using read-across and grouping approaches to address data gaps (Oomen et al. 2017).

Quantitative risk assessment of ENMs has been furthered by the incorporation of computational approaches and mathematical models (QSARs), but more work is still needed in this area as well (Gajewicz et al. 2012, Gajewicz et al. 2017). In a recent review, the currently available (Q)SAR models for regulatory risk assessment of ENMs were analyzed and the results revealed that several of the reviewed QSARs did not fully comply with OECD validation principles and/or did not consider relevant endpoints. Hence, the use of these models for regulatory purposes was only recommended in a weight of evidence approach and therefore in conjunction with other in vitro and in vivo information. The results of this study suggest that additional data is required from standardized testing protocols and complemented with metadata (Burello 2017).

As the analysis and development of novel risk assessment approaches has increased, so has the need for adequate data and tools to assess data sufficiency to conduct such assessments (**Challenge #11**). To address data requirements, several methodologies (e.g., scientific reviews, multi-criteria decision analysis (MCDA), weight of evidence (WOE), and integrated approaches to testing and assessment) have been proposed to address the identification and prioritization of critical hazard and exposure data.

One approach to assessing whether sufficient information is available to conduct risk assessments of ENMs has been to conduct scientific reviews of the existing literature on the health and safety of specific categories of ENMs. The project ENHRES (Engineered nanoparticles: review of health and environmental safety) in the EU addressed the availability of scientific

information to assess potential risks for four classes of ENMs (fullerenes, carbon nanotubes (CNT), metals and metal oxides) via a comprehensive and critical scientific review (Aschberger et al. 2010, Aschberger et al. 2010, Christensen et al. 2010, Aschberger et al. 2011, Christensen et al. 2011). As part of ENHRES results, key recommendations were developed in the context of informing policymakers in the development of methods to address exposure as it relates to the potential hazards posed by ENMs (Aschberger et al. 2010, Aschberger et al. 2010, Christensen et al. 2010, Aschberger et al. 2011, Christensen et al. 2011).

Additionally, a weight of evidence (WOE) approach, which has been used in traditional hazard and risk assessment of chemicals, has been adapted to evaluate individual sources of information related to ENMs and form conclusions (Hristozov et al. 2014, Hristozov et al. 2014). By incorporating a WOE method with a weighted sum (MCDA technique), hazard is estimated on the basis of data for three (sets of) criteria: material properties, toxicity and data quality. This approach was tested on a case study involving nano TiO_2, where data from 29 papers reporting toxicity endpoints and highlighted conflicting results/data quality concerns were analyzed (Hristozov et al. 2014).

Approaches such as WOE and the ENHRES project that have focused on identifying whether adequate data exists to conduct risk assessments on ENMs have also been paralleled by a drive to prioritize of research needs. One approach for prioritizing research was developed by Linkov and coworkers (Linkov et al. 2011), which focuses on identifying and ranking how research resources should be directed to produce effective information to manage the risk and impacts of ENMs on the environment and health, based on MCDA and a value of information approach. The second example has been developed by the EU "NanoSafety Cluster" and aims at identifying key areas for further research on risk assessment procedures for ENMs. As part of this project, integrated

approaches for the (eco-)toxicological testing and assessment (IATA) of ENMs are used in a tiered manner to retrieve necessary information by starting at determining concerns, i.e., specific information needs for a given ENM based on realistic exposure scenarios (Oomen et al. 2014). In addition, the Intelligent Testing Strategy (ITS) Nano (also in the EU) was developed based on expert opinions (government, industry, academia, funders and NGOs). ITS Nano proposed the following elements as priorities for current and future risk assessment of ENMs, physicochemical characterization, exposure identification, hazard identification and modelling approaches (Stone et al. 2014).

To ensure sustainable development of nano-enabled products, some studies in the Nano EH&S have incorporated life-cycle assessment considerations, which allows for more holistic estimates of the potential environmental impacts of ENMs (**Challenge #12**). The importance of Life Cycle Analysis (LCA) in assessing the environmental and human health impacts of ENMs has been highlighted in several studies (Linkov and Seager 2011, Hischier and Walser 2012) and the incorporation of LCA and RA for ENMs has been assessed in terms of key ‘‘lessons learned’’ from previous experience with chemicals (Grieger et al. 2012). While the studies addressing LCA of ENMs are limited (Hischier and Walser 2012) and differ from the scope of Risk Assessment (RA), the inclusion of life cycle perspectives has regarded as an important parameter in the applicability of RA frameworks to ENMs (Grieger et al. 2012). At this point, there appears to be fairly high consensus among the scientific community that environmental and health risks should be considered over the entire life cycle of ENMs or nano-products (Grieger et al. 2012). Advances in the field include the development of methodologies where LCA and RA inform each other (Walker et al. 2015). One example is a method that draws upon insights produced by risk assessment to better estimate potential harms associated with expected releases from a hypothetical

(or actual) process in LCA models (Walker et al. 2015). While, there remain challenges in the adaptation of LCA to ENMs and in communicating results for scientific as well as policy audiences, the influence of LCA offers considerable insights into potential consequences of ENMs as an emergent technology (Walker et al. 2015).

In addition to the development of frameworks to assess risk potential of ENMs and the incorporation of life cycle perspectives, a major milestone has been the translation of the effects see in experimental assays to human population via epidemiological studies (**Challenge #13**). The relevance of epidemiological studies to Nano EH&S is critical, given that these studies integrate the assessment of ENMs hazards and human exposure resulting in human health risks. In this regard, the first wave of epidemiological studies conducted over the last decade was analyzed in 2015 (Liou et al. 2015). A recent review of the epidemiological literature identified 15 studies (Liou et al. 2015), of which 11 were cross-sectional, 4 were longitudinal (prospective), and 1 was a descriptive pilot study. These studies focused on occupational exposure by assessing biomarkers of exposure (the assessment of exposure was made by mass concentration in 10 out of the 15 studies) as the dependent variables. Eleven cross-sectional studies showed a positive relationship between various biomarkers and ENM exposures (Liou et al. 2015). Only three of the four longitudinal studies showed a negative relationship between the biomarker and the exposure. Most studies involved a small sample size (from 2 to 258 exposed workers). In conclusion, the exposure levels reported were not very high in comparison to those in human inhalation chamber studies (Liou et al. 2015).

Finally, one of the most important applications of risk information and methodologies has been the development and implementation of risk reduction strategies to protect human health and the environment (**Challenge #14**). As a result of the focus on occupational safety of ENMs, some

advances have been made in the last decade to develop risk management guidance. Important achievements in the mitigation of occupational risks related to ENMs, include determination of risk exposure levels and implementation of industrial hygiene (IH) control measures by a NIOSH research group (Kuempel et al. 2012).

To mitigate risks, important advances in the last decade include determination of occupational exposure limits (OELs) (Kuempel et al. 2012) and prevention strategies (e.g., modification of ENMs physicochemical properties) (Yan et al. 2011). A grouping approach to develop OELs was proposed by Kuempel et al. (Kuempel et al. 2012) and consists on the identification of relevant benchmark (reference) particles, which can be defined as substances with adequate data on dose–response relationships and biological mode of action (MOA) for use in quantitative risk estimation. Using this approach, Kuempel and co-workers developed OELs for ultrafine TiO_2, Diesel exhaust particles and Carbon black (Kuempel et al. 2012). Additional studies have also proposed OELs for ENMs such as CNTs (single and multi-walled), nano TiO_2, nano Ag and Fullerenes (Lee et al. 2011, Van Broekhuizen et al. 2012). However, existing studies suggest a need for further development of more scientifically robust OELs that are based on long term inhalation and epidemiological studies (Gordon et al. 2014). In the absence of OELs for specific nanomaterials, occupational health specialists consider that the best strategy to mitigate risks is to minimize exposures to ENMs by using engineering controls and personal protective equipment (PPE) (Schulte et al. 2013). Other prevention strategies include ENMs that still have desirable materials properties but have lower hazard potential. For instance, in the case of carbonaceous ENMs, effective strategies include: chemical modification (e.g., regulating one or more of the factors such as reactivity, purity and solubility), modification of surface chemistry (e.g., transform the surface of carbon ENMs from hydrophobic to hydrophilic by attaching different water-soluble and

functional moieties on the surfaces or adding a coating material to reduce hazard (Wang et al. 2015)), and modification of the ENM structure (e.g., replacing single-walled CNTs for double-walled CNTs in applications lead to safer products) (Yan et al. 2011).

Progress Related to Ensuring the Safety Next Generation ENMs

Over the last two decades, the development of new ENMs and nano-enabled products, such as those applied in medicine has led to a higher level of complexity in assessing their safety (**Challenge #15**). The development of novel and complex ENMs (and their commercial applications) has led to a critical need for scientific evidence to support their submission for approval by regulatory agencies. With nanotechnology medical applications already in the market (e.g., liposomes in Doxil used to treat certain types of cancer) (Chang and Yeh 2012) and continuous growth, it is expected that the need for novel testing strategies will also increase.

Highlights of new nano-enabled products include medical applications such as ENMs used as diagnostics (e.g., imaging), drug delivery agents and cancer therapies (Bobo et al. 2016). In the case of diagnostics, magnetic nanoparticles (MNP) have been used for disease imaging via passive targeting, and recently they have been used for to cellular-specific targeting, drug delivery, and multi-modal imaging (Veiseh et al. 2010). In order to work effectively, MNPs must be able to bypass in vivo barriers, which is highly influenced by the physicochemical properties of ENMs (size, shape, and surface chemistry all can dictate in vivo behavior, including biodistribution, biocompatibility, and pharmacokinetics) (Veiseh et al. 2010). Other relevant examples in nanomedicine include silica particles, which have been developed into improved treatments for pancreatic cancer (Liu et al. 2016), Aluminum based ENMs have been used as vaccine adjuvants

(Sun et al. 2013) and gold based ENMs have been used for dermal formulations (Bessar et al. 2016).

As of 2015, a significant number or nanomedicine applications had been approved by the FDA (Bobo et al. 2016). Bobo et al. identified 51 FDA-approved nanomedicines (i.e., therapeutic or imaging agents which included a nanoparticle in order to control the biodistribution, enhance the efficacy, or otherwise reduce toxicity of a drug or biological agent) and 77 additional products in clinical trials (Bobo et al. 2016). While most of the FDA-approved materials include polymeric, liposomal, and nanocrystal formulations, the field is moving towards the inclusion of more complex materials including micelles, protein-based NPs, and a variety of inorganic and metallic particles.

However, to advance the progress of nanomedicine applications from research stage into the market, a key challenge for researchers, industry, and regulators is how to classify new materials and what safety testing is required before products become available. Advances in terms of testing include work conducted by the Nanotechnology Characterization Laboratory (NCL) (U.S.) in 2004 to develop and perform characterization standards for nanomedicines (e.g., the use of multiple methods based on different principles to measure each physicochemical and performance property of a nanomedicine) that reach the clinical-trial stage (Tinkle et al. 2014). In Europe, The European Medicines Agency (EMA) evaluates and supervises medicines designed for use in the EU for protecting and promoting public (and animal) health (Hafner et al. 2014). As part of these evaluations, the EMA monitors the safety of nanotherapeutic products and regulates them within a conventional regulatory framework. However, additional expert evaluations are necessary to confirm the quality, safety, and efficacy of nanotherapeutics because of their complexity. In this regard, recent EMA activities aiming to provide regulatory guidance (i.e., implementing the needs

of nanotherapeutic-specific properties) and assistance (i.e., scientific advice on the appropriate tests and studies) in developing high-quality, effective, and safe nanotherapeutics have been reviewed in the literature (Hafner et al. 2014). Furthermore, the regulatory frameworks in Europe and elsewhere are still adapting to the needs of nanomedicine and incorporating new scientific data addressing the safety of novel applications (Sainz et al. 2015). Consequently, a series of reflection papers has been drafted on principles for the development and evaluation of nanomedicines off-patent. These have been developed largely with reference to first-generation nanomedicines and provide the principles to be considered when generating supporting evidence to changes made to the manufacture and control of these products, as well as principles for the development and evaluation of emerging nanomedicines (second-generation nanomedicines) progressing towards first-in-man studies (Ehmann et al. 2013).

As this field keeps evolving, the current regulatory gaps that exist in nanomedicine must be addressed and a general recommendation among experts is that existing and future efforts are balanced between innovation (e.g., R&D) and public health protection (Tinkle et al. 2014). In the U.S., the need for adequate standardization and characterization of nano-based systems is currently being addressed by a specific initiative (i.e., the NCL). Thus, novel challenges in regulatory science (e.g., related to personalized medicine) questions could be met in the US within this initiative (i.e., the NCL) by integrating materials science with validation of adequate models (e.g. preclinical human cells and tissues in appropriate setting to foster clinical translation and better outcomes within clinical phase), and targeting an adequate disease stage and disease evolution conditions (Tinkle et al. 2014).

Overall, the development of next generation ENMs (3^{rd} and 4^{th} generation) is growing at a fast pace, which means that procedures developed for relatively simple ENMs to be validated as

suitable for these next generation ENMs (**Challenge #16**). Over the last decade, major advances have been accomplished in terms of EH&S and risk assessment approaches for passive structures (e.g., 1st generation ENMs, including aerosols, colloids, nanoparticles, polymers, etc. and 2nd generation ENMs, including targeted drugs and biodevices). However, emerging 3rd generation ENMs (systems of nanosystems, including guided assembling; 3D networking and new hierarchical architectures, robotics, evolutionary Biosystems) and 4th generation ENMs (molecular nanosystems, including molecular devices 'by design', atomic design, emerging functions) still warrant tailored safety assessment approaches.

As nanotechnology improvement milestones (e.g., better control of molecular self-assembly, quantum behavior, creation of new molecules, and interaction of nanostructures with external fields in order to build materials, devices, and systems by modeling and computational design (Roco et al. 2011)) are coming to reality, a framework has been developed to assess the safety of complex ENMs (von Gleich et al. 2008). In this approach, the step from the stage of self-organization towards self-replication is considered critical with respect to precautionary risk management measures. This framework will likely be important in cases where nanotechnology is combined with biotechnology or robotics. However, the steps of the proposed approach (i.e., technology characterization, development of eco-profiles through life cycle and development of vision statements that integrate health, safety and environment) do not include a differentiation between first/second generation ENMs and novel generations. More recently, a call for changes/improvements in EH&S oversight/regulatory actions was part of a NSF 4-year project on "Evaluating Oversight Models for Active Nanostructures and Nanosystems: Learning from Past Technologies in a Societal Context'' (Ramachandran et al. 2011). This call includes three major features:

- More stringent regulatory oversight is needed that is adapted to the changes of nanotechnology in a timely manner;
- Inputs from all stakeholders must be integrated, with strong public engagement in decision-making; and
- An overarching coordinating entity should be considered to assure strong inter-agency coordination and communication.

Despite their activity, certain challenging features/characteristics of next generation ENMs are still to be addressed by safety assessments and warrant further research (Subramanian et al. 2010). Important classes of next generation ENMs for which safety research is needed include:

- Remote-actuated active nanostructures (nanotechnology based on the principle of remote activation of sensing),
- Environmentally-responsive nanostructures (nanotechnology that is sensitive to stimuli like pH, temperature, light, oxidation-reduction, etc.),
- Hybrid active nanostructures (nanotechnology involving uncommon combinations of biotic, abiotic, organic, inorganic materials), and
- Transforming active nanostructures (nanotechnology that changes irreversibly during some stage of its life cycle).

Progress Related to Communicating the Risks of ENMS

Among the elements considered by the Nano EH&S community in addressing long-term implications of nanotechnology, the Ethical, Legal and Societal Issues (ELSI) related to nanotechnology and the involvement of stakeholder groups have remained as a priority (**Challenge #17**). In the U.S., the NNI has incorporated ELSI into Nano EH&S since 2010 (Roco et al. 2011).

The NNI aims to achieve collaboration from different communities, such as consumers, engineers, ethicists, manufacturers, nongovernmental organizations, regulators, and scientists. As these stakeholder groups provide their perspectives on new research directions, this process is critical to ensure public trust in nanotechnology and to promote innovation and commercialization of ENMs. Stakeholder engagement activities such as meetings, workshops and symposia have resulted in an active/ongoing participation of members from all fields related to Nano EH&S (e.g., scientists, regulators, industry, various interest groups, representatives of media and the public at large) (Linkov et al. 2009, NNI 2012, Nel et al. 2013, NNI 2013, NRC 2013, Godwin et al. 2015). Dialogue in this regard has been encouraged by U.S. and European funding and government agencies to disseminate reliable information on nanosafety, and outreach to various stakeholder groups in order to assure that health and environment aspects are being taken into account (Savolainen 2013).

In the United States, the NNI has been actively involved in stakeholder engagement activities since 2006, when the NNI's first comprehensive workshop on public participation in Nanotechnology was held. This NNI workshop was intended as a guidance to the Nanotechnology Public Engagement and Communications (NPEC) group in the development of strategic plans for ongoing participation activities. This workshop brought together people with a wide range of interests and expertise, from diverse backgrounds in academia, industry, government and NGOs, along with members of the general public, to explore ideas on how to engage the public in nanotechnology policy development and decision making (NNI 2012). A subsequent effort was the workshop on Stakeholder Perspectives on Perception, Assessment, and Management of the Potential Risks of Nanotechnology (the "R3 Workshop"), which was held in 2014. The goal of this workshop was to assess the status of Nano EH&S risk science three years after the

development of the 2011 NNI EH&S Research Strategy and to identify the tools and best practices used by risk assessors to address the implications of nanotechnology. At this event, a wide range of stakeholders including Federal and State regulators, small and large businesses, insurance companies, academic researchers, occupational safety specialists, and public and environmental advocacy groups shared their perspectives on ENM risk management and discussed strategies and approaches for improving risk science methods for ENMs. Another important outcome was the discussion of ways that NNI agencies can assist stakeholders in the responsible development of nanotechnology (NNI 2013). Since their first report, the U.S. National Research Council (NRC) "A Research Strategy for Environmental, Health and Safety aspects of Engineered Nanomaterials", the agency worked on improving agency interaction, accountability and stakeholder involvement (NRC 2013). In a 2012 workshop, federal agency and foreign officials, academic researchers, and representatives of nongovernment organizations and industry discussed scientific and regulatory progress on Nano EH&S (NRC 2013). As part of the report, successful collaboration efforts were highlighted including the NIOSH's work with the NNI and external partners in the private, academic, government, and international sectors aimed at stakeholder engagement; and direct engagement with the ENM industry through the site-visit program for ENM manufacture and use and through evaluation of materials and processes that are under development. Additional efforts by NIOSH consist of public–private partnerships, including publication of research results from NIOSH and development of memoranda of understanding at key research and development centers (NRC 2013).

Other initiatives involving stakeholder involvement include a series of meetings held by the OECD nanomaterial working party (OECD 2013) and the development of Nano Risk framework for ENMs by DuPont (DUPONT 2007). Starting in 2006, the meetings organized by OECD

nanomaterial working have provided a platform for OECD delegates to describe national initiatives related to the safety of ENMs (OECD 2006). During their first meeting, the OECD delegates reported the milestones related to:

1. Any national regulatory developments on human health and environmental safety including recommendations or discussions related to adapting existing regulatory systems or the drafting of laws/ regulations/ guidance materials;

2. Developments related to voluntary or stewardship schemes;

3. Information on any risk assessment decisions;

4. Information on any developments related to good practice documents;

5. Research programs or strategies designed to address human health and/ or environmental safety aspects of nanomaterials; and

6. Information on any public/stakeholder consultation (OECD 2006).

Groups from academia have also led important activities to promote stakeholder participation in Nano EH&S. In 2014, during a meeting of the Sustainable Nanotechnology Organization, a wide-ranging discussion concerning nano-manufacturing environmental health and safety, between industry and government representatives, insurers and litigators, and experts in life cycle and risk analysis, was transformed into a sharing experience of the participants' expertise and concerns. In this meeting, key understandings emerged about a broad range of factors influencing industry decision-making and investment, public perception, and government regulation (Isaacs et al. 2015). A separate workshop convened by the UC-CEIN brought together national and international leaders from government, industry, and academia. Using CNTs as a case study, participants were able to discuss the utility of ATS for decision-making analyses of ENMs. As a result of this interaction, a short list of generally shared viewpoints on this topic was generated,

including a general view that ATS approaches for ENMs can significantly benefit chemical safety analysis (Nel 2013). A second workshop convened by the UC-CEIN resulted in the articulation of a process for categorization of ENMs according to risk potential and a description of how such an approach could facilitate regulatory decision-making in the future (Godwin et al. 2015).

Beyond international meetings and academic efforts, active participation and engagement of stakeholders has been part of an enhanced implementation of and compliance with the Nano EH&S regulations. For example, in the EU, the relevant stakeholders have actively participated not only in the design but in the implementation of nano-regulations (Justo-Hanani and Dayan 2016). Given the high attention to the regulatory and public engagement aspects of nanotechnology in Europe several efforts have emerged. The Department for Environment, Food and Rural Affairs (DEFRA) in the UK and the Europe Nanotechnology Trade Alliance (ENTA) have organized policy workshops for stakeholders and published research reports with a clear focus on understanding the social implications of nanotechnology (Matsuda and Hunt 2009).

In addition to stakeholder involvement programs, the dissemination of knowledge regarding the benefits and potential risks of ENMs is another factor affecting the public perception, and potential "acceptance" of ENMs (Savolainen 2013) (**Challenge #18**). Hence, work carried out by federal agencies and research groups to communicate risks/develop risk communication strategies to inform relevant stakeholders is critical (Bostrom and Löfstedt 2010, Gibson et al. 2012). Effective risk communication strategies must consider public perception, which may have a critical effect on the development, uptake and exploitation of ENMs. Hence, targeted, neutral and reliable communication by the different stakeholders (regulators, industry, various interest groups, representatives of media and the public at large) associated with Nano EH&S can influence the acceptability of safe ENMs (Savolainen 2013).

One of the challenges in the dissemination of knowledge in Nano EH&S is that not all of the long-term risks associated with ENMs are not fully understood (Priest 2011). In this regard, efforts by the European NanoSafety Cluster have been directed towards the identification of priority areas for Nano EH&S research (e.g., understanding of ENM features, exposure to them, hazard mechanisms of ENM, and risk assessment and management) (Savolainen 2013) and a description of best practices for stakeholder engagement in these areas. The NanoSafety Cluster report highlights that effective communication and knowledge dissemination should include the integration of all relevant key stakeholders and dialogue in focus groups to gain added value via detailed in-depth discussion. Such stakeholder groups should involve representatives of all key stakeholder groups within European Union, North America, Asian countries and other global interest groups in active communication with press/media to facilitate science-based information transfer to the general public (Savolainen 2013). The European Agency for Safety and Health at Work (EU-OSHA) has also published a report aimed at identifying principles risk perception and communication of nanotechnology in the workplace and the public understanding of the risks (Gibson et al. 2012). At the time of publication this report (2012), the state of risk communication regarding ENM safety was deemed as limited, given the many uncertainties of ENMs' hazards. It was observed that workers had minimal knowledge and understanding of ENMs. As for the initiatives (e.g., exhibitions, web-based materials or events) available at the time, only a few of them were considered suitable to engage the public or promote dialogue and some of them were aimed at researchers or the public as consumers rather than at people potentially exposed in an occupational setting (Gibson et al. 2012).

Key elements of Nano EH&S risk communication

Good risk communication practice in Nano EH&S should involve planning, delivering and evaluating new initiatives. Different risk communication approaches should be used depending on whether risks are routine, highly uncertain or potentially controversial and tailored to the target audiences (user-centered) considering the audience's information needs and preferences, concerns and value systems. An additional parameter for successful strategies is whether the intended audience retains key information and takes action based on such information (Gibson et al. 2012). In addition to these activities, the dissemination of knowledge to the public has been identified as critical. In the U.S., surveys conducted in 2004-2005 showed little to limited knowledge of nanotechnology by the general public and a need for further public awareness of nanotechnology (Bostrom and Löfstedt 2010). Media coverage regarding nanotechnology showed a significant rise during the 2000-2009 period (Bostrom and Löfstedt 2010). Based on these results, Bostrom et al. concluded that regulators and industry needed to play a more active role in proactive risk communication strategies, as most of the available risk communication documents had been developed by NGOs and other third parties, among whose interests the science may sometimes be secondary (Bostrom and Löfstedt 2010).

Other studies have addressed the need to communicate Nano EH&S findings to academics and decision-makers. For example Handy et al. (Handy and Shaw 2007) have provided a toxicologists perspective, outlining possible routes of uptake by humans, environmental concentrations, known or suspected toxic effects, and the practical implication for human health risk assessments and public perception (Handy and Shaw 2007). Massawe et al. (Massawe 2013) conducted a survey among state agencies responsible for the environment, safety, and public health to understand their current and future information needs and capabilities to regulate ENMs. The results showed that,

at the time of the survey, participants considered there were significant data gaps on the toxicity and ecological impacts of nanomaterials and hence precautionary measures should be taken. These researchers suggested that research to develop techniques for exposure assessments, surveillance and monitoring, databases, and characteristics of workplaces where ENMs are used was warranted based on the results of the survey (Massawe 2013). Innovators across disciplines such as engineering, biology, medicine, and public health should collaborate in order to minimize potential negative impact on health for individuals and populations. Knowledge gaps regarding the potential health and safety implications of exposure to engineered nanomaterials need to continue to be addressed and actively researched (Pautler and Brenner 2010).

Finally, significant milestones have also been reached in providing education and outreach about Nano EH&S to the public. Important initiatives led by the NSF include academic programs, student fellowship programs, combined research-educational curriculum development and public education programs (Roco 2002). With the continuous growth of nanotechnology, projections of work force needed in the next decade suggest that 2 million workers will be needed worldwide for the nano industry. Hence, the U.S. NSF has supported education and training activities (directly by education awards, or in conjunction with research projects) in the areas of nanoscale science and engineering. Key areas of focus have included courses offered in universities; student fellowship programs; education and training in centers and networks; combined research – curriculum development (CRCD); local and long-distance outreach education; technological education; public education (non-technical audiences); and international education exchanges (Roco 2002). In addition to workforce training, informal education events have also been developed on Nano EH&S topics. For example, the National Informal STEM Education Network (NISE) organized annual "Nano Days" during the period of 2008 – 2015. NanoDays was an

annual, nationwide festival of educational programs about nanoscale science and engineering and their impact on society. NanoDays events were organized by participants in the Nanoscale Informal Science Education Network and took place at science museums, research centers, and universities across the country from Puerto Rico to Hawaii (NISE 2015).

Although major accomplishments have been accomplished in terms of outreach and risk communication, one group of stakeholders that have been largely overlooked, but who are important to the sustainability of nanotechnology, is the public health community. The contribution of nanotechnology and nanomedicine to public health is important because a wide range of innovations in nanomedicine have the potential to impact nearly every medical specialty and unveil novel ways to improve the quality and extend the duration of life (Pautler and Brenner 2010). For example, heart disease and cancer combined make up approximately half of all deaths in the United States per year, and advances in nanomedicine demonstrate great potential to reduce rates of morbidity and mortality due to these diseases. Most of the publications on Nano EH&S and public health have exclusively focused on the potential roles of ENMs and nanomedicine in improving global health (Faunce and Watal 2010, Pautler and Brenner 2010). Public health applications of nanomedicine such as rapid and portable diagnostics and more effective vaccinations do have the potential to revolutionize global health (Pautler and Brenner 2010), but there are other areas where nanotechnology can be leveraged to further public health. One example is a study published in 2010, where nano Ag in waste water was listed by a team of public health experts as one of 15 nascent issues that could deleteriously affect the conservation of biological diversity (Faunce and Watal 2010). The position of these action was that, while the application of nano Ag to water treatment presented considerable benefits, major concerns were related to its high in vitro toxicity for aquatic organisms and capacity to environmentally persist. The

researchers were also concerned about potential effects from direct poisoning of humans or the production of bacterial resistance in hospital settings (Faunce and Watal 2010).

THE NEED FOR INTEGRATIVE ENVIRONMENTAL IMPACT ASSESSMENT FRAMEWORKS FOR ENGINEERED NANOMATERIALS

ENMs are ubiquitous as they are applied in various commercial and domestic products and technology, which include catalysis, imaging, medical treatments and equipment, and environmental applications (Hotze et al. 2010). The promise of remarkable benefits through the potential applications of ENMs has reached all aspects of cutting-edge technology and everyday life. Such implications mark a huge milestone in terms of development since the current and expected applications are present in medicine and pharmaceuticals, personal care and consumer products, food and agriculture, energy, computing, the environment and public health. Currently, nanomaterials used in commerce include the application of nano sized TiO_2 in sunscreen lotions to provide transparency and a less toxic alternative to certain organic sunscreens (Coussens and Goldman 2005). Other ENMs are also incorporated in sporting equipment, clothing, and even three-dimensional printing (Coussens and Goldman 2005) and pharmaceuticals such as Doxil (e.g., in treatment for certain types of cancer) (Chang and Yeh 2012).

However, this rapid introduction of existing and new engineered nanomaterials (ENMs) into the market and their increased production levels (Roco 2011) has led to increased concern about potential exposure (e.g., in the workplace, among consumers, and in the environment (Som et al. 2010)) and potential impacts. Various studies have reported that certain ENMs possess properties that may lead to biological hazard (Elder et al. 2009, Oberdoester 2010, Pietroiusti 2012).

However, the elucidation of the general principles governing the toxicity potential and the long-term environmental health and safety impact of ENMs is yet to be determined (SCENIHR 2009).

Thus, an imperative step to address the increasing concerns about ENM safety is to develop data/information, modeling/analysis, tools and frameworks to conduct Environmental Impact Assessment (EIA) of ENMs for various release and exposure scenarios and communicate the results back to the Nano EH&S community. To accomplish the above, the scientific community is faced with critical needs and challenges, including:

- Insufficient data/information to conduct quantitative environmental impact/risk assessment,
- A need for data storage and integration techniques for seamless integration with analysis tools/models,
- A need for methods to handle uncertainties associated with potentially conflicting published data and meta-analysis based on multiple sources, and
- The complexities involved in integrating qualitative and quantitative information to identify and rank ENMs with respect to their environmental concentrations and toxicity outcomes.

To date, a substantial amount of work has been published in efforts to address the needs in this field (as shown in **Figures 1.2** and **1.3**). However, the field of environmental impact/risk assessment of ENMs is at present not advanced enough to accomplish all of the criteria needed for the development of an internationally accepted nanomaterial decision framework (Hjorth 2017).

Given the current information gaps and complexity in analyses faced by the Nano EH&S community and the fact that waiting until all the elements needed for an ideal framework is unfeasible, one way to design a practical and rapid EIA strategy is via an integrative process

divided in tiers (e.g., sequence of logical/incremental steps). For this purpose, one can think of such an approach, geared towards the EIA of ENMs, as capable of handling data uncertainty and identify conditional dependence of various attributes affecting ENM toxicity, exposure and ENMs release scenarios. Thus, prior to commencing with the process of assessing the potential impacts of ENMs, the objectives of the targeted assessment must be formulated, and the process divided into a sequence of organized steps.

DESCRIPTION OF A PROPOSED INTEGRATIVE PROCESS FOR ENVIRONMENTAL IMPACT ASSESSMENT OF ENMS AND ORGANIZATION OF THE THESIS

The primary goal of this thesis is to present a proposed tiered process to conduct the EIA of ENMs and provide a methodology to enable this process. The tiered EIA process we propose includes the following steps (**Figure 1.4**): 1) Identify the intent/purpose of the analysis; 2) Identify frameworks /approaches available to conduct the assessment; 3) Assess the adequacy/sufficiency of information available to conduct the assessment and 4) Select the framework for the environmental impact assessment and conduct the analysis for the desired scenario(s).

1. **Identify the intent/purpose of the analysis.** The first step in the analysis is to identify the *intent for making the decisions* regarding the potential risk of ENMs and the level of decision making, if any (i.e., who is and/or what is the authority of the decision maker/analyst?). Another relevant element is to identify the analysis type (e.g., if the purpose is to assess hazard, assess exposure or assess risk potential of the target ENM or all or some of the above).

2. **Identify frameworks/approaches available to conduct the assessment.** One way of identifying suitable EIA/risk assessment frameworks is to identify potential decision-making contexts. In **Chapter 2**, a critical review and analysis (Romero-Franco et al. 2017) is presented to provide an overview of the state of the art of existing frameworks and approaches developed to conduct EIA of ENMs and to illustrate how such frameworks can be applied for different purposes (Steps 1 and 2). This chapter covers six plausible decision scenarios with the types of information involved and potential solutions/tools development to address potential challenges. These scenarios were designed based on the most common and pressing needs by critical stakeholders to arrive at decisions regarding the environmental health and safety of ENMs. The first four of the selected scenarios were related to manufacturing ENMs and occupational health and safety concerns, and the last two scenarios were related to registration of new ENMs and establishment of maximum allowable exposure levels. This chapter also highlights the gaps that currently exist between the needs of decision makers, and the abilities of present frameworks and tools to meet those needs.
3. **Assess the adequacy/sufficiency of information available to conduct the assessment.** A critical step in any EIA or risk assessment process is problem formulation, since a deficient problem formulation may lead to insufficient clarity regarding the purpose and the use of data being collected (EPA 2004). In the context of EIA for ENMs, the assessment of the adequacy of information during a problem formulation stage (i.e., prior to conducting a full EIA), can help the analyst in the identification of critical information and data gaps before too much time is invested in the EIA. This step is of particular importance in order to quantify the availability or lack of information so as to avoid, to the extent possible, "paralysis by analysis" where the decision process is slow or "frozen" (Hansen and Baun 2012). One

possible way to assist EIA analysts and decision makers is to provide a decision support tool (DST) that can integrate elements from multi-criteria decision analysis, whereby different studies are organized systematically and assigned to specific information parameters. These parameters (divided into categories, sub-categories and attributes, as shown in **Chapter 3**) can be given weights to reflect their relevance to the EIA process (or parts of the process). Next, the information elements, including the individual studies, are aggregated to estimate quantitative scores that represent the adequacy of information and so the analyst can proceed with the selection of a suitable method/framework to conduct an EIA. Major challenges in developing this type of approach are the integration of heterogeneous information (i.e., quantitative and qualitative) and handling data uncertainties.

In **Chapter 3**, we present the design, development and implementation of a tool to assess the adequacy of available information to conduct EIA of ENMs (IANano) (**Step 3**). Also, in **Chapter 3**, we discuss how regardless of the level of EIA complexity, the application of literature data mining and knowledge extraction approaches to heterogeneous datasets must consider the relevance and significance of various ENMs physicochemical and experimental conditions (i.e., attributes) with respect to their environmental and health impacts.

4. **The final step consists of selection of the suitable framework/method for the environmental impact assessment and conduct the analysis for the desired scenario(s).** By assessing the adequacy of the body of available information for EIA, the analyst is able to apply a selected framework and conduct an impact assessment. If the results of the previous step indicate that additional information is required, then the analyst can proceed

with gathering additional information or selecting a different approach/framework based on the purpose of the analysis.

Finally in **Chapter 4**, an analysis is provided of the advances in Nano EH&S in a relevant context to the public health community, including key milestones and changes in policy related to Nano EH&S research over the last two decades. In **Chapter 4**, the scientific advances highlighted in **Chapters 1, 2** and **3** are analyzed and presented in a way that illustrates how these advances have been matched by changes in policies and regulations at the local, national, and international level. Furthermore, this analysis is used to provide answers to key questions that are of importance to the public health community.

In **Chapter 5,** key messages are presented showing how specific frameworks are suitable to (partly) meet the needs of potential decision makers in a set of decision making scenarios (based on the results shown in **Chapter 2**); the elements needed to assess the sufficiency/adequacy of information to conduct an EIA of ENMs (as discussed in **Chapter 3**); and how the main advances identified in the field of Nano EH&S research can be used to answer key questions posed by the public health community (**Chapter 4**). In **Chapter 5**, a brief description is also included on how a suitable method can be implemented to conduct an EIA using exposure and hazard potential datasets curated for a group of ENMs included in a case study presented in **Chapter 3** and future research steps recommended towards this goal.

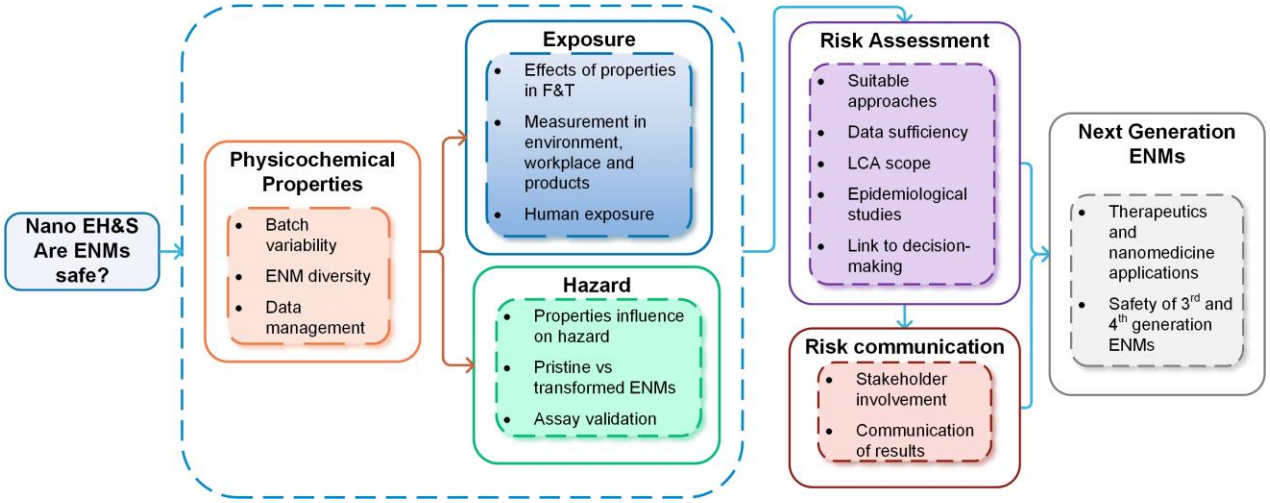


Figure 1.1 Challenges in Nano EH&S

Derived from a literature review of the field of Nano EH&S, the above-mentioned challenges in the field are classified into information elements needed to assess the safety of ENMs. These challenges also reflect novel concerns derived from next generation ENMs, as well as societal and ethical concerns surrounding the potential risks of ENMs.

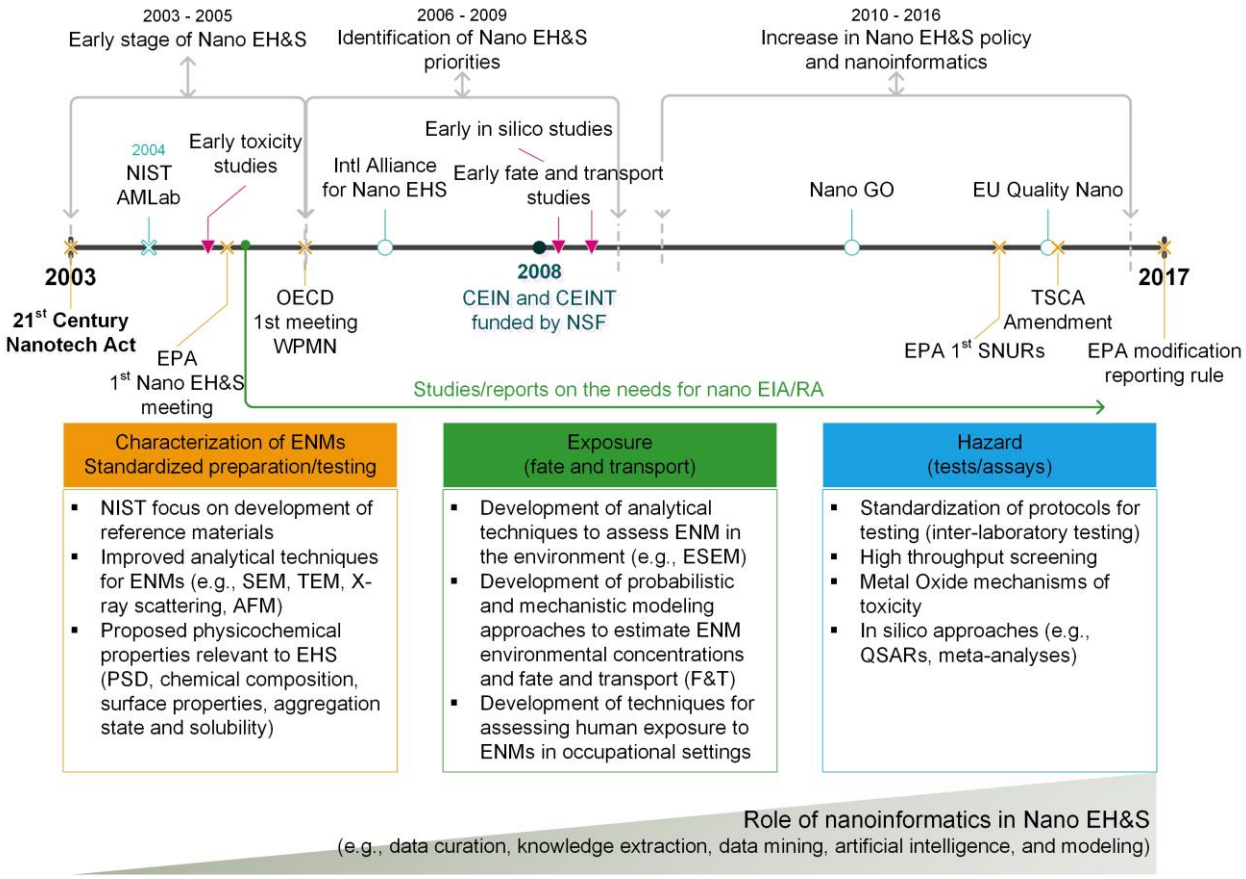


Figure 1.2 Infographic of progress in the field of Nano EH&S over the last decade and a half
The brackets above the timeline represent time periods showcasing the main information needs/priorities related to Nano EH&S research. The elements marked in the timeline (blue marks) represent events relevant to advances in characterization of ENMs and inter-laboratory cooperation efforts. The boxes below the timeline include examples of research advances in various aspects relevant to environmental impact assessment of ENMs.

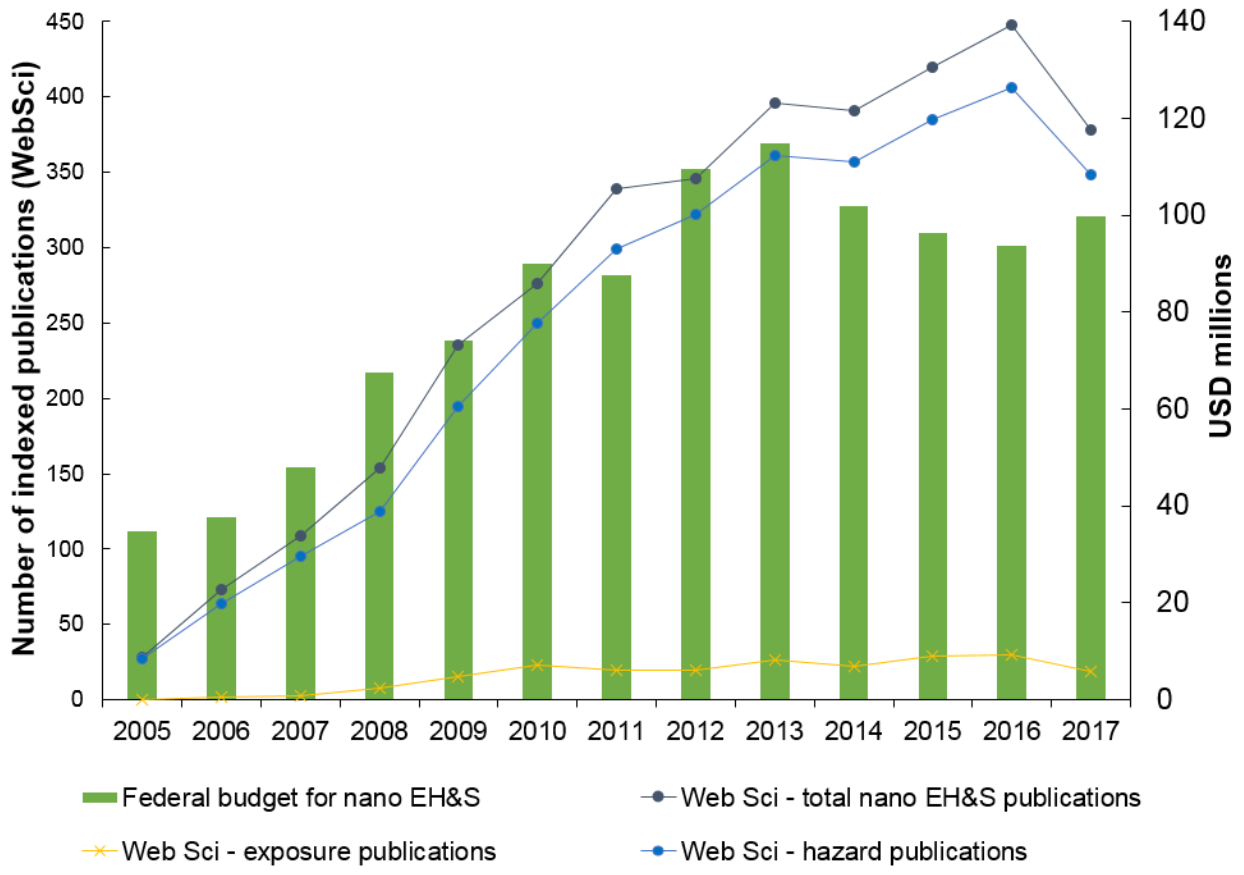


Figure 1.3 U.S. Federal budget allocated to Nano EH&S research per fiscal year according to the National Nanotechnology Initiative and number of publications, in the U.S., available in Web of Science from a 2017 literature search.

The dark blue circles represent the total number of Nano EH&S publications identified on the Web of Sciences database filtered by country for the U.S. (search terms used: "nanomaterial", "nanoparticle", "nanotechnology", AND "environmental health", "environmental health and safety", "risk assessment", "risk management", "fate and transport", "environmental release", "exposure assessment", "hazard", "toxicity", "nanotoxicity", "occupational health", "occupational risk", "worker health"). The light blue circles represent the number of publications identified for ENM hazard related topics only. The yellow cross marks represent the number of publications identified for ENM exposure related topics only.

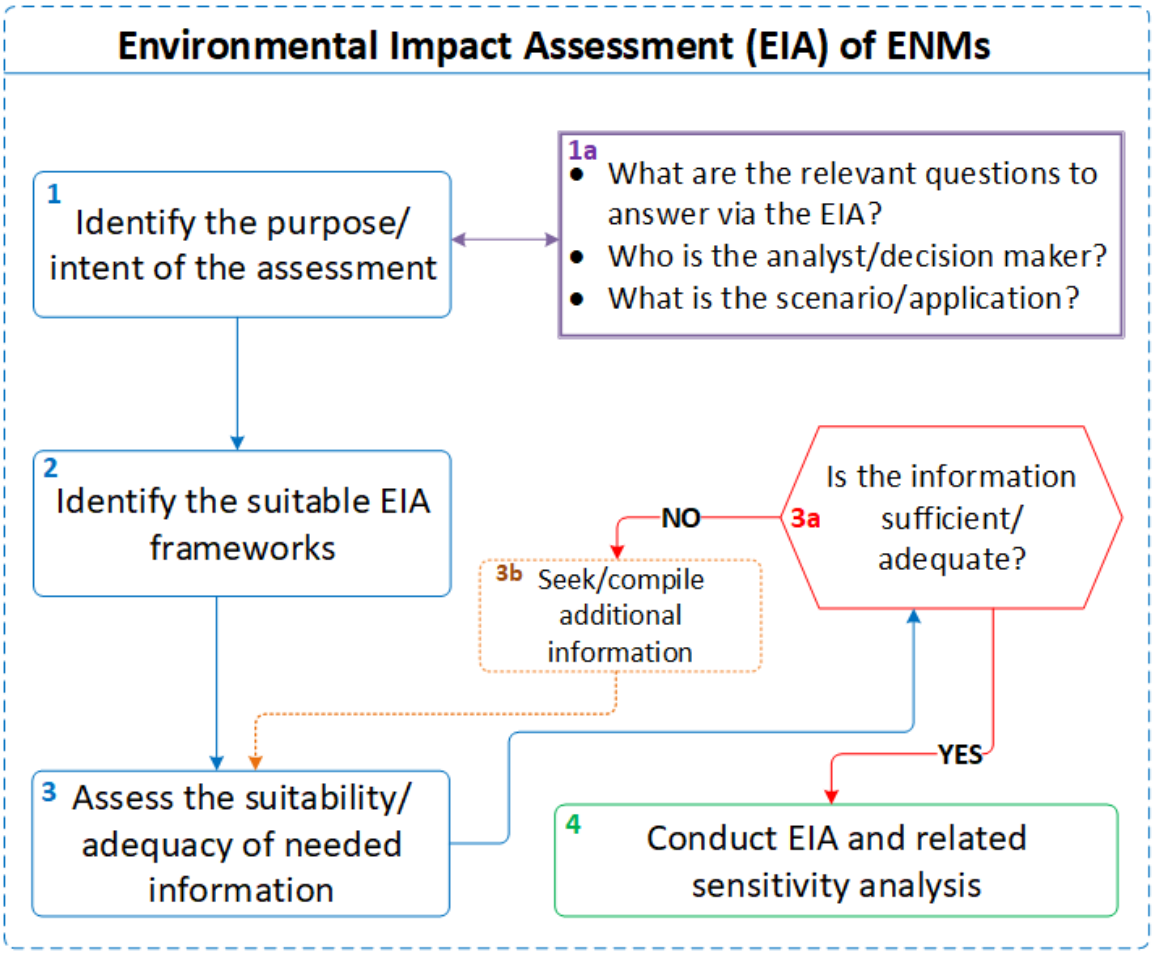


Figure 1.4 Tiered process to conduct an environmental impact assessment of Engineered Nanomaterials (ENMs)

The steps shown in this process are proposed to assess the potential environmental impacts of ENMs. As discussed throughout **Chapter 1**, it is envisioned that the process illustrated here can be adopted to assess potential impacts of emerging chemicals and technologies. This process can also be used in environmental health decision-making to support the evaluation of scientific evidence.

REFERENCES FOR CHAPTER 1

Arts, J. H. E., M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, W. Wohlleben and R. Landsiedel (2015). "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (Df4nanogrouping)." Regulatory Toxicology and Pharmacology **71**(2, Supplement): S1-S27.

Aschberger, K., H. J. Johnston, V. Stone, R. J. Aitken, S. M. Hankin, S. A. Peters, C. L. Tran and F. M. Christensen (2010). "Review of Carbon Nanotubes Toxicity and Exposure--Appraisal of Human Health Risk Assessment Based on Open Literature." Critical Reviews in Toxicology **40**(9): 759-790.

Aschberger, K., H. J. Johnston, V. Stone, R. J. Aitken, C. L. Tran, S. M. Hankin, S. A. Peters and F. M. Christensen (2010). "Review of Fullerene Toxicity and Exposure--Appraisal of a Human Health Risk Assessment, Based on Open Literature." Regulatory Toxicology and Pharmacology **58**(3): 455-473.

Aschberger, K., C. Micheletti, B. Sokull-Kluttgen and F. M. Christensen (2011). "Analysis of Currently Available Data for Characterising the Risk of Engineered Nanomaterials to the Environment and Human Health - Lessons Learned from Four Case Studies." Environment International **37**(6): 1143-1156.

Bessar, H., I. Venditti, L. Benassi, C. Vaschieri, P. Azzoni, G. Pellacani, C. Magnoni, E. Botti, V. Casagrande, M. Federici, A. Costanzo, L. Fontana, G. Testa, F. F. Mostafa, S. A. Ibrahim, M. V. Russo and I. Fratoddi (2016). "Functionalized Gold Nanoparticles for Topical Delivery of Methotrexate for the Possible Treatment of Psoriasis." Colloids and Surfaces B: Biointerfaces **141**(Supplement C): 141-147.

Bilal, M., H. Liu, R. Liu and Y. Cohen (2017). "Bayesian Network as a Support Tool for Rapid Query of the Environmental Multimedia Distribution of Nanomaterials." Nanoscale **9**(12): 4162-4174.

Bobo, D., K. J. Robinson, J. Islam, K. J. Thurecht and S. R. Corrie (2016). "Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date." Pharmaceutical Research **33**(10): 2373-2387.

Bonner, J. C., R. M. Silva, A. J. Taylor, J. M. Brown, S. C. Hilderbrand, V. Castranova, D. Porter, A. Elder, G. Oberdorster, J. R. Harkema, L. A. Bramble, T. J. Kavanagh, D. Botta, A. Nel and K. E. Pinkerton (2013). "Interlaboratory Evaluation of Rodent Pulmonary Responses to Engineered Nanomaterials: The NIEHS Nano Go Consortium." Environmental Health Perspectives **121**(6): 676-682.

Bostrom, A. and R. E. Löfstedt (2010). "Nanotechnology Risk Communication Past and Prologue." Risk Analysis **30**(11): 1645-1662.

Boverhof, D. R. and R. M. David (2010). "Nanomaterial Characterization: Considerations and Needs for Hazard Assessment and Safety Evaluation." Analytical and Bioanalytical Chemistry **396**(3): 953-961.

Burello, E. (2017). "Review of (Q) Sar Models for Regulatory Assessment of Nanomaterials Risks." NanoImpact **8**: 48-58.

Buzea, C., I. I. Pacheco and K. Robbie (2007). "Nanomaterials and Nanoparticles: Sources and Toxicity." Biointerphases **2**(4): MR17-MR71.

Card, J. W. and B. A. Magnuson (2010). "A Method to Assess the Quality of Studies That Examine the Toxicity of Engineered Nanomaterials." International Journal of Toxicology **29**(4): 402-410.

Cardinal, M. F., E. Vander Ende, R. A. Hackler, M. O. McAnally, P. C. Stair, G. C. Schatz and R. P. Van Duyne (2017). "Expanding Applications of Sers through Versatile Nanomaterials Engineering." Chemical Society Reviews **46**(13): 3886-3903.

Chang, H. I. and M. K. Yeh (2012). "Clinical Development of Liposome-Based Drugs: Formulation, Characterization, and Therapeutic Efficacy." International Journal of Nanomedicine **7**: 49-60.

Christensen, F. M., H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger (2010). "Nano-Silver - Feasibility and Challenges for Human Health Risk Assessment Based on Open Literature." Nanotoxicology **4**(3): 284-295.

Christensen, F. M., H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger (2011). "Nano-Tio_2--Feasibility and Challenges for Human Health Risk Assessment Based on Open Literature." Nanotoxicology **5**(2): 110-124.

Cohen, Y., R. Rallo, R. Liu and H. H. Liu (2012). "In Silico Analysis of Nanomaterials Hazard and Risk." Accounts of Chemical Research **46**(3): 802–812.

Colvin, V. (2003). Ntp Testing Nomination for Nanoscale Materials. N. T. Program/NIEHS. Houston, Texas.

Coussens, C. and L. Goldman (2005). Implications of Nanotechnology for Environmental Health Research, National Academies Press.

Dang, Y., Y. Zhang, L. Fan, H. Chen and M. C. Roco (2010). "Trends in Worldwide Nanotechnology Patent Applications: 1991 to 2008." Journal of Nanoparticle Research **12**(3): 687-706.

Debia, M., B. Bakhiyi, C. Ostiguy, J. H. Verbeek, D. H. Brouwer and V. Murashov (2016). "A Systematic Review of Reported Exposure to Engineered Nanomaterials." The Annals of Occupational Hygiene **60**(8): 916-935.

Denison, R. A. (2005). "A Proposal to Increase Federal Funding of Nanotechnology Risk Research to at Least $100 Million Annually." Washington, DC: Environmental Defense.

Domingos, R. F., M. A. Baalousha, Y. Ju-Nam, M. M. Reid, N. Tufenkji, J. R. Lead, G. G. Leppard and K. J. Wilkinson (2009). "Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes." Environmental Science & Technology **43**(19): 7277-7284.

Donaldson, K., X. Y. Li and W. MacNee (1998). "Ultrafine (Nanometre) Particle Mediated Lung Injury." Journal of Aerosol Science **29**(5): 553-560.

Donaldson, K., V. Stone, C. L. Tran, W. Kreyling and P. J. A. Borm (2004). Nanotoxicology, BMJ Publishing Group Ltd.

DuPont. (2007). "Nanorisk Framework." from http://www.nanoriskframework.org/.

Ehmann, F., K. Sakai-Kato, R. Duncan, D. Hernan Perez de la Ossa, R. Pita, J. M. Vidal, A. Kohli, L. Tothfalusi, A. Sanh, S. Tinton, J. L. Robert, B. Silva Lima and M. P. Amati (2013). "Next-Generation Nanomedicines and Nanosimilars: EU Regulators' Initiatives Relating to the Development and Evaluation of Nanomedicines." Nanomedicine (Lond) **8**(5): 849-856.

Elder, A., I. Lynch, K. Grieger, S. Chan-Remillard, A. Gatti, H. Gnewuch, E. Kenawy, R. Korenstein, T. Kuhlbusch, F. Linker, S. Matias, N. Monteiro-Riviere, V. R. S. Pinto, R. Rudnitsky, K. Savolainen and A. Shvedova (2009). "Human Health Risks of Engineered Nanomaterials: Critical Knowledge Gaps in Nanomaterials Risk Assessment." NATO Advanced Research Workshop on Nanomaterials: Environmental Risks and Benefits.

EPA (2004). Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. R. Jones, J. Leahy, M. Mahoney et al.**:** 92.

EPA (2005). Environmental Protection Agency Nanotechnology White Paper: External Review Draft, EPA.

Erbis, S., Z. Ok, J. A. Isaacs, J. C. Benneyan and S. Kamarthi (2016). "Review of Research Trends and Methods in Nano Environmental, Health, and Safety Risk Analysis." Risk Analysis **36**(8): 1644-1665.

Farre, M., K. Gajda-Schrantz, L. Kantiani and D. Barcelo (2009). "Ecotoxicity and Analysis of Nanomaterials in the Aquatic Environment." Analytical and Bioanalytical Chemistry **393**(1): 81-95.

Faunce, T. and A. Watal (2010). "Nanosilver and Global Public Health: International Regulatory Issues." Nanomedicine (Lond) **5**(4): 617-632.

Fröhlich, E. and E. Roblegg (2012). "Models for Oral Uptake of Nanoparticles in Consumer Products." Toxicology **291**(1): 10-17.

Fruijtier-Pölloth, C. (2012). "The Toxicological Mode of Action and the Safety of Synthetic Amorphous Silica—a Nanostructured Material." Toxicology **294**(2): 61-79.

Fubini, B., I. Fenoglio, M. Tomatis and F. Turci (2011). "Effect of Chemical Composition and State of the Surface on the Toxic Response to High Aspect Ratio Nanomaterials." Nanomedicine (Lond) **6**(5): 899-920.

Gajewicz, A., M. T. Cronin, B. Rasulev, J. Leszczynski and T. Puzyn (2015). "Novel Approach for Efficient Predictions Properties of Large Pool of Nanomaterials Based on Limited Set of Species: Nano-Read-Across." Nanotechnology **26**(1): 015701.

Gajewicz, A., K. Jagiello, M. T. D. Cronin, J. Leszczynski and T. Puzyn (2017). "Addressing a Bottle Neck for Regulation of Nanomaterials: Quantitative Read-across (Nano-QRA) Algorithm for Cases When Only Limited Data Is Available." Environmental Science: Nano.

Gajewicz, A., B. Rasulev, T. C. Dinadayalane, P. Urbaszek, T. Puzyn, D. Leszczynska and J. Leszczynski (2012). "Advancing Risk Assessment of Engineered Nanomaterials: Application of Computational Approaches." Advanced Drug Delivery Reviews **64**(15): 1663-1693.

Gao, X. and G. V. Lowry (2017). "Progress Towards Standardized and Validated Characterizations for Measuring Physicochemical Properties of Manufactured Nanomaterials Relevant to Nano Health and Safety Risks." NanoImpact.

Garner, K. L. and A. A. Keller (2014). "Emerging Patterns for Engineered Nanomaterials in the Environment: A Review of Fate and Toxicity Studies." Journal of Nanoparticle Research **16**(8).

Gibson, R., N. Stacey, E. Drais, H. Wallin, W. Zatorski and V. Virpi (2012). Risk Perception and Risk Communication with Regard to Nanomaterials in the Workplace. European Agency for Safety and Health at Work,. Luxembourg, EU-OSHA.

Godwin, H., C. Nameth, D. Avery, L. L. Bergeson, D. Bernard, E. Beryt, W. Boyes, S. Brown, A. J. Clippinger, Y. Cohen, M. Doa, C. O. Hendren, P. Holden, K. Houck, A. B. Kane, F. Klaessig, T. Kodas, R. Landsiedel, I. Lynch, T. Malloy, M. B. Miller, J. Muller, G. Oberdorster, E. J. Petersen, R. C. Pleus, P. Sayre, V. Stone, K. M. Sullivan, J. Tentschert, P. Wallis and A. E. Nel (2015). "Nanomaterial Categorization for Assessing Risk Potential to Facilitate Regulatory Decision-Making." ACS Nano **9**(4): 3409-3417.

Gordon, S. C., J. H. Butala, J. M. Carter, A. Elder, T. Gordon, G. Gray, P. G. Sayre, P. A. Schulte, C. S. Tsai and J. West (2014). "Workshop Report: Strategies for Setting Occupational Exposure Limits for Engineered Nanomaterials." Regulatory Toxicology and Pharmacology **68**(3): 305-311.

Gottschalk, F., E. Kost and B. Nowack (2013). "Engineered Nanomaterials in Water and Soils: A Risk Quantification Based on Probabilistic Exposure and Effect Modeling." Environmental Toxicology and Chemistry **32**(6): 1278-1287.

Gottschalk, F., C. Lassen, J. Kjoelholt, F. Christensen and B. Nowack (2015). "Modeling Flows and Concentrations of Nine Engineered Nanomaterials in the Danish Environment." International Journal of Environmental Research and Public Health **12**(5): 5581-5602.

Gottschalk, F., R. W. Scholz and B. Nowack (2010). "Probabilistic Material Flow Modeling for Assessing the Environmental Exposure to Compounds: Methodology and an Application to Engineered Nano-TiO_2 Particles." Environmental Modelling & Software **25**(3): 320-332.

Gottschalk, F., T. Sonderer, R. W. Scholz and B. Nowack (2009). "Modeled Environmental Concentrations of Engineered Nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for Different Regions." Environmental Science & Technology **43**(24): 9216-9222.

Gottschalk, F., T. Sun and B. Nowack (2013). "Environmental Concentrations of Engineered Nanomaterials: Review of Modeling and Analytical Studies." Environmental Pollution **181**(Supplement C): 287-300.

Grieger, K. D., A. Laurent, M. Miseljic, F. Christensen, A. Baun and S. I. Olsen (2012). "Analysis of Current Research Addressing Complementary Use of Life-Cycle Assessment and Risk Assessment for Engineered Nanomaterials: Have Lessons Been Learned from Previous Experience with Chemicals?" Journal of Nanoparticle Research **14**(7): 23.

Grieger, K. D., I. Linkov, S. F. Hansen and A. Baun (2012). "Environmental Risk Analysis for Nanomaterials: Review and Evaluation of Frameworks." Nanotoxicology **6**(2): 196-212.

Guzan, K. A., K. C. Mills, V. Gupta, D. Murry, C. N. Scheier, D. A. Willis and M. L. Ostraat (2013). "Integration of Data: The Nanomaterial Registry Project and Data Curation." Computational Science & Discovery **6**(1): 014007.

Hafner, A., J. Lovrić, G. P. Lakoš and I. Pepić (2014). "Nanotherapeutics in the EU: An Overview on Current State and Future Directions." International Journal of Nanomedicine **9**: 1005-1023.

Handy, R. D. and B. J. Shaw (2007). "Toxic Effects of Nanoparticles and Nanomaterials: Implications for Public Health, Risk Assessment and the Public Perception of Nanotechnology." Health, Risk & Society **9**(2): 125-144.

Hansen, S. F. and A. Baun (2012). "When Enough Is Enough." Nature Nanotechnology **7**(7): 409-411.

Harper, S. L., J. E. Hutchison, N. Baker, M. Ostraat, S. Tinkle, J. Steevens, M. D. Hoover, J. Adamick, K. Rajan, S. Gaheen, Y. Cohen, A. Nel, R. E. Cachau and M. Tuominen (2013). "Nanoinformatics Workshop Report: Current Resources, Community Needs, and the Proposal of a Collaborative Framework for Data Sharing and Information Integration." Computational Science & Discovery **6**(1): 14008.

Hischier, R. and T. Walser (2012). "Life Cycle Assessment of Engineered Nanomaterials: State of the Art and Strategies to Overcome Existing Gaps." Science of the Total Environment **425**: 271-282.

Hjorth, R. (2017). "The Shortfall of Risk Assessment for Decision-Making." Nature Nanotechnology **12**(12): 1109.

Holbrook, R. D., A. A. Galyean, J. M. Gorham, A. Herzing and J. Pettibone (2015). Chapter 2 - Overview of Nanomaterial Characterization and Metrology. Frontiers of Nanoscience. M. Baalousha and J. R. Lead, Elsevier. **8:** 47-87.

Holden, P. A., J. L. Gardea-Torresdey, F. Klaessig, R. F. Turco, M. Mortimer, K. Hund-Rinke, E. A. Cohen Hubal, D. Avery, D. Barcelo, R. Behra, Y. Cohen, L. Deydier-Stephan, P. L. Ferguson, T. F. Fernandes, B. Herr Harthorn, W. M. Henderson, R. A. Hoke, D. Hristozov, J. M. Johnston, A. B. Kane, L. Kapustka, A. A. Keller, H. S. Lenihan, W. Lovell, C. J. Murphy, R. M. Nisbet, E. J. Petersen, E. R. Salinas, M. Scheringer, M. Sharma, D. E. Speed, Y. Sultan, P. Westerhoff, J. C. White, M. R. Wiesner, E. M. Wong, B. Xing, M. Steele Horan, H. A. Godwin and A. E. Nel (2016). "Considerations of Environmentally Relevant Test Conditions for Improved Evaluation of Ecological Hazards of Engineered Nanomaterials." Environmental Science & Technology **50**(12): 6124-6145.

Hole, P., K. Sillence, C. Hannell, C. M. Maguire, M. Roesslein, G. Suarez, S. Capracotta, Z. Magdolenova, L. Horev-Azaria, A. Dybowska, L. Cooke, A. Haase, S. Contal, S. Mano, A. Vennemann, J. J. Sauvain, K. C. Staunton, S. Anguissola, A. Luch, M. Dusinska, R. Korenstein, A. C. Gutleb, M. Wiemann, A. Prina-Mello, M. Riediker and P. Wick (2013). "Interlaboratory Comparison of Size Measurements on Nanoparticles Using Nanoparticle Tracking Analysis (NTA)." Journal of Nanoparticle Research **15**: 2101.

Hotze, E. M., T. Phenrat and G. V. Lowry (2010). "Nanoparticle Aggregation: Challenges to Understanding Transport and Reactivity in the Environment." Journal of Environmental Quality **39**(6): 1909-1924.

Howard, J. (2013). "Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers." DHHS (NIOSH) Publication(2013-145).

Hristozov, D., S. Gottardo, E. Semenzin, A. Oomen, P. Bos, W. Peijnenburg, M. van Tongeren, B. Nowack, N. Hunt, A. Brunelli, J. J. Scott-Fordsmand, L. Tran and A. Marcomini (2016). "Frameworks and Tools for Risk Assessment of Manufactured Nanomaterials." Environment International **95**(Supplement C): 36-53.

Hristozov, D. R., S. Gottardo, M. Cinelli, P. Isigonis, A. Zabeo, A. Critto, M. Van Tongeren, L. Tran and A. Marcomini (2014). "Application of a Quantitative Weight of Evidence Approach for Ranking and Prioritising Occupational Exposure Scenarios for Titanium Dioxide and Carbon Nanomaterials." Nanotoxicology **8**(2): 117-131.

Hristozov, D. R., S. Gottardo, A. Critto and A. Marcomini (2012). "Risk Assessment of Engineered Nanomaterials: A Review of Available Data and Approaches from a Regulatory Perspective." Nanotoxicology **6**(8): 880-898.

Hristozov, D. R., A. Zabeo, C. Foran, P. Isigonis, A. Critto, A. Marcomini and I. Linkov (2014). "A Weight of Evidence Approach for Hazard Screening of Engineered Nanomaterials." Nanotoxicology **8**(1): 72-87.

Huo, L. L., R. Chen, X. F. Shi, R. Bai, P. Wang, Y. Z. Chang and C. Y. Chen (2015). "High-Content Screening for Assessing Nanomaterial Toxicity." Journal of Nanoscience and Nanotechnology **15**(2): 1143-1149.

Isaacs, J. A., C. L. Alpert, M. Bates, C. J. Bosso, M. J. Eckelman, I. Linkov and W. C. Walker (2015). "Engaging Stakeholders in Nano-Ehs Risk Governance." Environment Systems & Decisions **35**(1, Sp. Iss. SI): 24-28.

Jeliazkova, N., C. Chomenidis, P. Doganis, B. Fadeel, R. Grafstrom, B. Hardy, J. Hastings, M. Hegi, V. Jeliazkov, N. Kochev, P. Kohonen, C. R. Munteanu, H. Sarimveis, B. Smeets, P. Sopasakis, G. Tsiliki, D. Vorgrimmler and E. Willighagen (2015). "The eNanoMapper Database for Nanomaterial Safety Information." Beilstein Journal of Nanotechnology **6**: 1609-1634.

Ji, Z., X. Jin, S. George, T. Xia, H. Meng, X. Wang, E. Suarez, H. Zhang, E. M. Hoek, H. Godwin, A. E. Nel and J. I. Zink (2010). "Dispersion and Stability Optimization of TiO_2 Nanoparticles in Cell Culture Media." Environmental Science & Technology **44**(19): 7309-7314.

Justo-Hanani, R. and T. Dayan (2016). "Explaining Transatlantic Policy Divergence: The Role of Domestic Politics and Policy Styles in Nanotechnology Risk Regulation." Global Environmental Politics.

Kuempel, E. D., V. Castranova, C. L. Geraci and P. A. Schulte (2012). "Development of Risk-Based Nanomaterial Groups for Occupational Exposure Control." Journal of Nanoparticle Research **14**(1029): 1-15.

Kuempel, E. D., C. L. Geraci and P. A. Schulte (2012). "Risk Assessment and Risk Management of Nanomaterials in the Workplace: Translating Research to Practice." Annals of Occupational Hygiene **56**(5): 491-505.

Lee, J. H., M. Kwon, J. H. Ji, C. S. Kang, K. H. Ahn, J. H. Han and I. J. Yu (2011). "Exposure Assessment of Workplaces Manufacturing Nanosized TiO_2 and Silver." Inhalation Toxicology **23**(4): 226-236.

Li, N., S. Georas, N. Alexis, P. Fritz, T. Xia, M. A. Williams, E. Horner and A. Nel (2016). "A Work Group Report on Ultrafine Particles (American Academy of Allergy, Asthma & Immunology): Why Ambient Ultrafine and Engineered Nanoparticles Should Receive Special Attention for Possible Adverse Health Outcomes in Human Subjects." Journal of Allergy and Clinical Immunology **138**(2): 386-396.

Li, N., C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines and A. Nel (2003). "Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage." Environmental Health Perspectives **111**(4): 455-460.

Lin, S., Y. Zhao, Z. Ji, J. Ear, C. H. Chang, H. Zhang, C. Low-Kam, K. Yamada, H. Meng and X. Wang (2013). "Zebrafish High-Throughput Screening to Study the Impact of Dissolvable Metal Oxide Nanoparticles on the Hatching Enzyme, Zhe1." Small **9**(9-10): 1776-1785.

Linkov, I., M. E. Bates, L. J. Canis, T. P. Seager and J. M. Keisler (2011). "A Decision-Directed Approach for Prioritizing Research into the Impact of Nanomaterials on the Environment and Human Health." Nature Nanotechnology **6**(12): 784-787.

Linkov, I. and T. P. Seager (2011). "Coupling Multi-Criteria Decision Analysis, Life-Cycle Assessment, and Risk Assessment for Emerging Threats." Environmental Science & Technology **45**(12): 5068-5074.

Linkov, I., J. Steevens, G. Adlakha-Hutcheon, E. Bennett, M. Chappell, V. Colvin, J. M. Davis, T. Davis, A. Elder, S. Hansen, P. B. Hakkinen, S. M. Hussain, D. Karkan, R. Korenstein, I. Lynch, C. Metcalfe, A. B. Ramadan and F. K. Satterstrom (2009). "Emerging Methods and Tools for Environmental Risk Assessment, Decision-Making, and Policy for Nanomaterials: Summary of Nato Advanced Research Workshop." Journal of Nanoparticle Research **11**(3): 513-527.

Liou, S. H., C. S. Tsai, D. Pelclova, M. K. Schubauer-Berigan and P. A. Schulte (2015). "Assessing the First Wave of Epidemiological Studies of Nanomaterial Workers." Journal of Nanoparticle Research **17**: 413.

Liu, H. H. and Y. Cohen (2014). "Multimedia Environmental Distribution of Engineered Nanomaterials." Environmental Science & Technology **48**(6): 3281-3292.

Liu, R. and Y. Cohen (2015). "Nanoinformatics for Environmental Health and Biomedicine." Beilstein Journal of Nanotechnology **6**: 2449-2451.

Liu, R., H. H. Liu, Z. Ji, C. H. Chang, T. Xia, A. E. Nel and Y. Cohen (2015). "Evaluation of Toxicity Ranking for Metal Oxide Nanoparticles Via an in Vitro Dosimetry Model." ACS Nano **9**(9): 9303-9313.

Liu, R., H. Y. Zhang, Z. X. Ji, R. Rallo, T. Xia, C. H. Chang, A. Nel and Y. Cohen (2013). "Development of Structure-Activity Relationship for Metal Oxide Nanoparticles." Nanoscale **5**(12): 5644-5653.

Liu, X., A. Situ, Y. Kang, K. R. Villabroza, Y. Liao, C. H. Chang, T. Donahue, A. E. Nel and H. Meng (2016). "Irinotecan Delivery by Lipid-Coated Mesoporous Silica Nanoparticles Shows Improved Efficacy and Safety over Liposomes for Pancreatic Cancer." ACS Nano **10**(2): 2702-2715.

Massawe, E. (2013). "Nanomaterials for Environmental Remediation: Investigating the Role of Nanoinformatics in Support of Environmental, Health, and Safety Oversight of Nanotechnologies at the Local Level." J Environ Health **76**(1): 8-17.

Matsuda, M. and G. Hunt (2009). "Research on the Societal Impacts of Nanotechnology: A Preliminary Comparison of USA, Europe and Japan." Bio-Medical Materials and Engineering **19**(2-3): 259-267.

Maynard, A. D. (2006). "A Research Strategy for Addressing Risk." Nanotechnology, Woodrow Wilson International Center for Scholars **444**: 267-269.

Maynard, A. D. and R. J. Aitken (2016). "'Safe Handling of Nanotechnology' Ten Years On." Nature Nanotechnology **11**(12): 998-1000.

Meesters, J. A. J., A. A. Koelmans, J. T. K. Quik, A. J. Hendriks and D. van de Meentt (2014). "Multimedia Modeling of Engineered Nanoparticles with Simplebox4Nano: Model Definition and Evaluation." Environmental Science & Technology **48**(10): 5726-5736.

Meng, H., T. Xia, S. George and A. E. Nel (2009). "A Predictive Toxicological Paradigm for the Safety Assessment of Nanomaterials." ACS Nano **3**(7): 1620-1627.

Methner, M., L. Hodson, A. Dames and C. Geraci (2010). "Nanoparticle Emission Assessment Technique (Neat) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials—Part B: Results from 12 Field Studies." Journal of Occupational and Environmental Hygiene **7**(3): 163-176.

Methner, M., L. Hodson and C. Geraci (2010). "Nanoparticle Emission Assessment Technique (Neat) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials—Part A." Journal of Occupational and Environmental Hygiene **7**(3): 127-132.

Money, E. S., L. E. Barton, J. Dawson, K. H. Reckhow and M. R. Wiesner (2014). "Validation and Sensitivity of the Fine Bayesian Network for Forecasting Aquatic Exposure to Nano-Silver." Science of the Total Environment **473-474**: 685-691.

Money, E. S., K. H. Reckhow and M. R. Wiesner (2012). "The Use of Bayesian Networks for Nanoparticle Risk Forecasting: Model Formulation and Baseline Evaluation." Science of the Total Environment **426**: 436-445.

Morris, J. and J. Willis (2007). "US Environmental Protection Agency Nanotechnology White Paper." US Environmental Protection Agency, Washington, DC.

Napierska, D., L. C. Thomassen, D. Lison, J. A. Martens and P. H. Hoet (2010). "The Nanosilica Hazard: Another Variable Entity." Particle and Fibre Toxicology **7**(1): 39.

National Nanotechnology, I. (2014). "NNI Supplement to the President's 2014 Budget." Washington, DC: National Science and Technology Council.

Nazarenko, Y., T. W. Han, P. J. Lioy and G. Mainelis (2011). "Potential for Exposure to Engineered Nanoparticles from Nanotechnology-Based Consumer Spray Products." Journal of Exposure Science & Environmental Epidemiology **21**(5): 515-528.

Nazarenko, Y., H. Zhen, T. Han, P. J. Lioy and G. Mainelis (2012). "Potential for Inhalation Exposure to Engineered Nanoparticles from Nanotechnology-Based Cosmetic Powders." Environmental Health Perspectives **120**(6): 885-892.

Nel, A., D. Grainger, P. J. Alvarez, S. Badesha, V. Castranova, M. Ferrari, H. Godwin, P. Grodzinski, J. Morris, N. Savage, N. Scott and M. Wiesner (2011). Nanotechnology Environmental, Health, and Safety Issues. Nanotechnology Research Directions for Societal Needs in 2020: Retrospective and Outlook. M. C. Roco, M. C. Hersam and C. A. Mirkin. Dordrecht, Springer Netherlands**:** 159-220.

Nel, A., T. Xia, L. Mädler and N. Li (2006). "Toxic Potential of Materials at the Nanolevel." Science **311**(5761): 622-627.

Nel, A. E. (2013). "Implementation of Alternative Test Strategies for the Safety Assessment of Engineered Nanomaterials." Journal of Internal Medicine **274**(6): 561-577.

Nel, A. E., L. Mädler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova and M. Thompson (2009). "Understanding Biophysicochemical Interactions at the Nano–Bio Interface." Nature Materials **8**(7): 543-557.

Nel, A. E., L. Maedler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova and M. Thompson (2009). "Understanding Biophysicochemical Interactions at the Nano-Bio Interface." Nature Materials **8**(7): 543-557.

Nel, A. E., E. Nasser, H. Godwin, D. Avery, T. Bahadori, L. Bergeson, E. Beryt, J. C. Bonner, D. Boverhof, J. Carter, V. Castranova, J. R. DeShazo, S. M. Hussain, A. B. Kane, F. Klaessig, E. Kuempel, M. Lafranconi, R. Landsiedel, T. Malloy, M. B. Miller, J. Morris, K. Moss, G. Oberdorster, K. Pinkerton, R. C. Pleus, J. A. Shatkin, R. Thomas, T. Tolaymat, A. Wang and J. Wong (2013). "A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment." ACS Nano **7**(8): 6422-6433.

Nelson, B. C., E. J. Petersen and P. Jaruga (2015). GC/MS Measurement of Nanomaterial Induced Genotoxicity in Isolated DNA.

NIOSH, U. S. (2005). "Approaches to Safe Nanotechnology: An Information Exchange with Niosh." USA: NIOSH.

NIOSH, U. S. (2005). Strategic Plan for Niosh Nanotechnology Research and Guidance: Filling the Knowledge Gaps. Draft.

NISE. (2015). "Nanodays." Retrieved November 30, 2017, from http://www.nisenet.org/nanodays.

NIST. (2017). "Nano-Measurement Protocols." Retrieved November 1, 2017, from https://www.nist.gov/mml/nano-measurement-protocols.

NIST. (2017). "Standard Reference Materials." from https://www-s.nist.gov/srmors/index.cfm.

NNI (2012). Public Participation in Nanotechnology Workshop. Arlington, VA.

NNI (2013). Stakeholder Perspectives on Perception, Assessment, and Management of the Potential Risks of Nanotechnology.

Nowack, B. (2017). "Evaluation of Environmental Exposure Models for Engineered Nanomaterials in a Regulatory Context." NanoImpact **8**: 38-47.

Nowack, B., M. Baalousha, N. Bornhöft, Q. Chaudhry, G. Cornelis, J. Cotterill, A. Gondikas, M. Hassellöv, J. Lead and D. M. Mitrano (2015). "Progress Towards the Validation of Modeled Environmental Concentrations of Engineered Nanomaterials by Analytical Measurements." Environmental Science: Nano **2**(5): 421-428.

NRC (2003). The Measure of Star: Review of the U.S. Environmental Protection Agency's Science to Achieve Results (Star) Research Grants Program. Washington, DC, The National Academies Press. **2017:** 192.

NRC (2012). A Research Strategy for Environmental, Health, and Safety Aspects of Engineered Nanomaterials, The National Academies Press.

NRC (2013). Research Progress on Environmental, Health, and Safety Aspects of Engineered Nanomaterials, National Academies Press.

NSET (2006). Environmental, Health and Safety Research Needs for Engineered Nanoscale Materials. R. Rusell and R. Cresanti. Washington, D.C., Nanoscale Science, Engineering and Technology Subcommittee Committee on Technology.

NSF. (2006). "NSEC: Center for Biological and Environmental Nanotechnology." Retrieved September 12, 2017, from https://www.nsf.gov/awardsearch/showAward?AWD_ID=0118007.

NSF (2008). NSF and EPA Establish Two Centers for Environmental Implications of Nanotechnology. https://www.nsf.gov/news/news_summ.jsp?cntn_id=112234

NSF (2015). Public Access Plan: Today's Data, Tomorrow's Discoveries: Increasing Access to the Results of Research Funded by the National Science Foundation**:** 31. https://www.nsf.gov/publications/pub_summ.jsp?ods_key=nsf15052

Oberdoester, G. (2010). "Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles (Vol 113, Pg 823, 2005)." Environmental Health Perspectives **118**(9): A380-A380.

Oberdörster, G., R. M. Celein, J. Ferin and B. Weiss (1995). "Association of Particulate Air Pollution and Acute Mortality: Involvement of Ultrafine Particles?" Inhalation Toxicology **7**(1): 111-124.

Oberdorster, G., E. Oberdorster and J. Oberdorster (2005). "Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles." Environmental Health Perspectives **113**(7): 823-839.

OECD (2006). Current Developments/ Activities on the Safety of Manufactured Nanomaterials. Tour de Table at the 1st Meeting of the Working Party on Manufactured Nanomaterials. London, UK., OECD.

OECD (2013). Current Developments in Delegations on the Safety of Manufactured Nanomaterials - Tour de Table at the 10th Meeting of the Working Party on Manufactured Nanomaterials. London, UK.

OECD (2015). "Data-Driven Innovation: Big Data for Growth and Well-Being." http://www.oecd.org/sti/ieconomy/data-driven-innovation.htm

Oh, E., R. Liu, A. Nel, K. B. Gemill, M. Bilal, Y. Cohen and I. L. Medintz (2016). "Meta-Analysis of Cellular Toxicity for Cadmium-Containing Quantum Dots." Nature Nanotechnology **11**(5): 479-486.

Oomen, A. G., E. A. Bleeker, P. M. Bos, F. van Broekhuizen, S. Gottardo, M. Groenewold, D. Hristozov, K. Hund-Rinke, M. A. Irfan, A. Marcomini, W. J. Peijnenburg, K. Rasmussen, A. S. Jimenez, J. J. Scott-Fordsmand, M. van Tongeren, K. Wiench, W. Wohlleben and R. Landsiedel (2015). "Grouping and Read-across Approaches for Risk Assessment of Nanomaterials." International Journal of Environmental Research and Public Health **12**(10): 13415-13434.

Oomen, A. G., P. M. J. Bos, T. F. Fernandes, K. Hund-Rinke, D. Boraschi, H. J. Byrne, K. Aschberger, S. Gottardo, F. von der Kammer, D. Kühnel, D. Hristozov, A. Marcomini, L. Migliore, J. Scott-Fordsmand, P. Wick and R. Landsiedel (2014). "Concern-Driven Integrated Approaches to Nanomaterial Testing and Assessment – Report of the Nanosafety Cluster Working Group 10." Nanotoxicology **8**(3): 334-348.

Oomen, A. G., K. G. Steinhäuser, E. A. J. Bleeker, F. van Broekhuizen, A. Sips, S. Dekkers, S. W. P. Wijnhoven and P. G. Sayre (2017). "Risk Assessment Frameworks for Nanomaterials: Scope, Link to Regulations, Applicability, and Outline for Future Directions in View of Needed Increase in Efficiency." NanoImpact **9**: 1-13.

Paik, S. Y., D. M. Zalk and P. Swuste (2008). "Application of a Pilot Control Banding Tool for Risk Level Assessment and Control of Nanoparticle Exposures." Annals of Occupational Hygiene **52**(6): 419-428.

Pal, A. K., C. Y. Watson, S. V. Pirela, D. Singh, M. C. Chalbot, I. Kavouras and P. Demokritou (2015). "Linking Exposures of Particles Released from Nano-Enabled Products to Toxicology: An Integrated Methodology for Particle Sampling, Extraction, Dispersion, and Dosing." Toxicological Sciences **146**(2): 321-333.

Panneerselvam, S. and S. Choi (2014). "Nanoinformatics: Emerging Databases and Available Tools." International Journal of Molecular Sciences **15**(5): 7158-7182.

Pautler, M. and S. Brenner (2010). "Nanomedicine: Promises and Challenges for the Future of Public Health." International Journal of Nanomedicine **5**: 803-809.

Peters, A., H. E. Wichmann, T. Tuch, J. Heinrich and J. Heyder (1997). "Respiratory Effects Are Associated with the Number of Ultrafine Particles." American Journal of Respiratory and Critical Care Medicine **155**(4): 1376-1383.

Petersen, E. J., S. A. Diamond, A. J. Kennedy, G. G. Goss, K. Ho, J. Lead, S. K. Hanna, N. B. Hartmann, K. Hund-Rinke and B. Mader (2015). "Adapting OECD Aquatic Toxicity Tests for Use with Manufactured Nanomaterials: Key Issues and Consensus Recommendations." Environmental Science & Technology **49**(16): 9532-9547.

Pettitt, M. E. and J. R. Lead (2013). "Minimum Physicochemical Characterisation Requirements for Nanomaterial Regulation." Environment International **52**: 41-50.

Pietroiusti, A. (2012). "Health Implications of Engineered Nanomaterials." Nanoscale **4**(4): 1231-1247.

Powers, C. M., K. A. Mills, S. A. Morris, F. Klaessig, S. Gaheen, N. Lewinski and C. Ogilvie Hendren (2015). "Nanocuration Workflows: Establishing Best Practices for Identifying, Inputting, and Sharing Data to Inform Decisions on Nanomaterials." Beilstein Journal of Nanotechnology **6**: 1860-1871.

Priest, S. H. (2011). Nanotechnology and the Public: Risk Perception and Risk Communication, CRC Press.

Ramachandran, G., S. M. Wolf, J. Paradise, J. Kuzma, R. Hall, E. Kokkoli and L. Fatehi (2011). "Recommendations for Oversight of Nanobiotechnology: Dynamic Oversight for Complex and Convergent Technology." Journal of Nanoparticle Research **13**(4): 1345-1371.

Rashba, E., D. Gamota, D. Jamison and J. Miller (2004). "Standards in Nanotechnology." Nanotechnology Law & Business **1**: 185.

Raunio, H. (2011). "In Silico Toxicology – Non-Testing Methods." Frontiers in Pharmacology **2**: 33.

Roco, M. (2011). The Long View of Nanotechnology Development: The National Nanotechnology Initiative at 10 Years. Nanotechnology Research Directions for Societal Needs in 2020, Springer Netherlands. **1:** 1-28.

Roco, M. C. (2002). "Nanoscale Science and Engineering Education Activities in the United States (2001–2002)." Journal of Nanoparticle Research **4**(3): 271-274.

Roco, M. C., C. A. Mirkin and M. C. Hersam (2011). Nanotechnology Research Directions for Societal Needs in 2020: Retrospective and Outlook, Springer Science & Business Media.

Roco, M. C., C. A. Mirkin and M. C. Hersam (2011). Nanotechnology Research Directions for Societal Needs in 2020: Summary of International Study, Springer.

Roebben, G., H. Emons and G. Reiners (2011). Nanoscale Reference Materials. Nanotechnology Standards. V. Murashov and J. Howard. New York, NY, Springer New York**:** 53-75.

Roebben, G., S. Ramirez-Garcia, V. A. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, C. M. Garner, A. Rawle and A. Elder (2011). "Interlaboratory Comparison of Size and Surface Charge Measurements on Nanoparticles Prior to Biological Impact Assessment." Journal of Nanoparticle Research **13**(7): 2675.

Roebben, G., S. Ramirez-Garcia, V. A. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, C. M. Garner, A. Rawle, A. Elder, V. L. Colvin, W. Kreyling, H. F. Krug, Z. A. Lewicka, S. McNeil, A. Nel, A. Patri, P. Wick, M. Wiesner, T. Xia, G. Oberdorster and K. A. Dawson (2011). "Interlaboratory Comparison of Size and Surface Charge Measurements on Nanoparticles Prior to Biological Impact Assessment." Journal of Nanoparticle Research **13**(7): 2675-2687.

Roebben, G., K. Rasmussen, V. Kestens, T. P. J. Linsinger, H. Rauscher, H. Emons and H. Stamm (2013). "Reference Materials and Representative Test Materials: The Nanotechnology Case." Journal of Nanoparticle Research **15**(3): 1455.

Rogers, E. J., S. F. Hsieh, N. Organti, D. Schmidt and D. Bello (2008). "A High Throughput in Vitro Analytical Approach to Screen for Oxidative Stress Potential Exerted by Nanomaterials Using a Biologically Relevant Matrix: Human Blood Serum." Toxicology in Vitro **22**(6): 1639-1647.

Romero-Franco, M., H. A. Godwin, M. Bilal and Y. Cohen (2017). "Needs and Challenges for Assessing the Environmental Impacts of Engineered Nanomaterials (Enms)." Beilstein Journal of Nanotechnology **8**: 989-1014.

Royal, Society, Engineering (2004). Nanoscience and Nanotechnologies : Opportunities and Uncertainties: Summary and Recomendations. London, Royal Society : Royal Academy of Engineering.

Sainz, V., J. Conniot, A. I. Matos, C. Peres, E. Zupancic, L. Moura, L. C. Silva, H. F. Florindo and R. S. Gaspar (2015). "Regulatory Aspects on Nanomedicines." Biochemical and Biophysical Research Communications **468**(3): 504-510.

Salata, O. V. (2004). "Applications of Nanoparticles in Biology and Medicine." Journal of Nanobiotechnology **2**(1): 3.

Santamaria, A. (2012). "Historical Overview of Nanotechnology and Nanotoxicology." Nanotoxicity: Methods and Protocols: 1-12.

Savolainen, K. (2013). Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations. Finland, Finnish Institute of Occupational Health. www.ttl.fi/en/publications/electronic_publications/pages/default.aspx

SCENIHR (2005). Opinion On: The Appropriateness of the Risk Assessment Methodology in Accordance with the Technical Guidance Documents for New and Existing Substances for Assessing the Risks of Nanomaterials. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003.pdf

SCENIHR (2007). Opinion On: The Appropriateness of the Risk Assessment Methodology in Accordance with the Technical Guidance Documents for New and Existing Substances for Assessing the Risks of Nanomaterials. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_012.pdf

SCENIHR (2009). Opinion On: The Appropriateness of Existing Methodologies to Assess the Potential Risks Associated with Engineered and Adventitious Products of Nanotechnologies. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf

Schulte, P. A., C. L. Geraci, L. L. Hodson, R. D. Zumwalde, E. D. Kuempel, V. Murashov, K. F. Martinez and D. S. Heidel (2013). "Overview of Risk Management for Engineered Nanomaterials." Journal of Physics: Conference Series **429**(012062): 1-10.

Som, C., M. Berges, Q. Chaudhry, M. Dusinska, T. F. Fernandes, S. I. Olsen and B. Nowack (2010). "The Importance of Life Cycle Concepts for the Development of Safe Nanoproducts." Toxicology **269**(2-3): 160-169.

Stone, V., S. Pozzi-Mucelli, L. Tran, K. Aschberger, S. Sabella, U. Vogel, C. Poland, D. Balharry, T. Fernandes, S. Gottardo, S. Hankin, M. G. J. Hartl, N. Hartmann, D. Hristozov, K. Hund-Rinke, H. Johnston, A. Marcomini, O. Panzer, D. Roncato, A. T. Saber, H. Wallin and J. J. Scott-Fordsmand (2014). "Its-Nano - Prioritising Nanosafety Research to Develop a Stakeholder Driven Intelligent Testing Strategy." Particle and Fibre Toxicology **11**: 9-9.

Subramanian, V., J. Youtie, A. L. Porter and P. Shapira (2010). "Is There a Shift to "Active Nanostructures"?" Journal of Nanoparticle Research **12**(1): 1-10.

Sun, B., Z. Ji, Y.-P. Liao, M. Wang, X. Wang, J. Dong, C. H. Chang, R. Li, H. Zhang, A. E. Nel and T. Xia (2013). "Engineering an Effective Immune Adjuvant by Designed Control of Shape and Crystallinity of Aluminum Oxyhydroxide Nanoparticles." ACS Nano **7**(12): 10834-10849.

Sun, T. Y., F. Gottschalk, K. Hungerbühler and B. Nowack (2014). "Comprehensive Probabilistic Modelling of Environmental Emissions of Engineered Nanomaterials." Environmental Pollution **185**: 69-76.

Taurozzi, J. S., V. A. Hackley and M. R. Wiesner (2013). "A Standardised Approach for the Dispersion of Titanium Dioxide Nanoparticles in Biological Media." Nanotoxicology **7**(4): 389-401.

Tervonen, T., I. Linkov, J. R. Figueira, J. Steevens, M. Chappell and M. Merad (2009). "Risk-Based Classification System of Nanomaterials." Journal of Nanoparticle Research **11**(4): 757-766.

Thomas, D. G., S. Gaheen, S. L. Harper, M. Fritts, F. Klaessig, E. Hahn-Dantona, D. Paik, S. Pan, G. A. Stafford, E. T. Freund, J. D. Klemm and N. A. Baker (2013). "ISA-TAB-Nano: A Specification for Sharing Nanomaterial Research Data in Spreadsheet-Based Format." BMC Biotechnology **13**(1): 2.

Tinkle, S., S. E. McNeil, S. Muhlebach, R. Bawa, G. Borchard, Y. C. Barenholz, L. Tamarkin and N. Desai (2014). "Nanomedicines: Addressing the Scientific and Regulatory Gap." Annals of the New York Academy of Sciences **1313**: 35-56.

Totaro, S., G. Cotogno, K. Rasmussen, F. Pianella, M. Roncaglia, H. Olsson, J. M. Riego Sintes and H. P. Crutzen (2016). "The JRC Nanomaterials Repository: A Unique Facility Providing Representative Test Materials for Nanoehs Research." Regulatory Toxicology and Pharmacology **81**(Supplement C): 334-340.

U.S.C. (2003). 21st Century Nanotechnology Research and Development Act. t. C. (2003-2004). Public Law No: 108-153.

Van Broekhuizen, P., W. I. M. Van Veelen, W.-h. Streekstra, P. Schulte and L. Reijnders (2012). "Exposure Limits for Nanoparticles: Report of an International Workshop on Nano Reference Values." Annals of Occupational Hygiene **56**(5): 515-524.

Veiseh, O., J. W. Gunn and M. Zhang (2010). "Design and Fabrication of Magnetic Nanoparticles for Targeted Drug Delivery and Imaging." Advanced Drug Delivery Reviews **62**(3): 284-304.

von Gleich, A., M. Steinfeldt and U. Petschow (2008). "A Suggested Three-Tiered Approach to Assessing the Implications of Nanotechnology and Influencing Its Development." Journal of Cleaner Production **16**(8): 899-909.

Walker, W. C., C. J. Bosso, M. Eckelman, J. A. Isaacs and L. Pourzahedi (2015). "Integrating Life Cycle Assessment into Managing Potential Ehs Risks of Engineered Nanomaterials: Reviewing Progress to Date." Journal of Nanoparticle Research **17**(8): 344.

Wang, X., M. C. Duch, N. Mansukhani, Z. Ji, Y.-P. Liao, M. Wang, H. Zhang, B. Sun, C. H. Chang and R. Li (2015). "Use of a Pro-Fibrogenic Mechanism-Based Predictive Toxicological Approach for Tiered Testing and Decision Analysis of Carbonaceous Nanomaterials." ACS Nano **9**(3): 3032-3043.

Wiesner, M. R., G. V. Lowry, K. L. Jones, M. F. Hochella, R. T. Di Giulio, E. Casman and E. S. Bernhardt (2009). "Decreasing Uncertainties in Assessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials." Environmental Science & Technology **43**(17): 6458-6462.

Xia, T., R. F. Hamilton, J. C. Bonner, E. D. Crandall, A. Elder, F. Fazlollahi, T. A. Girtsman, K. Kim, S. Mitra, S. A. Ntim, G. Orr, M. Tagmount, A. J. Taylor, D. Telesca, A. Tolic, C. D. Vulpe, A. J. Walker, X. Wang, F. A. Witzmann, N. Q. Wu, Y. M. Xie, J. I. Zink, A. Nel and A. Holian (2013). "Interlaboratory Evaluation of in Vitro Cytotoxicity and Inflammatory Responses to

Engineered Nanomaterials: The NIEHS Nano Go Consortium." Environmental Health Perspectives **121**(6): 683-690.

Yan, L., F. Zhao, S. Li, Z. Hu and Y. Zhao (2011). "Low-Toxic and Safe Nanomaterials by Surface-Chemical Design, Carbon Nanotubes, Fullerenes, Metallofullerenes, and Graphenes." Nanoscale **3**(2): 362-382.

Zhang, H., Z. Ji, T. Xia, H. Meng, C. Low-Kam, R. Liu, S. Pokhrel, S. Lin, X. Wang and Y.-P. Liao (2012). "Use of Metal Oxide Nanoparticle Band Gap to Develop a Predictive Paradigm for Oxidative Stress and Acute Pulmonary Inflammation." ACS Nano **6**(5): 4349-4368.

CHAPTER 2: NEEDS AND CHALLENGES FOR ASSESSING THE ENVIRONMENTAL IMPACTS OF ENGINEERED NANOMATERIALS (ENMS)

(A modified version of this chapter has been published in Beilstein J. Nanotechnol. 2017, 8, 989–1014. doi:10.3762/bjnano.8.101)

ABSTRACT

The potential environmental impact of nanomaterials is a critical concern and the ability to assess these potential impacts is of top priority for the progress of sustainable nanotechnology. Risk assessment tools are needed to enable decision makers to rapidly assess the potential risks that may be imposed by engineered nanomaterials (ENMs), particularly when confronted by the reality of limited hazard or exposure data. In this review, we examine a range of available risk assessment frameworks considering the contexts in which different stakeholders may need to assess the potential environmental impacts of ENMs. Assessment frameworks and tools that are suitable the different decision analysis scenarios are then identified. In addition, we identify the gaps that currently exist between the needs of decision makers, for a range of decision scenarios, and the abilities of present frameworks and tools to meet those needs.

INTRODUCTION

Engineered nanomaterials (ENMs) are increasingly being used in numerous industrial products and processes owing to their unique physicochemical properties. There are over 1000 nano-enabled consumer products (Hristozov et al. 2012), representing an estimated global and U.S. markets of $1 trillion and $800 billion, respectively (Roco 2011). Applications of ENMS include,

for example, nanomedicine (e.g., drug delivery, early diagnosis and therapy for chronic diseases) (Dekkers et al. 2010) and environmental remediation (Mauter and Elimelech 2008).

Given the rapid growth of the market for ENMs, there is concern about potential adverse impacts from possible exposures to ENMs during production, distribution, use, and disposal (Som et al. 2010). Human exposure to ENMs can occur through inhalation, ingestion or dermal absorption. Despite the fact that chronic health effects of ENMs have not been conclusively identified in human populations (WHO 2013), animal studies suggest that the ENMs exhibit mechanisms of respiratory toxicity similar to those of ambient ultrafine particles (UFPs) (Xia et al. 2009). Furthermore, *in-vitro* and *in-silico* studies (Maynard et al. 2011, Gajewicz et al. 2012) suggest that inhalation of some ENMs may cause additional adverse outcomes, such as damage to the respiratory tract, inflammation, and activation of signaling pathways. For additional routes of exposure, such as dermal absorption, existing evidence suggests that certain ENMs may penetrate the skin (e.g., cobalt nanoparticles in human volunteers and quantum dots 'QDs' in rat skin) and cause irritation (e.g., nano ZnO in zebrafish models) (Teow et al. 2011). Oral exposure to ENMs can result in subsequent absorption in the GI tract and organ damage (e.g., nano Cu in mice via oral gavage damaged liver, spleen and kidneys, and nano ZnO caused necrosis of liver tissues and severe renal damage) (Teow et al. 2011). Given the above concerns, decision-makers and relevant stakeholders are confronted with the need to identify and utilize reliable methods to ascertain environmental impacts related to the production, use and disposal of ENMs.

The default process for evaluating the potential impacts of ENMs would be to use existing frameworks that were developed to assess the environmental health and safety (EHS) impacts of new chemicals and new industrial technologies more broadly. One such general framework is Environmental Impact Assessment (EIA), which was promoted within the National Environmental

Policy Act (NEPA) in the early 1970's (NRC 1994) as a holistic approach that considers the environmental, social and economic implications of planned projects. Another existing general framework is "risk assessment" (RA) (National Research and Committee on the Institutional Means for Assessment of Risks to Public 1983), which was developed to estimate human health related risks in a systematic manner based on toxicity, dose response curves and quantitative exposure assessment. These frameworks (i.e., EIA and RA) have also been incorporated into ecological risk assessment (ERA) (Liu et al. 2012), which is used to evaluate the likelihood of adverse environmental effects with focus on ecological receptors (e.g., biota, environmental compartments) (EPA 1998). However, application of these existing frameworks to ENMs is not straightforward. For example, although RA methods for chemicals are well established, their adoption and/or adaptation for ENMs would require consideration of various issues that include, but are not limited to the: behavior of ENMs in various media (e.g., dissolution, agglomeration/aggregation, adsorption); persistence (techniques to predict aspects of degradation of certain ENMs; transportation/distribution; predicted environmental concentrations (PECs) and transformation products and impurities; bioaccumulation; effects/predicted no effect concentration (PNEC) (Cohen et al. 2012, OECD 2012) (Figure 2.1). As a result, the implementation of RA for ENMs would be extremely costly and time consuming. Additionally, challenges such as the lack of information on background levels of naturally occurring nanoparticles and needed monitoring data on environmental concentrations of ENMs (David 2013) restrict the application of traditional RA and EIA to ENMs. Furthermore, the adaptation of chemical RA to ENMs would require the development of data on: (i) ENMs hazard properties, (ii) ENMs dose-response and dosimetry metrics, (ii) production volume and emission rates (including modes of release) of ENMs, (iii)

environmental transformations, and (iv) distribution of ENMs in the environment and associated multimedia exposure levels (Cohen et al. 2012, OECD 2012).

Due to data gaps, RA of ENMs that have been performed to date have frequently had to rely on expert judgment, which can result in ongoing debates about the validity of the results obtained from this approach (NRC 2009, Cohen et al. 2012). Furthermore, the complexities of ENMs transformation (e.g., agglomeration, complex formation) make it difficult to quantify the relevant ENM absorbed and/or effective doses and complicate the development of dose-response relationships.

As defined by the United States Environmental Protection Agency (U.S. EPA), "Risk characterization is an integral component of the risk assessment process for both ecological and health risks, i.e., it is the final, integrative step of risk assessment…risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers" (EPA 2000). Evidence regarding the harmful impacts of chemicals has been traditionally addressed via toxicity and epidemiological studies, which allow risk assessors to establish causal relationships between exposure and effects. Risk characterization for chemicals entails quantitative integration of exposure assessment and dose-response information, and the metrics include the establishment of reference doses (e.g., to protect the susceptible population from harmful effects), hazard index and cancer potency factors. By contrast, the bulk of available toxicity data for ENMs are mostly based on in-vitro assays and modeling approaches (e.g., quantitative structure activity relationships (QSARs)). For exposure assessment, the RA process for chemicals has been traditionally performed via laboratory studies, field monitoring, use of biomarkers, or fate and transport modeling. The RA process for ENMs, on the other hand, involves the additional complexity

associated with having to address the impact of particle size distribution and agglomeration on the fate and transport and bio-uptake of ENMs by ecological receptors.

Given the complexity of quantifying environmental exposures to ENMs and the scarcity of toxicity data at the organism level, several alternative approaches have been proposed (as an alternative to RA or EIA) for assessing the potential environmental impacts of ENMs. Previous reviews of the above approaches (Grieger et al. 2012, Hristozov et al. 2012, Hendren et al. 2013) have provided insight into the various elements of the assessment methods, but did not assess whether they meet the needs for ENMs RA and the associated challenges faced by the multiplicity of stakeholders for specific decision-making scenarios. Here, we provide a critical assessment of: (1) the types of decisions that different stakeholders in regulatory and non-regulatory environments need to make about the risk potential of ENMs and what types of tools they require; (2) which of the existing frameworks may be most suitable to address these needs; and (3) the gaps that exist between the needs of decision makers and the RA tools that are currently available.

METHODS

Delineation of Decision-Making Scenarios. To assess the relevance of the different risk assessment approaches, six different plausible decision-making scenarios were considered. The term "scenario" has been used across different fields and practices with different meanings and uses (Ramirez et al. 2015), ranging from management and planning (e.g., a description of future events to identify key variables and questions, trends and actors to identify strategic options) (Godet and Roubelat 1996) to software design (e.g., envision of potential problems related to the use of the developed product) (Carroll) and environmental assessment (e.g., assessment of pathways of events under a set of key assumptions ('what if'?)) (van Vuuren et al. 2012). A

common ground for the different uses of scenario analysis is in its application as a tool to study multi-disciplinary problems. Within the context of framework analysis in this review, "scenarios" are defined as a set of equally plausible contexts in a narrative form (Ramirez et al. 2015). In the present review the example scenarios were selected with a focus on the United States landscape considering various frameworks reported in the published literature. The first four scenarios are related to manufacturing ENMs and occupational health and safety concerns, and the last two scenarios are related to registration of new ENMs and establishment of maximum allowable exposure levels. Evaluation of RA frameworks within the context of decision-making scenarios is particularly instructive in assessing the utility of specific RA methods (Beaudrie and Kandlikar 2011). The specific information needs for each decision-making scenario include: (1) definition of the intent of the analysis (e.g., selection of hazard identification, exposure assessment or risk characterization); (2) the level of resolution/type of the analysis result (e.g., qualitative categorization/prioritization of needed research or testing or quantitative information, for example, a permissible exposure limit (PEL)); (3) the typical level of expertise of stakeholders who would be making the decision; and (4) the type of data accessible to stakeholders (e.g., data reported from the literature, publicly available production reports, material safety data sheets (MSDS), etc.).

Scenario I reflects a process by which a company must decide whether to control exposure to workers during manufacturing or processing of ENMs. This is a common assessment carried out in industry to ensure occupational health and safety standards, require information about process details and potential exposures, and establish control practices.

Scenario II is for the establishment of safe exposure levels related to occupational health by a regulatory body (e.g., the U.S. Occupational Safety and Health Administration (OSHA)). OSHA requires information to drive risk management (e.g., establishment and enforcement of occupational exposure limits (OELs) and permissible exposure limits (PELs) and guidance for compliance). The main needs of this scenario are to establish OELs/PELs for a specific class of ENMs for which the agency is required to provide evidence based exposure limits.

Scenario III is for a company manufacturing ENMs that has to decide whether risk associated with such ENMs or products containing ENMs is manageable and how to manage any potential risks. This decision-making scenario requires information/data regarding the potential for exposure throughout the ENM's life cycle and the hazards it may pose to humans and the environment.

Scenario IV addresses the need for arriving at a decision by a company or regulatory agency for choosing the safest ENM out of a group of alternatives (ENMS or chemicals). In this scenario, the assessment of alternatives requires information regarding hazard posed to humans and the environment for all different alternatives as well as technical performance of the material for the intended application.

Scenario V focuses on decisions made by a regulatory body (i.e., the U.S. EPA) about whether to control the use, release, or emissions of an ENM via a Significant New Use Rule (SNUR). This decision entails gathering substantial evidence that indicates any unreasonable risk to people or the environment given information about production volume, release, exposure potential and

anticipated hazards. The SNUR must be justified considering: (1) the projected ENM volume of manufacturing and processing; (2) the extent to which ENM use changes the type or form of human and ecological receptors' exposure to the ENM; (3) the extent to which the ENM use increases the exposure level and period; and (4) the reasonably anticipated manner and methods of manufacturing, processing, distribution in commerce and disposal of a chemical substance (EPA 2016).

Scenario VI focuses on decisions involving food, drugs or personal care products. In this scenario, a regulatory body (i.e., the U.S. Food and Drug Administration (FDA)) needs to decide whether to allow registration of a new nano-enabled product in food, drugs and personal care products. In Scenario VI, examples are explored that pertain to cosmetics and new drugs containing ENMs (FDA 2016). While safety assessment is required for both product types, new drugs require a detailed Risk Evaluation and Mitigation Strategy (REMS) (Craig 2010), including an estimation of population exposed to the drug, benefits from treatment with the drug, potential health risks, and if the drug represents a new molecular entity (Duvall 2012).

Review of Available Frameworks in the Context of Specific Decision-Making Scenarios

Eighteen existing frameworks, identified from a literature review finalized in 2015, that are potentially useful for assessing the impacts of ENMs were evaluated. The literature review focused on peer review publications available through the UCLA library system. These frameworks can be categorized as follows: (1) hazard identification frameworks, (2) frameworks for environmental risk/impact characterization, and (3) frameworks for occupational risk characterization. The hazard identification frameworks that were evaluated were the Swiss Precautionary Matrix (SPM)

(Höck J. et al. 2013), Risk Classification System based on Multi Criteria Decision Analysis (MCDA risk classification) (Linkov et al. 2007, Linkov et al. 2009, Tervonen et al. 2009), NanoRiskCat (Hansen et al. 2014), the Decision-making framework for the grouping and testing of nanomaterials (DF4Nano grouping) (Arts et al. 2015), and the modified GreenScreen (Sass et al. 2016). The evaluated frameworks for environmental risk/impact characterization were Life Cycle Analysis (LCA) (Eckelman et al. 2012, Gavankar et al. 2012, Hischier and Walser 2012), DuPont's NanoRisk (DUPONT 2007), U.S. EPA's Comprehensive Environmental Assessment (CEA) (Powers et al. 2012, Powers et al. 2014), NanoHAZ (O'Brien and Cummins 2010), Nanomaterial risk screening tool (NRST) (Beaudrie et al. 2015), Engineered Nanoparticles - Review of Health and Environmental Safety: Human health and Ecological Risk Assessment (ENRHES RA) (Aschberger et al. 2011), Risk Quantification based on Probabilistic Mass Flow Modeling Analysis (PMFA Risk Assessment) (Gottschalk et al. 2013), Forecasting of the Impacts of Nanomaterials in the Environment (FINE) based on Bayesian Networks (BN) (Money et al. 2012), and Life Cycle Risk Analysis for nanomaterials (Nano LCRA) (Shatkin 2008, Shatkin and Kim 2015). The assessed frameworks for occupational risk characterization were Risk based classification for occupational exposure control (Risk based OEL) (Kuempel et al. 2012), Risk Classification based on an Industry Insurance Protocol (RCIP) (Robichaud et al. 2005), CB Nanotool (Paik et al. 2008) and the Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012).

The potential for using existing frameworks for environmental impact/risk assessment and other relevant health and safety assessment of ENMs was evaluated systematically (see **Appendix B Tables B1** and **B2**) by identifying the following characteristics for each framework:

(1) The intent of the analysis based on the framework's main elements of the risk assessment process (e.g., hazard identification, exposure assessment, and risk characterization);

(2) The inputs required to conduct the analysis (e.g., environmental fate and transport data, physicochemical properties; toxicological information including dose-response information);

(3) The outputs/results obtained from the analysis such as description of the outcome (e.g., predicted values of environmental concentration, probability of risk) and its category (e.g., quantitative value/magnitude, qualitative classification) relative to the intent of the analysis;

(4) The intrinsic characteristics of the applied methodologies including, for example, the basis for the analysis (e.g., conceptual model, questionnaire, statistical model), settings or conditions for which the framework was designed (e.g., a specific geographical location, a particular working environment) and data used to support the design of the framework (e.g., experimental data, mechanistic studies, authors' assumptions);

(5) The capability of a framework to address data gaps (e.g., via consideration of expert judgment or modeling tools incorporated in the framework); and

(6) The availability of software tools specifically designed to conduct the analysis.

Upon analysis of the reviewed frameworks, the process and criteria used to identify their suitability for each of the specific decision-making scenarios is illustrated in **Figure B1** and **Table B4** on Appendix B. Finally, a discussion is provided of the potential opportunities for improving and/or adapting current frameworks and further to develop recommendations for the development of future tools. Moreover, the reviewed frameworks and the corresponding required information

were further evaluated within the context of the selected decision-making scenarios in order to identify remaining major challenges.

REVIEW

The basic characteristics of the 18 frameworks that were evaluated are summarized in **Table 2.1**. Below, we summarize the intent, inputs, outputs, intrinsic characteristics, ability to address data gaps, and availability of software tools for each of the frameworks. The frameworks were evaluated, as detailed in the following sections, according to their intended applications for hazard identification, characterization of environmental risk, and characterization of occupational risk.

(1) *Hazard Identification Frameworks*

The following four frameworks for identifying hazards associated with engineered nanomaterials were assessed: (a) the Swiss Precautionary Matrix, (b) "Risk" Classification Systems Based on Multi Criteria Decision Analysis, (c) NanoRiskCat, and (d) Decision-making framework for the grouping and testing of nanomaterials. Although several of the above approaches include "risk" in their titles, in practice they have been used either to solely assess hazard and/or do not yield a combined risk score. As a result, the above frameworks that are summarized below are considered in the present review as a category separate from those frameworks that have been used to identify risks associated with ENMs.

(a) The Swiss Precautionary Matrix (SPM) was designed as a response to the Swiss Action Plan on Synthetic Nanomaterials (SAPSN) to use existing information to identify potential harmful impacts of synthetic nanoparticles on health and the environment (Höck J. et al. 2013). The SPM,

which is available as a web-tool (Health 2013) is not designed to be a comprehensive risk assessment framework, but rather to provide an initial screening approach to determine the required measures for safe handling of nanomaterials in Switzerland (Höck J. et al. 2013). Prior to evaluating a specific ENM or a nano-enabled product using the SPM, the analyst has to assess whether that material or product meets the definition of "nano-relevant" using the European Union regulatory recommendation of 2011 (EU 2011). According to this recommendation, a nanomaterial is defined as "an unknown material containing primary particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the primary particles in the number size distribution, one or more external dimensions is in the size range 1 nm -100 nm or if the number size distribution is unknown". A material is also considered to be "nano-relevant" by this definition if its specific surface area per unit volume is greater than 60 m^2/cm^3, or a material that consists of fullerenes, graphene flakes or single wall carbon nanotubes.

For materials considered to be nano-relevant, the SPM tool can be used to develop scores representing levels of concern for the following parameters: *potential effect* (W), *potential exposure of humans or environmental release* (E), and *available information on the material's life cycle* (I). Threshold values and limits for each of these parameters (W, E, I), as determined by the peer-reviewed literature are specified in the SPM guidance document, which is provided online by the tool developers (Höck J. et al. 2013). The potential effect (W) is a score assigned on the basis of the ENMs' reactivity (e.g., redox activity, catalytic activity, oxygen radical formation potential or induction potential for inflammation reactions) and stability (e.g., half-life of the nanomaterial in the human body, or under environmental conditions). For example, a metal oxide nanoparticle with a conduction band energy that is much lower relative to the redox potential of biological molecules would be given the lowest score (1) on a scale of 1-9 (where 1 is low, 5 is medium and

9 is high). Likewise, a score is assigned for the ENM period of stability (e.g., 1, 5 and 9 for hours, days-weeks, and months respectively). The potential for human exposure to a given ENM and its environmental release (E) is assessed based on: *(a) the carrier material of the ENM and availability of the ENM for release, (b) the maximum possible extent of human exposure* via inhalation based on the daily amount of nanomaterial with which a worker comes into contact; *(c) the maximum ENM input into the environment* during manufacture via wastewater, exhaust gases or solid waste; and *(d) the available information on the ENM life cycle.* Finally, the score for *(d) the available information on life cycle (I)* is assigned based on the answers the user provides to the following questions: (i) is the ENM's origin (i.e., starting material(s)) is known? (ii) Is sufficient information available to complete the SPM based on the ENM starting materials? (iii) Are users of the ENM known? And (iv) is the composition/purity of the ENM known or can be estimated?

Once the categories for each of the assessed parameters has been determined, an overall score for the nano-relevant material is calculated, which is expressed as a *Precautionary need = f (N, W, E, I)* (Höck J. et al. 2013). The resulting score represents a measure of the need to review existing measures or evaluate new measures of risk management related to the nanomaterial. It should be noted that the SPM framework allows for updating data/information given that calculated scores can be high when data gaps exist. Overall, the SPM is a useful approach for setting priorities for action related to nano-relevant materials. However, the SPM tool does not identify specific control measures for risk reduction nor quantifies the risk/impact posed by the nano-relevant materials in question.

(b) "Risk" Classification Systems Based on Multi Criteria Decision Analysis (MCDA) has been used as an analytical framework for environmental hazard assessment and/or management

for ENMs, which can also aid in decision support or decision analysis (Linkov et al. 2013). Although this approach has been termed "Risk Classification by MCDA", to date, use of this approach has primarily focused on hazard identification; hence for the purpose of this review it is categorized as a "hazard identification" approach (Linkov et al. 2007, Linkov et al. 2013). The MCDA process involves (Linkov et al. 2007) identification of stakeholders and evaluation criteria, elicitation of MCDA parameters (e.g., establishment of weights and thresholds), model execution using available software tools (Mustajoki and Marttunen 2013), and interpretation of results. MCDA is often limited with regard to addressing data gaps, since scores must be provided by the assessor or via expert elicitation. Integration of MCDA with Life Cycle Analysis (LCA) and RA has been proposed to compare the impact of ENMs across life cycle stages (Linkov and Seager 2011). However, case studies based on this integrative approach have not yet been reported (as of September 2016).

An interesting MCDA example of classifying the risk potential of ENMs is the "stochastic multi-criteria acceptability analysis" (SMAA-TRI) approach (Tervonen et al. 2009). In the above approach, ENMs are classified into "risk" categories (e.g., very low, low, medium, high and extremely high risk) to provide recommendations for additional testing prior to ENM use in consumer products (Tervonen et al. 2009). In the SMAA-TRI method, the highest scoring category (i.e., extremely high risk) is assigned to ENMs that have high scores for the majority of the criteria described below. The SMAA-TRI was utilized (Tervonen et al. 2007) to compare a set of ENMs (called "alternatives" in the framework) based on selected hazard and toxicity criteria (e.g., extrinsic ENM properties such as agglomeration, reactivity/charge, critical functional groups, contaminant dissociation and size; evidence of toxicity; and other factors related to toxicity such as bioavailability and bioaccumulation). This outranking method has the advantage, when criteria

metrics are not easily aggregated, of providing qualitative metrics for ENM ranking (e.g., "most-least" favorable) (Linkov et al. 2007). The SMAA-TRI approach has been demonstrated for ranking of C_{60}, Multi-walled Carbon Nanotubes (MWCNTs), CdSe, Ag nanoparticles (NP), and Al NP according to the following scales: *size* (quantitative scale 1-100 based on literature review for the studied ENMs); *agglomeration, reactivity/charge, critical functional groups and contaminant dissociation* (qualitative scale of 1-5, where scores are assigned based on expert judgement with 1 representing the most favorable score as judged based on the perceived hazard/toxicity (i.e., lower score for less harmful/toxic ENM) and 5 the least favorable/more toxic); and *toxicity evidence, bioavailability, and bioaccumulation* (scale of 0-100 of a "subjective" probability scale constructed by the authors based on their expert judgment). In the proposed approach, the authors followed the scoring with a Monte-Carlo simulation to sample from a given probability distribution for each parameter to arrive at probabilities for the ranked ENMs for each of the categories. Such analysis suggested that CdSe was of greatest concern among the analyzed ENMs, ranking in the high-risk category with a 98% probability. In contrast, the ranking for C_{60} was fairly evenly divided between medium risk (51% probability) and high risk (49% probability), and Al NP was fairly evenly divided between medium risk (34% probability) and low risk (33% probability) (Tervonen et al. 2009). As illustrated by the above case study, the Risk Classification MCDA framework is useful in a context where the ENMs hazards are known and can be reasonably or rationally grouped within categories for the intended ranking. However, the assignment of ENMs' properties or hazard traits involves subjective expert analysis and therefore may be biased depending on the knowledgebase available to the assessor.

(c) NanoRiskCat is roadmap/flowchart designed as a first-tier approach to assess and communicate the hazard and exposure potential of ENMs that are used in consumer products (Hansen et al. 2014). In the above approach, hazard and exposure potential are assessed individually and are not combined to yield a risk score. Therefore, in the current review, NanoRiskCat is categorized as a "hazard identification framework". This framework typically requires expert judgement in order to interpret the available data. In addition, use of this framework by individuals other than the developers is currently limited given the present unavailability of a software implementation of the framework. Nonetheless, this framework could serve to aid companies and regulators for assessing the potential exposure, human health and environmental hazards associated with specific ENMs.

The NanoRiskCat framework leads the assessor through a series of questions that guide through the process of qualitatively classifying the hazard and exposures potential of the ENM of concern. Qualitative Classification is expressed in terms of a color code where red, yellow, green and gray indicate high, medium, and low potential hazard/exposure, respectively, while gray signifies that data are insufficient for an assessment. Questions are then posed to allow one to classify the hazard and exposure potential; such questions also include queries regarding the physical form of the ENM and potential receptors (e.g., professional-end users, consumers and/or environment) that could be exposed to the ENM. The framework includes questions about the potential hazards of the ENM with respect to human health (e.g., evidence of acute toxicity, germ cell mutagenicity, carcinogenicity, reproductive toxicity) and environmental hazards (e.g., adverse outcomes to aquatic and terrestrial species). Based on answers to the posed questions, NanoRiskCat bins ENMs into three categories of potential exposure (high, medium and low) (Hansen et al. 2007, Hansen et al. 2008). The potential for ENM human hazard is also evaluated

based on answers to questions about the ENM aspect ratio (e.g., a high aspect ratio ENM is categorized immediately as high), evidence of adverse outcomes related to acute and chronic effect posed by the ENM (e.g., evidence to support genotoxicity, neurotoxicity, carcinogenicity, and/or cardiovascular, respiratory toxicity). Environmental impacts are also assessed based on bioaccumulation, persistence, as well as dispersibility and other "warning signs" of potential hazard (Hansen et al. 2013). Given the above, it can be stated that qualitative results obtained via NanoRiskCat are intended to be a tool for risk communication strategies.

The use of NanoRiskCat was demonstrated for the categorization of the following ENM containing products: cleansing soap (containing nano Ag), tennis rackets (CNTs), automotive oil (Fullerene C_{60}), and sunblock (nano ZnO), among others (Hansen et al. 2014). NanoRiskCat analysis concluded that sunblock and cleansing soap were in the category of overall red/high exposure potential for human and for environmental hazards (Hansen et al. 2014). The tennis racket, as a source of ENMs, on the other hand, was categorized as being of low potential exposure. However, since the tennis racket contained CNTs it was designated in the medium/high category for human and environmental hazard. As demonstrated in the case study (Hansen et al. 2014), the NanoRiskCat framework can be a useful tool to qualitatively identify areas of concern (e.g., ecological and/or human health hazards) through the analysis of published information. However, the approach does is not built for direct analysis of quantitative data or handling of areas of missing information.

(d) The **Decision-making framework for the grouping and testing of nanomaterials (DF4Nano grouping)** was designed by the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC) "Nano Task Force" as a regulatory framework to guide the users on

grouping ENMs to make human health hazard assessment and identify information needs/research priorities for inhaled ENMs (Arts et al. 2015). This framework leverages the concept of "read-across", which allows data gaps to be filled assuming that ENMs with similar structures and/or physicochemical properties will exhibit similar hazard profiles (Arts et al. 2015). The Nano Task Force proposed that this framework could be useful for categorizing substances into common groups based on similarity of structural and physicochemical properties that induce similar patterns of toxicity.

In DF4Nano, ENMs are grouped into four main categories: soluble ENMs, biopersistent high aspect ratio (HAR) ENMs, passive ENMs, and active ENMs. *Soluble ENMs* are defined as ENMs with a water solubility that exceeds 100 mg/L or not water-soluble but soluble in biological media and/or if the ENM has a pulmonary half-life of less than 40 days. For soluble ENMs, no further nano-specific sub-grouping is specified and read-across of the properties of the dissolved materials to the corresponding bulk materials is applied. *Biopersistent high aspect ratio (HAR) ENMs* are defined as ENMs with an aspect ratio less than 3:1, a length greater than 5 μm, a diameter less than 3 μm, and an aqueous dissolution rate (suggesting biopersistence) greater than 100 mg/L or a pulmonary half-life upon intratracheal instillation greater than or equal to 40 days. *Passive ENMs* are those materials considered to be of very low or no hazard potential by virtue of containing less than 0.1% toxic components, low surface reactivity (e.g., based on ferric reducing ability of serum or cytochrome C), high dispersibility (based on an average aggregation number (AAN) ≥ 3), no cellular effects observed at a surface area ≤ 10 μg/cm^2, and low toxic potency (i.e., a no adverse effect concentration (NOAEC) in short-term inhalation studies (STIS) > 10 mg/m^3). Active ENMs are those that either do not meet the criteria for soluble ENMs, biopersistent high aspect ratio ENMs, or passive ENMs, or that meet the criteria for multiple categories, assuming that the

NOAEC for the ENM in STIS is ≥ 610 mg/m^3. For ENMs of group 4, further sub-grouping is required according to the degree of mobility in air (dustiness) and in physiological fluids (dispersibility), as well as on the uptake, biopersistence, and biodistribution as determined *in vitro* and *in vivo* short-term inhalation studies (STIS).

The DF4Nano Grouping is based on data provided by the analyst for the ENMs of interest through "tiers" or information filters, where specific thresholds are set for intrinsic material properties (e.g., water solubility, primary particle size, surface area, composition, crystallinity, and surface chemistry); system-dependent properties (e.g., dissolution rate in biological simulation fluid (BSF), release of toxic ions, size in relevant media and dispersibility); biopersistence (e.g., property of the ENM to persist in a cell, tissue, organ or organism as a proxy of pulmonary retention); uptake and biodistribution (e.g., evidence of alveolar uptake and subsequent distribution through the pulmonary system); and cellular (e.g., membrane damage including cationic phagolysosome damage, generation of reactive oxygen species (ROS), oxidative stress, redox activities, etc.) and apical toxic effects (e.g., respiratory effects shown in short-term inhalation studies).

In the initial tier (0) of information focuses on gathering data regarding intrinsic material properties (e.g., water solubility, primary particle size (PPS), surface area, composition, crystallinity, and surface chemistry). In tier 1, the ENM can be assigned into one of the following groups of intrinsic material properties: water solubility, particle morphology (PPS and shape, including aspect ratio and surface area) and chemical composition. Tier 2 focuses on the ENM's i) intrinsic properties and those linked to the ENMs functionality in the environment, (e.g., surface reactivity, dissolution rate, and dispersibility), ii) intended use, release and exposure, iii) uptake, biodistribution and biopersistence, and iv) biophysical interactions and cellular effects (Arts et al.

2015) to assign non-soluble ENMs to one of the following groups: biopersistent high aspect ratio (HAR) ENMs, passive ENMs, or active ENMs. Analysis within tier 2 is meant to indicate whether the ENM should be classified as either a biopersistent HAR ENM or an active ENM. Tier 3 is reached if the ENM has not been classified within any of the groups of tiers (1) and (2) or to confirm/revise the assignment of ENMs to the resulting category. Tier 3 includes a confirmation of *in vivo* toxic effects, which are considered higher in ranking than *in vitro* effects, to define and refine additional information needs. The specific toxicological information assessed in tier 3 includes: lung burden, systemic uptake, in vivo biopersistence, biodistribution, apical toxic effects and toxic potency, as assessed by STIS, in addition to *ex vivo* genotoxicity screening.

The application of the DF4Nano Grouping has been proposed (Arts et al. 2015) as a resource where physicochemical characterization and toxicity data are available for the ENM under consideration, or for those ENMs with similar properties to those for which toxicological information is available. In cases involving novel ENMs or where physicochemical characterization data are lacking, the application of DF4Nano requires additional ENM characterization. Also, given that exposure assessment is not performed in this framework the applicability of DF4Nano is suitable where qualitative assessments may suffice.

(e) A **modified GreenScreen** tool (Action 2017) was recently developed (Sass et al. 2016) following the original GreenScreen approach advanced by the Clean Production Action Group to assist in conducting chemical hazard assessment. The approach incorporates aspects of the U.S. EPA's Design for the Environment (DfE) Alternatives Assessment Criteria for Hazard Evaluation and the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (Sass et al. 2016, Action 2017). GreenScreen was modified for application to ENMs by including collection of physicochemical properties of the target nanomaterials (e.g., agglomeration and or

aggregation, chemical composition, purity, shape, surface area, surface chemistry (including composition and reactivity)) (Sass et al. 2016). The various studies from which information on the nanomaterials (properties and toxicity endpoints) is compiled are then assessed with respect to the reliability of the provided information. Briefly, the application of the modified GreenScreen approach, which is available as an online software tool, entails the following steps: 1) collection of publicly available data for 18 parameters that are relevant to hazard outcome (both chronic and acute) associated with the hazard endpoints of the target chemical, 2) expert evaluation of the collected data to assign "Benchmark Scores" (e.g., low, medium or high concern) or "DG" for data gaps to each of those 18 hazard endpoints; 3) assigning an aggregated benchmark (BM) score to categorize recommendations with respect to the material use. The proposed five categories are as follows: BM1 is for a substance of very high concern as defined by U.S., Canadian and European regulatory bodies, BM2 and BM3 designate a material that can be continued to be used but safer substitutes are desirables as the nanomaterial may present human health concerns, BM4 is for a material that represent low hazards to humans and the environment, and a fifth category (BM-U) where information is insufficient to assign a score.

In a case study developed by Sass et al (Sass et al. 2016), two types of nano-sized silver, AGS-20 and low soluble nano silver, were compared to non nano silver (conventional silver). Analysis using the modified GreenScreen tool suggested that low soluble nano silver and conventional Silver were of category BM-1 given evidence of high persistence and high ecotoxicity. In contrast, the lack of data for AGS-20 suggested classification of BM-U. As the above study notes (Sass et al. 2016), the modified GreenScreen tool is not intended for quantitative risk assessment, but rather as a suitable means for rapid screening to identify data needs and to compare available hazard information for ENMs.

(2) Frameworks for characterization of environmental risk

In evaluating frameworks that were designed to explicitly assess both hazard and exposure potential and to yield a net measure of risk potential, the present review focused on first considering frameworks that were designed to characterize environmental risk (including risks to humans due to exposure through environmental media) and then those designed to characterize occupational risk. Nine different frameworks for characterization of environmental risk of ENMs were assessed: (a) Life Cycle Analysis, (b) DuPont's Nano Risk Framework, (c) the U.S. EPA's Comprehensive Environmental Assessment (CEA) Framework, (d) NanoHAZ, (e) Nanomaterial risk screening tool (NRST), (f) the Engineered Nanoparticles - Review of Health and Environmental Safety: Human Health and Ecological Risk Assessment (ENRHES RA), (g) Risk Quantification based on Probabilistic Mass Flow Analysis (PMFA risk quantification), (h) Forecasting of the Impacts of Nanomaterials in the Environment (FINE) based on Bayesian Networks (BN), and (i) Life Cycle Risk Analysis for Nanomaterials (Nano LCRA).

(a) Life Cycle Analysis (LCA) refers to a class of approaches that follow a product over its life stages, including: (a) material acquisition and purification, (b) manufacturing and fabrication, (b) commercial uses, and (d) end-of-life product management (Theis et al. 2011). LCA is rooted in assessing environmental impacts. Examples of impacts that have been assessed previously using LCA include climate change, smog creation, eutrophication, toxicological stress on human health and ecosystems, depletion of resources that occur as a consequence of releases into the environment, and consumption of resources (Rebitzer et al. 2004). According to the Society of Environmental Toxicology and Chemistry (SETAC), LCA consists of the following steps: i) goal scope and definition (e.g., establishment of the product under analysis and study objectives); ii)

life cycle inventory analysis (e.g., tabulation of emissions and consumption of resources at each life stage of the product); iii) life cycle impact assessment (e.g., assessment of the impacts at each life stage of the product, which depend on the scope of the LCA); and iv) life cycle improvement assessment (e.g., a review of the LCA results to reduce impacts related to the product under analysis) (Rebitzer et al. 2004).

The applicability of LCA to assess the environmental impacts of ENMs has been subject of different reviews (Gavankar et al. 2012, Grieger et al. 2012, Hischier and Walser 2012), and the integration of LCA with risk assessment has also been suggested as a tool that could inform development of nano-enabled products that are "safer by design" (Som et al. 2010). For instance, Grieger et al (Grieger et al. 2012) qualitatively analyzed published case studies of ENMs RA and LCA. Their analysis demonstrated the differences between these two approaches: LCA provides an assessment of environmental impacts of a product/system while RA provides an assessment a particular substance or component of a complex material. Hischier et al (Hischier and Walser 2012) reviewed LCA case studies of several ENMs (e.g., CNTs, single walled CNTs, fullerenes, quantum dots and TiO_2) and nano-enabled products (e.g., dye containing nanoTiO_2 and carbon powder, t-shirt with nano Ag coating, and polymer composite) to assess the potential contributions of material production to CO_2 emissions. Most of the reviewed studies focused primarily on inventory of CO_2 emissions or energy analysis (Hischier and Walser 2012). An exception was a partial LCA and aquatic ecotoxicity impact assessment of carbon nanotubes (CNTs) reported by Eckelman et al (Eckelman et al. 2012). This latter study compared the environmental impacts (in freshwater) of chemical releases resulting from the manufacture (e.g., arc ablation, chemical vapor deposition (CVD), and high-pressure carbon monoxide (HiPCO)) for a hypothetical scenario in which CNTs and chemical releases are associated with the production of CNTs. The environmental

impact of CNTs was quantified via a characterization factor (i.e., *CF = effect factor* x *fate factor* x *exposure factor*) calculated as per the methodology of the fate and transport module of the USEtox model (Rosenbaum et al. 2008). The aquatic environmental impact of the release of chemicals released to freshwater due to CNT manufacturing was assessed based on previously reported data (Healy et al. 2008) and LCA software (Sima Pro 7.3) (Eckelman et al. 2012). In the above approach, the effect factor was defined the ratio of the potentially affected fraction (PAF) of aquatic organisms and average EC_{50}'s for the evaluated aquatic species. The fate was quantified as the residence time (days) of the CNTs or related chemicals in freshwater expressed as per the USEtox model. USEtox was not developed to specifically describe the fate and transport of particles or particle-bound chemicals; thus, its extended application to ENMs was based on heuristic assumptions and approximations. In the presented case studies, two different hypothetical release scenarios were considered for two hypothetical scenarios in either 100% ("worst case") or 2% of the total produced CNTs were assumed to be released to freshwater. It was concluded that under the "worst case" scenario the expected environmental impacts of CNTs would be equivalent to that which would result from chemicals released to the environment during the manufacture of CNTs. However, under the 2% release scenario, the expected environmental impacts of CNTs were assessed to be several orders of magnitude lower than for chemicals released during the manufacture of CNTs. Hence, it was recommend that further research was required to develop safer manufacturing processes for CNTs (Eckelman et al. 2012). LCA is noteworthy because it offers a myriad of options for analyses of ENMs and that have to be considered on a case-by-case basis. Depending on the scope of the assessment when sufficient data are available regarding ENM properties, fate and transport parameters, emissions, and toxicity/hazard then LCA could be performed to a reasonably approximate level via the USEtox model. The results of such analysis

must be evaluated cautiously given that USEtox is a model designed for dealing with organic chemicals and does not consider the complex environmental fate and transport behavior and toxicity of ENMs.

(b) DuPont's NanoRisk Framework was developed based on a joint effort by the Environmental Defense Fund and DuPont (DUPONT 2007) is a guide to presenting questions and request for information that should be considered by an organization to evaluate the risks associated with specific ENM applications. NanoRisk is a qualitative framework that guides the development of informational profiles (e.g., properties, hazards and exposures associated with a nanomaterial and its application) for the target ENMs throughout their lifecycle. The output is a worksheet that includes information on: (1) material description and application (e.g., technical name, commercial name, common form), and (2) ENM Profile Lifecycle(s) which consists of ENM Lifecycle Properties (ELP), ENM Lifecycle Hazard (ELH), and ENM Lifecycle Exposure (ELE) Profiles.

The NanoRisk ELP Profile includes ENM physicochemical properties such as chemical composition, surface coating, molecular structure, crystal structure, physical, form/shape, particle size, size distribution and surface area, agglomeration state, particle density, ENM bulk density, porosity, dispersibility, solubility in water and biologically relevant fluids, surface charge, and surface reactivity. The ELH profile includes acute hazard/toxicity information for the target ENM, and the ELE profile focuses on workers' exposure to ENM during the industrial process.

NanoRisk is useful in guiding the analyst in gathering information needed to assess the potential risk associated with the ENM of interest following the Chemical Process Quantitative Risk Assessment (CPQRA) approach (AIChE 2000). CPQRA is a methodology applied in the

chemical, petrochemical and oil processing industries to evaluate the overall process safety rather than a specific chemical substance or ENM (AIChE 2000). CPQRA consists of 7 steps: 1) definition of the potential incidents (e.g., qualitative hazard analysis), 2) evaluation of the potential consequences of the incidents (e.g., via vapor dispersion modeling and fire and explosion effect modeling), 3) estimation of the potential incident frequencies (e.g., via databases), 4) estimation of the incident impacts on people, environment and property, 5) estimation of the risk (e.g., combination of the potential consequences for each incident with the incident frequency and summing over all events), and 6) evaluation of the risk (e.g., identify the major sources of risk and determination if there are cost-effective process or modifications to reduce risk). NanoRisk itself does not generate specific guidance regarding quantitative estimation of risk associated with ENMs and does not provide a stand-alone methodology for integrating quantitative and qualitative information related to risk potential. However, NanoRisk does document a series of possible risk management decisions that should be addressed and provides recommendation on how to document specific risk management options.

(c) The **U.S. EPA's Comprehensive Environmental Assessment (CEA) Framework** provides a high-level set of recommendations for approaching the subject of assessing the potential health and environmental impacts of nanomaterials (Powers et al. 2012). CEA recommends following a traditional risk assessment process (NRC 2009), but stresses the need for considering the complete product lifecycle, transport and transformation in the environment, and exposure potential or absorbed dose (by all exposure pathways), in addition to impact assessment. CEA recommends the construction of an information system that considers both an expert domain knowledge (including via meta-analysis) and utilization of various LCA methods, cost-benefit

analysis, and decision science methods, while engaging stakeholders in the CEA process. CEA was evaluated via a case study (Powers et al. 2014) in which stakeholder engagement (expert elicitation) served to collect information about the risk potential of using Multi-Walled Carbon Nanotubes (MWCNTs) in flame-retardant coatings in upholstery textiles. Expert opinions were elicited, via a web-based tool ("CEAWeb") (Powers et al. 2014) to prioritize the range of needed studies on MWCNT release across the product life cycle and human exposure or health impacts, which included, for example, defining/quantifying exposure scenarios, effects of MWCNT functionalization, developing techniques to quantify MWCNTs in air and other media, and estimation of safety thresholds (Powers et al. 2014). CEA case studies were also documented for nano Ag and nano TiO_2, in which information gaps were assessed to identify future research needs and priorities (EPA 2010). Overall, although CEA provides a useful roadmap for evaluating the potential impacts of ENMs, this "framework" is essentially a guidance document that falls short of providing or recommending specific quantitative methodologies for the integration and analysis of information/data to assess the risk potential of ENMs.

(d) NanoHAZ is an approach developed specifically for assessing the potential ecological risks associated with ENMs (including human risks associated with exposure to ENMs through environmental media) in Ireland (O'Brien and Cummins 2010). NanoHAZ is based on comparison of estimated ENMs concentrations with existing regulatory limits for specific ENMs or their chemical building blocks (O'Brien and Cummins 2010). The approach relies on probabilistic material flow analysis (MFA) with heuristics or assumptions based on empirical knowledge regarding the potential ENM exposure concentrations in the various media. The estimated exposure level in a given media (primarily air and water) for the target ENM is then compared to

a bench-mark exposure concentration or critical concentration at which a specific effect is observed (as determined from in vivo toxicological studies) for the target receptors (human or ecological). One limitation of the NanoHAZ approach is the paucity of regulatory limits for ENMs (O'Brien and Cummins 2010). As a result, the initial reported application of the approach, which focused on metal and metal oxide ENMs, utilized regulatory limits on exposure concentrations for dissolved metals or chemical building blocks of the ENMs as surrogates for the ENMs themselves (O'Brien and Cummins 2010). Specific NanoHAZ case studies were reported for nano TiO_2 in paints, nano Ag as an antimicrobial agent in food packaging, and nano CeO_2 as a fuel additive. It was concluded that the level of concern regarding inhalation exposure to airborne nano CeO_2, associated with its use as a fuel additive, was higher relative to concern regarding air releases of nano Ag and nano TiO_2. The level of concern for nano TiO_2 was considered moderate given its relative high score of potential exposure in drinking and surface (relative to nano Ag and nanoCeO_2), and low relative score of hazard (e.g., ecotoxicological and toxicological effects) compared to nano Ag. Finally, nano Ag as an antimicrobial agent in food packaging was considered of low concern given its low score for potential exposure (lower release expected in water compared to nanoTiO_2) despite its moderate and high scores for ecotoxicological and toxicological effects.

Overall, the application of NanoHAZ can be useful if information is available regarding environmental releases of ENMs and their potential toxic effects are known or can be predicted from suitable models. As described in the available case study (O'Brien and Cummins 2010) NanoHAZ can serve to compare and rank ENMs with regard to their potential exposure and hazard.

(e) The **Nanomaterial risk-screening tool (NRST)** was developed on the basis of expert opinions compiled at a nanotechnology workshop that focused on assessing the importance of various factors that may affect hazard, exposure and risk associated with ENMs. The framework was formulated as an excel spreadsheet in which the analyst can select qualitative "risk ratings" (scale of 1-5, where 1 represents the lowest concern) (Beaudrie et al. 2015). The *hazard rating* is then calculated as the linear aggregation (using weight factors) of scores assigned to each contributing ENM physicochemical attribute (e.g., ENM chemical composition, crystallinity, average size, aspect ratio, surface area and charge, reactivity, solubility, hydrophobicity, agglomeration and sorption tendency) and contributing ENM hazard indicators (e.g., ENM potential for inducing ROS and mobility through cells). The *exposure rating* is determined based on aggregation of individual scores assigned to factors linked to environmental and human exposure potential during product manufacturing, use and end-of-life. These factors include product characteristics (e.g., content of ENM in product and form, product type) and exposure indicators (e.g., ENM environmental release potential, frequency and duration of exposure, number of exposed individuals). The aggregation of scores follows an assumption of linear additivity with assumed weight factors and does not provide for the establishment of bi-directional cause-effect relationship pathways. Therefore, one cannot directly ascertain the reliability of the obtained ranking relative to the existing quantitative body of evidence. Overall, however, the approach is a useful first step in organizing information and opinions to arrive at an initial ranking of concerns as being high, medium or low.

(f) Engineered Nanoparticles - Review of Health and Environmental Safety: Human Health and Ecological Risk Assessment (ENRHES RA) is a framework developed as part of the European Union project "Engineered Nanoparticles: Review of Health and Environmental Safety (ENRHES)" (Aschberger et al. 2011) The goal of ENRHES is to facilitate estimation of ecological and human health impacts of ENMs and identification of data gaps for regulatory risk assessment under the European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) guidelines (Aschberger et al. 2011). While the focus on human risk assessment presented in the published case studies (Aschberger et al. 2011) is not occupational, the exposure profiles reviewed included human exposure via manufacturing, consumer products and contact with the environment. The first step of the analysis process entails hazard identification (e.g., obtaining indicative no effect concentrations (INEC) for ecological receptors and indicative of no effect levels (INELs) for human population from published data) (Aschberger et al. 2010, Aschberger et al. 2010, Christensen et al. 2010, Christensen et al. 2011). The second step consists of exposure assessment, performed on the basis of evaluating the occupational exposure for human receptors reported in the literature for the target ENM(s). Environmental exposures are qualitatively estimated using the expected or known ENM presence in environmental compartments based on estimates obtained from material flow analysis (MFA) (Mueller and Nowack 2008). When incorporated into the ENRHES RA, the MFA data are not based on fundamental modeling of multimedia fate and transport. Thus, mass balance inconsistencies may arise and violations of constraints imposed by intermedia transport mechanisms. The third step consists of risk characterization for human and ecological receptors. For human risk characterization, the measured and/or monitored occupational exposure concentrations were compared with the INELs, whereas for ecological risk assessment the modeled ENMs

concentrations (e.g., orders of magnitude ng/L, µg/L, and µg/m^3) were compared with the INEC values.

ENRHES RA was demonstrated in a case study (Aschberger et al. 2011) exploring the potential human risk of four ENMs (nano silver, nano titanium dioxide (TiO_2), nano zinc oxide (ZnO), fullerenes and carbon nanotubes (CNTs). The analysis revealed that the INELs of fullerenes, nano Ag and nano TiO_2 are lower than most of the reported occupational exposure concentrations for these materials. It was also suggested that the exposure concentrations of concern, for ecological receptors, are likely to be due to release of the ENMs into water in the following decreasing level of concern: ZnO >>nano Ag>> nano TiO_2 > (MWCNT=C_{60}) (Aschberger et al. 2011).

In summary, the application of ENRHES RA framework for ENMs is particularly useful as a roadmap for the REACH process. While the approach provides a conceptual based description of the analysis process, as illustrated by case studies, application of the ENRHES RA framework is at present limited by the availability of exposure and hazard information for the target ENMs.

(g) Risk Quantification Based on Probabilistic Mass Flow Analysis (PMFA risk Quantification) was proposed as a basis for risk-based classification system of ENMs present in water and soils with the goal of quantifying the probability of environmental risks (Gottschalk et al. 2010, Gottschalk et al. 2013). The approach relies on a probabilistic material-flow analysis (PMFA) (Gottschalk et al. 2010) to estimate the releases of ENMs to the environment on the basis of available data and expert judgement regarding production, use and disposal, along with heuristic and empirical assumptions to arrive at potential exposure concentrations in various media (Liu and Cohen 2014). Published toxicity data (e.g., terrestrial and aquatic species tested for no observed

effect concentrations, lowest observed effect concentrations and lethal concentrations for a 50% of the population) are used as inputs. The above compiled information is then used to build species sensitivity distribution (SSD) models (Gottschalk and Nowack 2013). SSD models have also been used by the U.S. EPA to summarize evidence for stressor-response relationships obtained from laboratory studies (EPA 2012). In such an approach, the risk probability metric is defined as the product of the probability distribution of the predicted environmental concentrations and the probability that one or more organisms would be negatively impacted as a function of environmental concentration. In such analysis, zero percent risk indicates that all predicted environmental concentrations are lower than the lowest limit of the probabilistic SSD, and a 100% risk means that all predicted environmental concentrations overlap with the probabilistic SSD. Using this approach, Gottschalk et al (Gottschalk et al. 2013) evaluated the relative environmental risk posed by selected ENMs in Switzerland. It was reported that the highest risk, due to releases from sewage treatment plants, was associated with nano Ag (40% overlap of the modeled environmental concentrations with the SSD for aquatic species), followed by nano TiO_2 (19% overlap) and nano ZnO (1% overlap). With regard to ENMs found in surface water, nano Ag was reported to present a higher risk (1% overlap) than nano TiO_2 (< 0.1% overlap). In contrast, the authors concluded that there was no measurable risk related to CNTs and fullerenes in any of the studied environmental compartments (e.g., water and soil) (Gottschalk et al. 2013).

The PMFA framework is useful if quantitative data/information are available to construct the SSD and to estimate environmental concentrations. However, the application of the PMFA framework also requires expertise to conduct the analysis and reliance on expert judgement in estimating exposure concentrations and the SSD.

(h) Forecasting of the Impacts of Nanomaterials in the Environment (FINE) Based on Bayesian Networks (BN) is an approach proposed to formally incorporate expert judgments to address data gaps and provide a probabilistic measure of potential environmental impacts of ENMs (Money et al. 2012). The above approach is suitable both for incremental learning and the propagation of uncertainties (Wiesner and Bottero 2011). The initial demonstration of this method was an assessment of the environmental impacts of nano Ag in water and sediment (Money et al. 2012). BN were used to integrate quantitative and qualitative information, address data gaps, quantify uncertainties, and provide bidirectional causal relationships. Briefly, the BN approach consists of two main parts: 1) development of the network structure (nodes and their connectivity), and 2) determination of baseline parameters for each node in the form of conditional probability tables (CPTs). In the test study reported for nano Ag (Money et al. 2012), the BN structure was developed on the basis of expert elicitation and consisted of nodes that were grouped into three categories: i) media parameters (e.g., temperature, pH, presence of organic matter), ENM properties (e.g., ENM coatings, zeta potential, fractal dimension, ENM diameter) and ENM transformations (e.g., ENM aggregation potential, attachment efficiency, biodegradation, dissolution and deposition); ii) exposure potential (e.g., ENM concentration entering system, concentration in sediment, water and dissolved concentration), and iii) hazard potential (e.g., bioavailability potential, biouptake, effects on biomass/mortality, effects on the ecosystem, such as decomposition, methanogenesis, eutrophication). In the case study reported by Money et al (Money et al. 2012), the CPT for the different nodes and individual variables (input values, units, ranges and categories) were established based on expert judgment, and the BN was applied to estimate ecological risks (e.g., probability distribution of risk being < 1 or ≥ 1) posed by nano Ag particles present in the aquatic environment. The case study suggested that the greatest potential

risk is expected when nano Ag is accumulated in sediments rather than in water (Money et al. 2012). Given that the FINE BN framework was tailored specifically for nano Ag in water, its applications is relevant to the aquatic environment. However, FINE BN can be tailored to different ENMs, and various environmental media, provided that the BN design includes the causal relationships governing the various aspects of the environmental fate and transport and toxicity behavior of the classes of ENMs under consideration. The FINE BN framework can be particularly useful for integrating quantitative and qualitative information and for enabling period update (i.e., as new data becomes available) via incremental learning.

(i) Life Cycle Risk Analysis for Nanomaterials (Nano LCRA) is a screening approach developed with the intent of identifying potential risks and data gaps over a nanoproduct's life cycle (Shatkin 2008, Shatkin 2013). Nano LCRA incorporates relevant data through the life cycle of the target ENM with the intent of informing risk management practices and prioritizing research strategies. The analysis consists of the following ten steps: 1) Description of the life cycle of the product; 2) Identification of the materials and assessment of the potential hazards in each life cycle stage; 3) Exposure assessment for each life cycle stage; 4) Identification of the life cycle sages in which exposure may occur; 5) Evaluation of potential human and nonhuman toxicity at the key life cycle stages; 6) Analysis of risk potential for the selected life cycle stages; 7) Identification of key uncertainties and data gaps and communication of findings; 8) Development of mitigation/risk-management strategies; 9) Gathering additional information (e.g., data that might have been identified as missing from the assessment); and 10) Evaluating the efficiency of the developed risk management strategies and identifying the next set of priorities (e.g., identify newly available data to update mitigation/risk-management strategies) (Shatkin 2013).

The Nano LCRA framework was applied by to assess the potential risks of using cellulose nanomaterials (CNs) as substitutes for resource-intensive materials, such as plastics, including those used in commercial applications such as packaging, composite polymers, paints, cosmetics, water and air filtration, and recyclable electronics, and to identify data gaps (Shatkin and Kim 2015). Case study results indicated that the highest priority for the development of new data is the need for information/data regarding occupational inhalation exposure associated with handling CNs as a dry powder. The authors also concluded that significant knowledge gap regarding the toxicity of CNs used in consumer use products, such as packaging, particularly for food contact limited the scope of the study.

The Nano LCRA framework appears to be useful for qualitative analysis. However, the available studies have not incorporated a method for integration of quantitative data (e.g., release amounts of ENMs to the environment, predicted/calculated environmental concentrations, toxicity thresholds, etc.) Evaluation of the reported LCRA case study suggests that use of the Nano LCRA framework would require extensive data collection and analysis expertise throughout the various steps.

(3) Frameworks for risk characterization in occupational settings

In general, the proposed frameworks to characterize risks in occupational settings reflect efforts to adapt existing environmental RA approaches for conventional chemicals to develop and implement effective risk management (RM) guidance for addressing the risks of occupational exposures to ENMs (Kuempel et al. 2012). Four different frameworks for characterizing the occupational risks of ENMs were evaluated: (a) the Risk-Based Classification for Occupational Exposure Control ("Risk-Based OEL") approach, (b) the Risk Classification Based on an Industry

Insurance Protocol (RCIP) approach, (c) CB Nanotool, and (d) the Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects ("Stoffenmanager Nano").

(a) The **Risk-Based Classification for Occupational Exposure Control ("Risk-Based OEC")** approach was proposed to facilitate the development of occupational exposure levels (OELs) to improve risk management (reduce workers' exposure) in the workplace (Kuempel et al. 2012, Kuempel et al. 2012). In the Risk-Based OEC approach, hazard of ENMs are evaluated and risk estimates (e.g., % of excess risk) are developed. In cases where limited hazard data are available for the ENMs, hazard data for reference (benchmark) materials are used. Reference materials are selected based on whether they exhibit similar chemical/materials properties and similar modes of action (MOA) to the ENM of interest (e.g., for nano TiO_2, data for fine and ultrafine TiO_2 were used). Examples of modes of action include ROS formation, genotoxicity, or interference with specific cellular functions. The risk potential for exposure to the new ENM(s) in occupational settings via inhalation is then systematically compared with those of benchmark material(s) in the same MOA class. For example, Kuempel et al (Kuempel et al. 2012) used the approach to assess the risk potential of exposure to a variety of airborne particles, including both fine and ultrafine materials. The following standard risk assessment process steps were followed (NRC 2009): 1) Identifying the relevant animal model, dose metric, and disease response; 2) Modeling the animal dose–response relationship and estimate the critical effect level (e.g., benchmark dose); 3) Extrapolating the animal critical effect level estimates to humans by adjusting for factors that influence the deposited or retained lung dose in each species, assuming equal response at equivalent dose; and 4) Estimating airborne exposures (8-h time weighted average, TWA) that would result in the human-equivalent dose. The authors then calculated the 1/1,000

excess risk of lung cancer based on animal-to human extrapolation of benchmark dose estimates ("BMD" is a dose associated with a specified increase in the probability of a given response known as the "benchmark response" (BMR)) using a multistage cancer model and the U.S. EPA's BMD software (EPA 2010). Four risk categories were established in the above case study, for ENMs and fine-sized particles in air based on information derived from previously reviewed control approaches (Zalk and Nelson 2008): (1) *Low risk bin/category* aimed at dusts at an airborne concentration range > 1 mg/m^3 and where exposure can be controlled with general ventilation measures (e.g., fine-sized particles TiO_2 and MoO_3 at concentrations between 1,000–4,000 $\mu g/m^3$ TWA (time weighted average)); (2) *moderate risk bin* for dusts at an airborne concentration range (0.1–1 mg/m^3), which can be controlled with local exhaust ventilation measures; (e.g., carbon black, diesel exhaust particulate (DEP), and ultrafine TiO_2 at TWA airborne concentration (90–250 $\mu g/m^3$); (3) *high risk bin* for dusts at an airborne concentration range (0.01–0.1 mg/m^3), which can be controlled through ventilated enclosures; (e.g., fine particles of NiO and soluble $CoSO_4$ at TWA 20–30 $\mu g/m^3$); and (4) *very high risk* [dusts at airborne concentrations 0.001–0.01 mg/m^3], which can be controlled with containment systems (e.g., fine particles Ni_3S_2 and GaAs at TWA 4–5 $\mu g/m^3$).

The above Risk-Based OEC is useful for grouping inhalable ENMs in occupational settings on the basis of workers' exposure to ENMs or their ultrafine counterparts. However, considerations of the latter also require adequate characterization and toxicity/hazard data.

(b) The **Risk Classification Based on an Industry Insurance Protocol (RCIP)** was designed to compare risks associated with specific steps in the manufacturing of ENMs (as opposed to overall occupational risk) with those of traditional chemicals used in current activities such as

petroleum refining, polyethylene production, and synthetic pharmaceutical production (Robichaud et al. 2005). This framework follows two major parts. The first part involves data collection for each of the steps of a particular manufacturing/synthesis process including, inventory of input or constituent materials, output materials, waste streams, and physical conditions of the manufacturing processes (e.g., temperature, pressure and enthalpy, if available, or representative synthesis methods including a full description of the processes in form of flowcharts). For each constituent material the data to be collected include: toxicity values (e.g., LC_{50} and/or LD_{50}), water solubility, octanol-water partition coefficient, flammability, expected emissions, molecular weight, and photolysis and degradation rates (e.g., photolysis and degradation rates are considered to predict mobility of a material). In the second part of the framework an actuarial tool ("XL tool") is used to assign and tabulate risk scores to the operating conditions of the chemical processes, as well as to hazardous properties and toxicity values of the constituent materials.

The XL tool follows a protocol that is routinely used by industry to calculate insurance premiums. In the RCIP framework, the XL analysis involves a series of arithmetic operations to calculate additive scores for the "risk" posed by a specific process, the "risk" posed by the hazard/toxicity of the constituent materials, and the "risk" posed by the amount of the material emitted. After additive scores for the individual parameters inventoried in the first step are obtained, an aggregated score is calculated (e.g., the sum of the "risk" posed by the process, the "risk" posed by the hazard/toxicity of the constituent materials and the "risk" posed by the amount of the material emitted). The aggregated score is calculated considering two scenarios: a) normal conditions of operation (e.g., assuming that none of the constituent materials are mobile and that photolysis and degradation do not occur); and b) an accident scenario (to account for what might occur if there was an accidental emission resulting in mobility of the constituent

materials/chemicals); photolysis and degradation rates are also considered along with process conditions (e.g., temperature, pressure and heat transfer). These two aggregated scores, for the *normal conditions* and *accident scenarios* are then added and normalized with respect to the highest score to yield an overall score, which is referred to as the *latent risk score*.

The above approach was demonstrated by Robichaud et al (Robichaud et al. 2005), for a case study that considered representative synthetic processes for selected ENMs (C_{60}, single-walled carbon nanotubes (SWCNT), multi-walled carbon nanotubes (MWCNT), Cadmium selenide (CdSe) and Zinc selenide (ZnSe) quantum dots, carbon black, aluminum and silver nanoparticles (nano Al and nano Ag)) that were compared to synthetic processes for traditional chemicals (petroleum refining, polyethylene production, and synthetic pharmaceutical production). The analysis suggested that the manufacturing of the ENMs studied might present lower risks than for the chemicals listed above.

(c) The Control Banding (**CB) Nanotool** (Paik et al. 2008) is an approach developed with the intent of identifying/prioritizing health risks in the workplace in order to assist in the implementation of exposure controls (Paik et al. 2008). Control banding is a term originated from the field of industrial hygiene (Paik et al. 2008) and represents a qualitative approach to assessing risks associated with chemicals with the goal of developing suitable control measures (e.g., via personal protective equipment, administrative or engineering controls). In CB Nanotool, categories or ‘‘bands’’ are established for health hazards of ENMs, which are then combined with exposure scenarios for the target ENMs, to determine recommended levels of control. An advantage of this approach is that it can be used even in the absence of toxicity data for the specific ENM of interest. The above is regarded as a practical approach in the field of ENMs occupational

risk management, given the need to provide recommendations for control measures in the absence of complete hazard profiles for the rapidly growing number of new ENMs (Paik et al. 2008).

The CB Nanotool (Paik et al. 2008) was designed specifically for inhaled ENMs to determine the level of risk of operations carried out in research laboratories. In this approach, the risk level band is assigned based on a matrix that combines two scores, one for *severity* (e.g., degree of biological response elicited by the ENM exposure via inhalation or presence in the bloodstream) and one for *probability* (e.g., the extent to which employees may be potentially exposed to ENMs throughout the handling processes). The severity score is calculated by adding individual scores (e.g., scores assigned via the guidelines recommended by the authors) for physicochemical properties of the ENM (e.g., surface chemistry, particle shape, particle diameter, solubility) and evidence of toxicity (e.g., reproductive, carcinogenic, mutagenicity, dermal and acute toxicity) available for the ENM and for the ENM bulk counterpart (main chemical substance in the composition of the ENM). A probability score is calculated by adding individual scores assigned to the estimated amount of handled ENM (i.e., by the worker), dustiness/mistiness, number of employees with similar exposure, and duration of operation. Similar to the severity score, the proposed approach provides guidelines for assignment of values to each of the parameters of the probability score. The final product is presented as a combined score of the *severity* and *probability* parameters which are assigned to control bands. The combined score or assignation to a control band (e.g., RL) is done qualitatively via a matrix in which the severity scores of low, medium, high and very high grouped by category as rows, while the probability scores of extremely high, less likely, likely and probable grouped by category as columns. For example, the box assigned to the combination of the highest probability score with the highest score of severity will result in the highest band of recommended control measures (e.g., Risk Level 4 (RL 4), “seek specialist

advice"). As the combination of scores decreases in value, the assigned bands correspond to lower recommended control measures. An Excel sheet for use with the above approach was reported by Paik et al (Paik et al. 2008) and later evaluated by Zalk et al (Zalk et al. 2009).

The CB Nanotool represents a framework that is useful for identifying potential control measures for workers' protection. Its utility, however, is predicated on the availability of information on the various activities/steps (e.g., handling ENMs in powder form) involved in the ENM manufacturing process, as well as the hazards posed by the ENMs.

(d) The **Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects ("Stoffenmanager Nano")** (Van Duuren-Stuurman et al. 2012) is a framework based on control banding, similar to the CB Nanotool. The Stoffenmanager Nano approach was with the aim of identifying control measures to reduce the likelihood of inhalation exposure in occupational settings. This framework requires both exposure and industrial process information (e.g., point or fugitive emissions during production, handling powdered ENMs, dispersion of ENMs and activities resulting in ENM release, such as sanding of surfaces) and hazard identification parameters (e.g., solubility of ENMs, nanofiber shape, toxicological data of the ENM or parent material) as inputs. The approach is divided into two steps: 1) an assignment of a hazard category for the ENM and 2) an assignment of an exposure category for the industrial process.

In the first step, one of five hazard categories (A-E, where A and E represent the lowest and highest hazards, respectively) is assigned based on available data. For example, hazard classification can be made based on the water solubility of the ENM (i.e., high water solubility would suggests lower hazard as an ENM and thus such ENM would be in category (A) or based on persistence of nanofibers (where persistent nanofibers would result in a high hazard category

of (E); other ENM hazard data can also be taken into account at this stage (e.g., a band (B) is given to those ENM considered as irritant, a band (C) is given to an irritant that also causes burns). A table built based on expert elicitation with pre-assigned hazard bands is provided in the Stoffenmanager Nano tool for selected ENMs (i.e., C_{60}, carbon black, Ag, Fe, Au, Pb, La, TiN, TiO_2, CeO_2, ZnO and others such as nanoclay and polystyrene) (Van Duuren-Stuurman et al. 2012). In general, however, the assignment of the hazard band in the Stoffenmanager Nano is dependent upon the assessor's judgement and/or the guidelines/thresholds provided by the tool developers.

In the second step, the user has to select an exposure band value (range of 1 to 4, where 1 and 4 represent the lowest and highest exposure, respectively). The exposure band is assigned via scores (termed "multipliers" in the Stoffenmanager Nano tool) which take on numerical values proposed by the authors based on previously published data and or expert elicitation (Van Duuren-Stuurman et al. 2012). The scores provided by Stoffenmanager Nano tool are for various factors that influence exposure (e.g., substance emission potential, handling/activity emission potential, localized controls, segregation, dilution/dispersion, personal behavior, separation/personal enclosure, surface contamination, and respiratory protective equipment) for the industrial process/setting under consideration. Scores are then assigned to 4 bands depending on their value range. Once the hazard and exposure bands are assigned, a matrix is built that qualitatively combines the hazard (columns A-E) and exposure bands (rows 1-4) to yield the priority band (scale of 1-3, where 1, 2 and 3 are for high medium and low priorities, respectively, for exposure control). Following the above approach, for example, the highest priority (band 3) is associated with ENMs having both the highest hazard and highest exposure bands.

The Stoffenmanager Nano framework is particularly suited to situations where the industrial processes involving ENMs are known and where there is potential for inhalation exposure. Application of Stoffenmanager Nano allows the user to rank/prioritize ENMs based on potential worker exposure, which can be useful in situations where decisions must be made with limited data.

(4) Evaluation of the different risk assessment frameworks

In order to assess the utility of available risk assessment frameworks for ENMs described above, the following questions were posed:

1) What is the intent of the framework and who are the potential users/decision makers for which the framework is designed?
2) What is the level of resolution/type of results needed by the potential decision makers to be able to make risk management decisions about the target ENMs?
3) What is the level of expertise that the user must possess to conduct the analysis using the framework?

When addressing the first question, each framework was evaluated to determine if it addresses one or more of the six different decision-making scenarios described in the **Methods Section** and in **Table 2.2**. Lastly, for each decision-making scenario the critical needs that are not met by any of the existing frameworks were identified. A summary of the above findings is provided below.

(a) Suitable Frameworks for Scenario I ("A Company needs to decide whether to control exposure to workers during manufacturing or processing of ENMs"). The most suitable existing frameworks for *Scenario I* are the Swiss Precautionary Matrix (SPM) (Höck J. et al. 2013), the DuPont NanoRisk (DUPONT 2007), Control Banding Nanotool (Paik et al. 2008), and

Stoffenmanager Nano (Van Duuren-Stuurman et al. 2012). Each of these frameworks has different capabilities that companies can use to assess the need to control workers' exposure to ENMs. For example, SPM can be used to identify hazards and/or the need for further actions in terms of risk management related to manufacturing processes of ENMs. SPM allows for rapid assessment of known/unknown information (first tier assessment). The questions posed in SPM are mostly qualitative and designed to determine whether or not the user is dealing with a material that is considered to be classified as "nano". SPM was developed by the Swiss Federal Office of the Environment and the Federal Office of Public Health. Therefore, SPM includes pertinent regulatory definitions for nanomaterials (relevant for Switzerland) and provides useful guidelines to industry users wishing to comply with environmental health and safety regulations. The downside is that this framework does not include a detailed analysis of industrial processes parameters related to worker safety (e.g., the number of employees exposed, frequency of exposure, control measures already in place). Another limitation is that SPM does not provide the analyst with specific recommendations for implementation of industrial hygiene controls.

The DuPont NanoRisk can address the specific needs for a risk management strategy through ENM life cycle profiles. This framework is suitable for decision making related to controlling worker exposures because the DuPont NanoRisk framework requires the analyst to provide lifecycle, exposure and hazard profiles for the material of interest. The challenge with the DuPont NanoRisk framework, however, is that it requires input of a significant body of information in addition to conducting a chemical process risk assessment (CPQRA).

Banding approaches such as the Control Banding Nanotool and Stoffenmanager Nano are useful for classifying ENMs and establishing risk management needs (e.g., reducing working exposure via engineering controls, personal protection equipment and other measures). Because

CB Nanotool involves the identification and quantification of extensive characteristics of the industrial processes (e.g., number of workers potential exposed, frequency of exposure, concentrations that the workers could be exposed to), it allows the user to tailor protective measures to the company's needs. Two primary disadvantages of CB Nanotool are that it requires significant user data input, and that the procedure or the decision regarding the "bands" is highly dependent on the knowledge/expertise of the assessor. The Stoffenmanager Nano also requires extensive input information input by the analyst. However, Stoffenmanager Nano takes into consideration the use of personal protective equipment (PPE) and current industrial hygiene (IH) practices; hence, this approach is useful for reviewing current practices and for leading the analyst to identify possible needs for modifying current practices. Another advantage is that Stoffenmanager Nano is an accessible web-based tool.

(b) Suitable Frameworks for Scenario II ("Regulatory body that has to decide whether to control exposure to workers during ENM manufacturing or processing"). In *Scenario II*, the stakeholders wish to establish an evidence based exposure limit (e.g., OSHA established safe exposure level setting) for an ENM of concern. Of the existing frameworks, the Risk-based OEL framework proposed (Kuempel et al. 2012) is most suitable for this scenario, particularly in cases where a benchmark dose for a reference material (e.g., for a corresponding bulk material or ultrafine material) is available. The Risk-based OEL framework also offers the advantage of identifying specific/minimum data required for conducting an assessment, which allows users to prioritize future research and data collection. However, the applicability of this framework is limited to assessment of ENMs for which well characterized ultrafine counterparts exist; therefore, the approach may have limited utility for next generation ENMs for which well-characterized

reference materials are not available. Additionally, given the SPM parameters (potential effect (W), potential exposure of humans or environmental release (E), and available information on the material's life cycle) (Höck J. et al. 2013) this framework could be useful for the regulatory agency to identify potential ENMs of concern, hence preventing workers' exposure.

(c) Suitable Frameworks for Scenario III ("A Company that needs to decide if the risk(s) associated with producing a nanoparticle or nano-enabled product is manageable"). In *Scenario III*, the stakeholders are individuals working for a company that needs to ascertain whether the risks associated with producing a nanoparticle or nano-enabled product can be reasonable managed. The frameworks that are most suitable for this scenario are the: Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano), Risk Classification based on an Industry Insurance Protocol (RCIP), and Life Cycle Risk Analysis (Nano LCRA). Each of these frameworks allows the analyst to assess impacts related to production of certain ENMs and to identify risk management/reduction strategies. Stoffenmanager Nano is suitable for this scenario as it allows one to design risk reduction/management strategies for each of the "risk bands", which can then be applied to any ENM that meets the classification criteria for each risk band. Stoffenmanager Nano requires detailed information about both the ENM and the associated industrial handling operations. The above is needed to arrive at informative strategies to manage risks associated with the material. However, the above framework only provides a mechanism for qualitative assessment and suggestion of control measures of occupational risks, and risks related to potential releases to the environment that might occur during manufacturing.

The risk classification based on an insurance protocol (RCIP) framework is also suitable for Scenario III since it considers an "incident/accidental release scenario". In this framework, a

measure of the overall risk is calculated and the potentials for accidents are considered. This framework provides a detailed protocol, with the pertinent mathematical expressions, to calculate aggregate scores for parameters that affect risk (i.e., hazard and exposure). One limitation of this framework is that the risks associated with an ENM are calculated based on emissions, exposure potential and hazards of the chemicals involved in the synthesis of ENMs, not those for the actual ENM itself. Admittedly, the above limitation could also be perceived as an advantage in situations where limited data is available for industry to assess the target ENM. Another limitation of the RCIP framework is that it does not address the development of risk management strategies.

Whereas both the Stoffenmanager Nano and RCIP frameworks focus primarily on risks related to manufacturing/synthesis of ENMs, NanoLCRA takes into account the potential risks attributed to the ENM throughout its lifecycle. A shortcoming of the NanoLCRA framework is that it does not provide a specific methodology for quantifying risk (e.g., steps for aggregation of scores, guideline tables to assign exposure/risk bands, or mathematical equations to derive reference values, and benchmark doses). Moreover, the framework relies entirely on an expert evaluation of the available information.

(d) Suitable Frameworks for Scenario IV ("Company that needs to decide as to which nanoparticle or nano-enabled product poses less risk than alternatives for a particular application"). In *Scenario IV***,** the stakeholders are individuals representing a company that desires to identify the safest ENM for a particular application. For this scenario, the most suitable existing frameworks are: Multi-Criteria Decision Analysis (MCDA), Life Cycle Analysis (LCA), BN FINE and modified GreenScreen. MCDA (Tervonen et al. 2009) is appropriate for Scenario IV because it allows comparison among alternatives. For example, MCDA was demonstrated for ranking the relative risk potential of a set of ENMs based on hazard related properties (e.g., agglomeration,

potential to form ROS, reactivity, etc.) (Tervonen et al. 2009). MCDA provides a framework for assessing properties related to hazard and, in doing so, allows the analyst to identify critical properties that could be modified to develop safer ENMs. The disadvantages of applying MCDA to ENMs, at least as is currently proposed in the literature (Linkov et al. 2007, Tervonen et al. 2009, Linkov and Seager 2011), are that the approach relies primarily on expert judgment and that MCDA does not consider causal relationships (e.g., relating a specific ENM property to an adverse outcome).

LCA (Hischier and Walser 2012) is also suitable for companies that need to consider Scenario (IV). This is because LCA provides a framework for assessing environmental impacts throughout the ENM life cycle (synthesis, use, and disposal). The use of LCA, however, requires a significant data (e.g., emission inventories for all chemicals involved in the manufacture of ENMs throughout their lifecycle).

BN FINE (Money et al. 2012) is another useful framework for companies that need to address the above Scenario IV. Given that BN FINE involves the use of an influence diagram, which incorporates causal relationships between ENM properties and risk parameters, the approach can assist in identifying the relevant ENM properties that can be tailored to manufacture safe ENMs (i.e., "safer-by-design") (Geraci et al. 2015). In the absence of quantitative data, expert judgement can be incorporated into the BN framework (Money et al. 2012); however, the framework developer must be able to identify the critical causal relationships (e.g., between ENM physicochemical properties, environmental conditions and risk outcomes). Although the BN approach is extremely powerful, the construction of a BN based framework requires ENM specific data for its construction.

The modified GreenScreen (Sass et al. 2016, Action 2017) and NanoRiskCat (Hansen et al. 2014) approaches can be partially suitable for Scenario IV as they allow analysts to perform rapid screening of potential hazards among a group or individual ENMs for which data are available. The scores provided by GreenScreen are designed to make recommendations regarding the need for additional information or for seeking safer ENMs. Whereas, Nano RiskCat qualitative scores can indicate on a color scale the level of hazard to ecological or human receptors. These frameworks are suitable for hazard assessment for Scenario IV but not as a substitute for risk assessment.

(e) Suitable Frameworks for Scenario V ("Regulatory body that needs to decide whether or not to control environmental use, release, or emissions of an ENM"). Several existing frameworks are suitable for *Scenario V*, including U.S. EPA's own Comprehensive Environmental Assessment CEA (Powers et al. 2012, Powers et al. 2014), Nano HAZ (O'Brien and Cummins 2011), a risk quantification based on probabilistic flow modeling analysis (PMFA RQ) (Gottschalk et al. 2013), BN FINE (Money et al. 2012), and the Nanomaterial Risk-Screening Tool (NRST). CEA is useful for regulatory decision analysis (e.g., regarding issuance of Significant New Use Rule (SNUR) for a new ENM) because it can be used to systematically organize information. CEA provides a framework that allows decision makers to assemble and review data that are critical for determining whether a SNUR should be issued; such data includes, for example, the projected volume of manufacturing and processing, extent to which the novel ENM changes the exposure of human beings or the environment, and the anticipated manner and methods of manufacturing, processing, distribution in commerce, and disposal of a chemical substance. Advantages of the CEA framework include the provision of list/guidelines regarding the information needed for a

comprehensive assessment, and the availability of a survey tool ("CEA web tool") as a platform for eliciting expert information.

NanoHAZ and PMFA RQ are also suitable for use by regulators who are confronted with the need to reduce the potential environmental and health impacts of a specific ENM via restrictions on its environmental releases and use. NanoHAZ is specifically designed to provide qualitative estimations of risks for metallic ENMs in water treatment plants, via mass balance estimation of concentrations and with use of literature derived hazard data. Risk can also be estimated quantitatively in the PMFA RQ framework. The PMFA RQ framework can also take into account local geographical and meteorological conditions and specific hazard data if these are available. Furthermore, both NanoHAZ and PMFA RQ require the analyst to provide judgment as to whether the calculated risk is significant or unreasonable; such a request for information essentially implicitly implies that the analyst is knowledgeable regarding the implications of the various assumptions made by the frameworks' developers.

Bayesian Network (BN) approaches like BN FINE can also be suitable for used under *Scenario V*. One advantage of the above approach is that it allows incorporation of both quantitative and qualitative (including expert knowledge) data. BN offer the additional advantage of the convenience by which one can refine/modify the BN as additional information becomes available (i.e., via incremental learning). Two additional advantages of BN FINE for regulators are that this framework can address ecological risks and can quantify uncertainties, thus assisting regulators in determining whether or not the calculated risk is significant/unreasonable.

Finally, the Nanomaterial Risk-Screening Tool (NRST) is a suitable framework for *Scenario V* because it takes into consideration both potential human and ecological risks associated with ENMs. However, given that this framework requires expert judgment regarding available

information (and does not incorporate quantitative data), there may be a concern that potential bias could be introduced.

(f) Suitable Frameworks for Scenario VI (Regulatory body deciding whether to allow nanoparticles to be included in food, drugs, personal care products). NanoRiskCat (Hansen et al. 2014), ENRHES RA (Aschberger et al. 2011) and DF4Nano (Arts et al. 2015) are the most suitable frameworks available for *Scenario VI* because they focus on safety assessment for consumer products (e.g., new cosmetics or drugs applications), but each of them has significant limitations. NanoRiskCat is particularly useful for identifying potential exposure scenarios related to use of consumer products. Analysis via this framework, however, requires access to data regarding the form in which the ENM is present in the consumer product (e.g., as a spray, embedded in a solid film), as well knowledge of potential scenarios that can lead to ENM release to the environment. NanoRiskCat is also a suitable screening approach for identifying the need for more specific safety assessments. A major limitation, however, is that NanoRiskCat does not meet the requirements of REMS (Risk Evaluation and Mitigation Strategy); thus, it is less suitable for formal regulatory risk evaluation.

The DF4Nano framework can also be used to conduct a rapid assessment of human health hazards. If sufficient ENMs characterization data are available to allow a new ENM to be grouped with existing (better characterized) ENMs based on its properties, then DF4Nano can be used to classify the ENM risk potential in the absence of extensive toxicity data. One major limitation is that DF4Nano does not account for other product components or transformation of ENMs during product manufacturing.

The Human Health and Ecological Risk Assessment framework within the project "Engineered Nanoparticles - Review of Health and Environmental Safety" (ENRHES RA) can also be suitable

for *Scenario VI*. One advantage of the ENRHES RA framework is that it can serve to estimate the risk potential of ENMs in consumer products, provided that data are available regarding ENMs properties and potential for release after incorporation into consumer products. A limitation of ENRHES RA is that it requires quantitative dose-response data and information regarding the potentially exposed population and exposure scenarios to be able to quantitatively assess the risks associated with a particular ENM.

CONCLUSIONS

Over the last decade, a number of different frameworks have been developed with the goal of providing evidence-based approaches to making practical decisions related to the potential risk associated with ENMs. The utility of these frameworks should be assessed based on the *intent for making the decisions* regarding the potential risk of ENMs and the level of decision making (i.e., who is and/or what is the authority of the decision maker?). Accordingly, the current review of existing frameworks for assessing the potential environmental and health impacts of ENMs evaluated the applicability of different frameworks based on six plausible decision scenarios. These scenarios were designed to describe the most common and pressing needs by critical stakeholders to arrive at decisions respecting the environmental health and safety of engineered nanomaterials **(Table 2.2)**. For each of the explored decision scenarios, at least one existing framework was identified as being capable of partly meeting the needs of potential decision makers. Limitations and advantages of the different frameworks and associated available tools were then identified in relation to the needs for decision analysis.

Several of the existing frameworks were assessed to partially meet the needs of manufacturers and regulatory bodies seeking to identify measures for reducing or controlling workers' exposure

to ENMs during manufacture and other industrial activities (*Scenarios I and II*). These include the Swiss Precautionary Matrix, DuPont NanoRisk, Control Banding (CB Nanotool), and the Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano)). Each of these frameworks focuses on evaluating different activities that may lead to ENM exposure and incorporates hazard information to help the analyst develop and prioritize risk and exposure control measures for ENMs. However, because the above frameworks consider inhalation as the sole exposure pathway (with the exception of NanoRisk), they are of limited applicability to decision makers who wish to assess other exposure pathways (e.g. oral and dermal exposures).

Several frameworks that companies can use to assess or compare risks associated with production of nano-enabled products (*Scenarios III and IV*) are available; however, each of these frameworks requires either expert judgement, proprietary software packages, and/or extensive hazard data for the ENMs of interest. For instance, MCDA, LCA, BN FINE, Stoffenmanager Nano, RCIP, Nano LCRA frameworks all require access to extensive ENM toxicity and/or exposure data. MCDA and Nano LCRA also require expert judgment, while RCIP and BN FINE require significant expertise and use of external software packages.

Several frameworks have been designed to meet the need of decision makers (e.g., U.S. EPA and U.S. FDA) who wish to assess the potential impact of environmental releases of ENMs and safety of commercial products (*Scenarios V and VI*). However, each of these frameworks also has significant limitations. For example, the ability of regulatory decision makers to use CEA, NanoHAZ, PMFA, BN FINE, NRST, NanoRiskCat, ENRHES RA frameworks is limited to the types of hazard and exposure data that are currently available for ENMs. Because hazard and exposure data are typically only available for ENMs as manufactured, the above tools are not

directly applicable for assessing risks posed by of ENMs that have been transformed through their incorporation in nano-enabled products or their transformation in the environment. Moreover, frameworks such as U.S. EPA's CEA and NRST do not include tools to integrate quantitative and qualitative information about hazard and exposure and rely heavily on expert judgment to identify the data needed for the analysis.

Despite significant advances that have been made in the area of risk assessment associated with ENMs, the currently available frameworks do not provide a pragmatic, flexible and comprehensive approach that would meet the needs of all the critical categories of decision makers and decision scenarios. Given the varied decision analysis objectives, different risk assessment frameworks have been proposed at different levels of complexity, different data needs and with different outcome objectives. At present, the existing frameworks do not provide a convenient and transparent mechanism for integrating results from modeling tools with experimental and industry reported data. As a result, each of the existing frameworks is limited by the relatively incomplete exiting hazard and exposure data for ENMs. Given the rapid developments in nanotechnology, it would be highly desirable to develop an integrated framework that could provide an efficient mechanism for managing and integrating quantitative and qualitative information while also accounting for the impact of missing information as part of the analysis. Ideally, such a framework would also provide guidance to decision makers (in the absence of expert judgement) regarding the information needed to conduct decision analysis for specific scenarios that are of interest.

In closure, based on the present review of various risk assessment frameworks further research should focus on the development of integrative frameworks for assessing the risk potential of ENMs that: a) address the complexities of ENMs and their transformations, b) integrate quantitative and qualitative data, c) allow use of modeling tools to fill data gaps, d) minimize

reliance on expert judgement, and e) enable quantification of uncertainties associated with the use of both quantitative and qualitative data/information. Such frameworks would not only be of practical use for decision makers in a variety of contexts but would also provide evidence-based approaches for prioritizing future research and manufacturing of ENMs and related products in support of environmentally sustainable nanotechnology.

Table 2.1 Summary of critical characteristics of risk assessment frameworks relevant to ENMs reviewed herein

Name of the framework and developer	General description	Main output of analysis
Swiss precautionary matrix (Höck J. et al. 2013) (Swiss Federal Office of Public Health)	Decision tree/questionnaire about the properties of the ENM under consideration (e.g., dimensions), effects (e.g., reactivity, stability), and exposure/release potential (e.g., physical form of the ENM), suitable for pre-screening.	Classification of the hazard posed by the ENM into two main groups: A) no need for review of (unspecified) risk management measures; B) need for review of (unspecified) risk management measures or need for additional information.
Risk Classification System based on Multi Criteria Decision Analysis (MCDA risk classification) (various institutions)(Linkov et al. 2007, Linkov et al. 2009, Tervonen et al. 2009)	Systematic comparison of alternatives (ENMs) via outranking by assigning scores (e.g., qualitative scale of least-most desirable (1-4), subjective probability (0-100%), and quantitative measurement of size (0-100)) for pre-determined criteria related to hazard, including intrinsic ENM properties (e.g., agglomeration, reactivity/charge, critical function groups, contaminant dissociation and size) and factors affecting toxicity (bioavailability and bioaccumulation).	Categorical classification of the hazard (e.g., toxic potential): very low, low, medium, high, and extremely high.
Hazard and exposure potential identification for ENMs in consumer products (NanoRiskCat) (Hansen et al. 2014) (University of Denmark)	Decision tree/flowchart, where user answers "yes", "no", or "no data" to questions about the ENM of interest (e.g., physical form of the ENM applied to products, toxicity evidence, high aspect ratio, potential of transport across ecosystems) .	Color-coded/categorical classification of the hazard posed by the ENM: the scale ranges from a grey color assigned to insufficient data, green-low hazard, yellow-medium and red-high.
DF4Nano grouping (Arts et al. 2015) (European Center for Ecotoxicology and Toxicology of Chemicals "ECETOC" (NGO))	Theoretical framework presented in tables (e.g., threshold values obtained from published data and expert elicitation) to guide the user in the classification/prioritization of ENMs for additional testing/risk assessment.	Categorical classification of ENMs in four main categories: 1) soluble ENMs, 2) biopersistent high-aspect ratio (for which no additional testing is required), 3) passive ENMs, and 4) active ENMs (which require a further analysis/risk assessment).
Modified GreenScreen (Sass et al. 2016) (Clean Production Action Group (NGO))	Hazard assessment framework designed to screen chemicals with based on a range of toxicity endpoints and ENM physicochemical properties.	Categorical classification of ENMs in 5 main categories of aggregated benchmark (BM) scores to designate specific recommendations regarding ENM use based on the potential environmental and human health concerns as supported by available data.
Life Cycle Analysis (LCA) (Eckelman et al. 2012, Gavankar et al. 2012, Hischier and Walser 2012) (Various institutions)	Class of approaches that follow a product over its life stages, including: (a) material acquisition and purification, (b) manufacturing and fabrication, (b) commercial uses, and (d) end-of-life product management. LCA is rooted in assessing environmental impacts of chemicals, but it has been adapted for ENMs.	Environmental impacts of the product under analysis (e.g., effects on ecological receptors, potential CO_2 emissions attributed to synthesis/manufacture of ENMs).

Name of the framework and developer	General description	Main output of analysis
DUPONT's Nanorisk (DUPONT 2007) (Environmental Defense Fund (NGO) and DuPont (Industry))	Collection and organization of information, that can include a chemical process risk assessment (CPQRA) following AICHE guidelines in cases where sufficient quantitative data are available. CPQRA focuses on acute rather than chronic hazards. Risk in this system is defined as a function of the hypothetical scenario, the estimated consequence(s) of exposure, and the estimated frequency of exposure.	Results for individual ENMs and scenarios are presented as lifecycle profiles that include information on physical-chemical properties, ecotoxicity, and environmental fate to be used for risk management strategies. In cases where quantitative data are available, the results include a quantitative risk analysis of the industrial processes related to the ENM.
U.S. EPA's Comprehensive Environmental Assessment CEA (Powers et al. 2012, Powers et al. 2014) (U.S. EPA)	Compilation of extensive information needed to inform a *"collective judgment"*. Experts must then analyze the information to provide guidance to decision makers such as research planners and risk managers. This framework is presented as a roadmap to guide the user in a systematic data collection and identification of critical data gaps.	Summary of available information regarding a specific ENM. Typically accompanied by an evaluation of the resulting information by a group of experts that provides recommendations for research priorities and risk management.
An Adaptive Screening-Level Life Cycle Risk-Assessment Framework for Nanotechnology (Nano LCRA) (Shatkin 2008, Shatkin and Kim 2015) (Vireo Advisors)	Systematic compilation of information (e.g., properties, potential exposure and hazard of ENMs through all life cycle stages for a particular product) guided by a "roadmap" that is further analyzed by experts.	Summary of information with main findings/expert judgment based on those findings and indication of further information needs.
Ranking initial environmental and human health risk: Nano HAZ framework (O'Brien and Cummins 2010) (University College Dublin)	Process for developing qualitative risk rankings, including ecological risk and/or human health risk, for ENMs. Risk rankings reflect Bench Mark Dose (BMD) calculations, which are based on published/available data.	Categorical classification of ENMs into relative risk ranking groups: 0–2 (low environmental or health risk on a relative basis), 3–4 (concentrations that require monitoring and potential action), 5 + (environmental concentration above those provisional regulatory and toxicological limits as set in this study).
Nanomaterial risk screening (Beaudrie et al. 2015) (University of British Columbia and Decision Research (non-profit organization))	The framework guides the user through the process of assigning risk groups to ENMs. The categories are determined based on comparisons between data for the ENM under analysis to a reference set of information (tables) provided by the framework.	Categorical classification of ENMs in risk groups, where lowest concern = 1 and highest concern = 5.
Engineered Nanoparticles - Review of Health and Environmental Safety: Human health and Ecological Risk Assessment (ENRHES RA) (Aschberger et al. 2011) (European Commission Institute for Health and Consumer Protection)	Risk assessment of specific ENMs based on 90-day exposure studies and likely environmental concentrations determined by probabilistic models.	Ratio of the predicted environmental concentration for ENM of interested to the (predicted) human no effect levels (PEC/INEC).

Name of the framework and developer	General description	Main output of analysis
A Risk Quantification based on Probabilistic Mass Flow analysis (PMFA risk quantification) (Gottschalk et al. 2013) (Swiss Federal Laboratories for Materials Science and Technology (EMPA))	Risk assessment for ENM of interest that combines predicted environmental concentrations (determined via probabilistic modeling) with a species sensitivity distribution (e.g., probability distribution of harmful effects shown at different concentrations for a given ENM).	Quantitative measure of risk calculated from the product of the probability of critical environmental concentrations and the probability that organisms would potentially be negatively impacted by such concentrations.
Bayesian Networks based FINE (Forecasting the Impacts of Nanomaterials in the Environment applied to nano Ag) (Money et al. 2012) (Center for the Environmental Implications of Nanotechnology (CEINT) at Duke University)	Method for calculating the probability of risk for an ENM of interest using a Bayesian Network designed with inputs from expert judgment.	Modified version of a deterministic risk quotient (quantitative measure of risk) in a probabilistic expression.
Risk based classification for occupational exposure control (Risk based OEL) (Kuempel et al. 2012) (Nanotechnology Research Center (NTRC) and National Institute for Occupational Safety and Health (NIOSH)).	Process for quantitatively assessing the risk of an ENM of interest by applying benchmark doses (BMD).	Percent of excess risk related to a specific health outcome as a result of exposure to the ENM under analysis.
Risk classification based on an Industry Insurance Protocol (RCIP) (Robichaud et al. 2005) (Rice University, Golder Associates Inc and XL insurance)	Comparison of scores assigned to characteristics of the industrial process with pre-established scores from an insurance protocol.	Relative risk ranking for the ENM process compared to conventional industrial chemical process.
Control Banding: CB Nanotool (Paik et al. 2008) (Delft University of Technology)	Classification based on characteristics of the potential for exposure during preparation the ENM of interest (e.g., estimated amount of ENMs, dustiness/mistiness, number of employees with similar exposure, frequency and duration of operation) and properties related to hazard of the ENM (e.g., surface chemistry, particle shape and diameter, solubility, carcinogenicity, reproductive toxicity, mutagenicity, dermal hazard potential).	Risk banding for occupational risk. The risk bands indicate recommendations to pursue a risk management strategy to control exposure (e.g., RL 1: general ventilation; RL 2: fume hoods or local exhaust ventilation; RL 3: containment; RL 4: seek advice of environmental health specialist).
Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012)	Classification based on the characteristic of the potential for exposure during preparation the ENM of interest (ENM size, aspect ratio, handling, background exposure, duration, frequency) properties related to hazard of the ENM (e.g., toxicity data).	Priority banding where the bands indicate the priority for risk management.

Table 2.2 Decision needs and recommended ENMs relevant risk assessment frameworks for selected regulatory decision-making scenarios

Scenario	Example and desired output of analysis	Potential framework for use/ currently available frameworks
Scenario I: Company deciding whether to control exposure to workers during manufacturing or processing of ENMs.	• A company is producing a new ENM and wants to know what controls to put into place to product their workers. • Internal risk management strategy including recommended engineering controls, administrative controls.	Swiss Precautionary Matrix; DuPont NanoRisk (DUPONT 2007); Control Banding (CB Nanotool) (Paik et al. 2008); Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012).
Scenario II: Regulatory body deciding whether to control exposure to workers during manufacturing or processing.	• OSHA deciding whether to establish Occupational Exposure Limits (OEL)/Permissible Exposure Limits (PEL) for a specific class of ENMs. • Evidence based recommendation or requirement for allowed exposure.	Risk based classification for occupational exposure control (Risk based OEL) (Kuempel et al. 2012), SPM (Höck J. et al. 2013).
Scenario III: Company deciding whether risk associated with producing a nanoparticle or nano-enabled product is manageable.	• Company needs to assess the potential impacts of the production of a nano-enabled product and how to manage risks if any. • Risk assessment of a particular ENM and risk management strategy.	Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012); Risk classification based on an Industry Insurance Protocol (RCIP) (Robichaud et al. 2005); An Adaptive Screening-Level Life Cycle Risk-Assessment Framework for Nanotechnology (Nano LCRA) (Shatkin 2008, Shatkin and Kim 2015).
Scenario IV: Company deciding which nanoparticle or nano-enabled product poses less risk than alternatives for a particular application.	• Company interested in a precautionary approach to make safe-by-design applications. • Assessment or comparison of alternatives in terms of environmental impacts and technical performance.	Multi Criteria Decision Analysis (MCDA) (Tervonen et al. 2009), Life Cycle Analysis (Hischier and Walser 2012), FINE (Forecasting the Impacts of Nanomaterials in the Environment) based on Bayesian Networks (Money et al. 2012), modified GreenScreen (Sass et al. 2016), NanoRiskCat (Hansen et al. 2014).
Scenario V: Regulatory body deciding whether to control environmental use, release, or emissions of an ENM.	• U.S. EPA deciding whether to issue a Significant New Use Rule (SNUR) under TSCA (Toxic Substances Control Act) for a particular type of ENM. • Substantial evidence to indicate that a specific ENM will present an unreasonable risk to people or the environment.	U.S. EPA's Comprehensive Environmental Analysis (CEA) (Powers et al. 2012); Risk Assessment Framework for Assessing Metallic Nanomaterials of Environmental Concern (NanoHAZ) (O'Brien and Cummins 2011); A risk quantification based on probabilistic flow modeling analysis (PMFA); Forecasting the Impacts of Nanomaterials in the Environment (FINE) based on Bayesian Networks (Money et al. 2012); Nano material risk-screening tool (NRST) (Beaudrie et al. 2015).
Scenario VI: Regulatory body deciding whether to allow nanoparticles to be included in food, drugs, personal care products.	• U.S. FDA deciding whether to allow registration of a new nano-enabled product in food (whole food, dietary supplement, food ingredient or additive), medical devices, drugs or cosmetics. • Safety assessment for cosmetic products or a Risk Evaluation and Mitigation Strategy (REMS) for a new drug (Duvall 2012).	NanoRiskCat (Hansen et al. 2014); Engineered Nanoparticles - Review of Health and Environmental Safety: Human health and Ecological Risk Assessment (ENRHES RA) (Aschberger et al. 2011); DF4Nano (Arts et al. 2015).

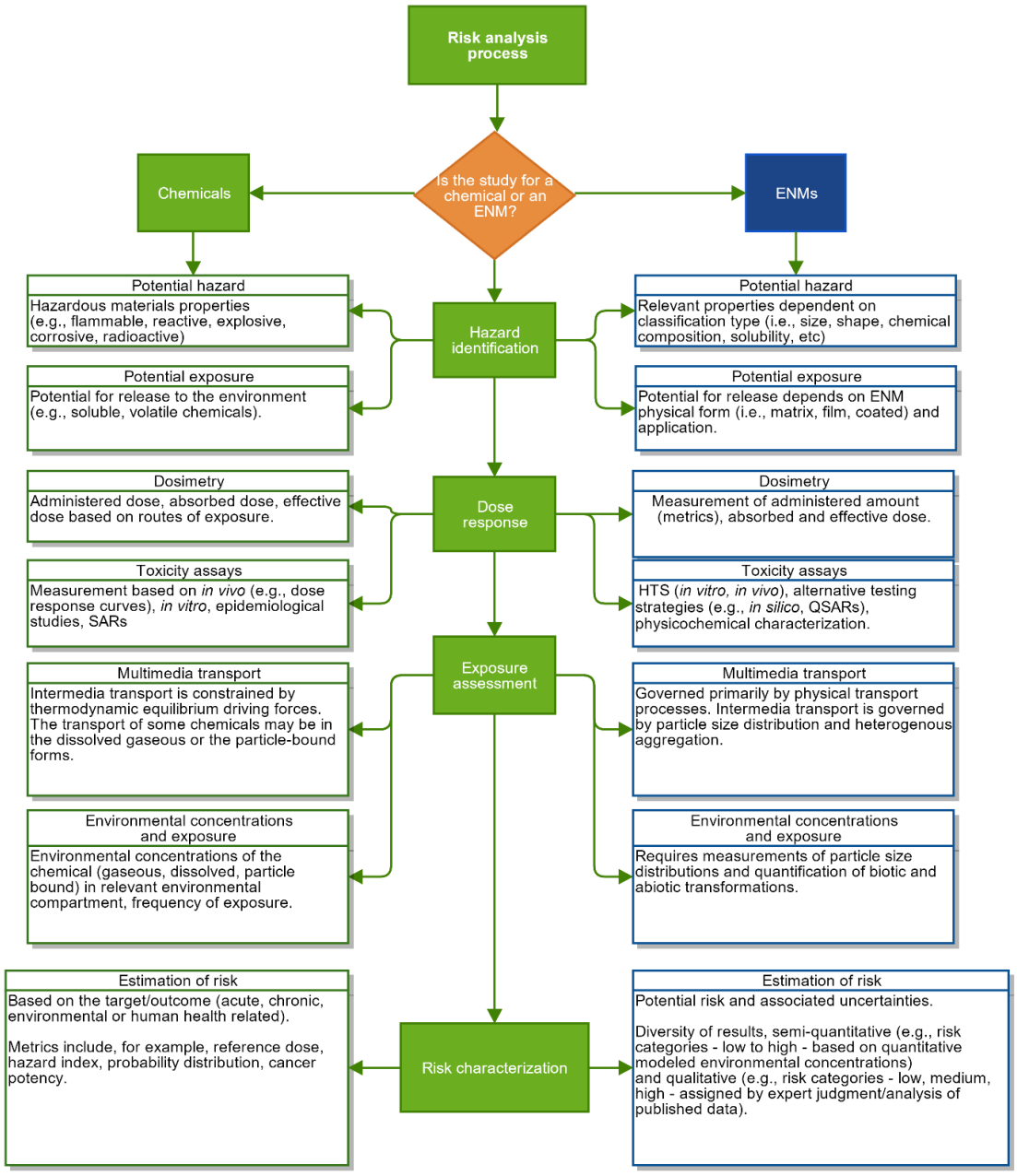


Figure 2.1 Challenges encountered at each step of the traditional risk assessment process for conventional chemicals and its relevance to ENMs

REFERENCES FOR CHAPTER 2

Action, C. P. (2017). "Greenscreen for Safer Chemicals." Retrieved March 14, 2017, from http://www.greenscreenchemicals.org/.

AIChE (2000). Guidelines for Chemical Process Quantitative Risk Analysis (2nd Edition), Center for Chemical Process Safety/AIChE.

Arts, J. H. E., M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, W. Wohlleben and R. Landsiedel (2015). "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (Df4nanogrouping)." Regulatory Toxicology and Pharmacology **71**(2, Supplement): S1-S27.

Aschberger, K., H. J. Johnston, V. Stone, R. J. Aitken, S. M. Hankin, S. A. Peters, C. L. Tran and F. M. Christensen (2010). "Review of Carbon Nanotubes Toxicity and Exposure--Appraisal of Human Health Risk Assessment Based on Open Literature." Critical Reviews in Toxicology **40**(9): 759-790.

Aschberger, K., H. J. Johnston, V. Stone, R. J. Aitken, C. L. Tran, S. M. Hankin, S. A. Peters and F. M. Christensen (2010). "Review of Fullerene Toxicity and Exposure--Appraisal of a Human Health Risk Assessment, Based on Open Literature." Regulatory Toxicology and Pharmacology **58**(3): 455-473.

Aschberger, K., C. Micheletti, B. Sokull-Kluttgen and F. M. Christensen (2011). "Analysis of Currently Available Data for Characterising the Risk of Engineered Nanomaterials to the Environment and Human Health - Lessons Learned from Four Case Studies." Environment International **37**(6): 1143-1156.

Beaudrie, C. E. H. and M. Kandlikar (2011). "Horses for Courses: Risk Information and Decision Making in the Regulation of Nanomaterials." Journal of Nanoparticle Research **13**(4): 1477-1488.

Beaudrie, C. E. H., M. Kandlikar, R. Gregory, G. Long and T. Wilson (2015). "Nanomaterial Risk Screening: A Structured Approach to Aid Decision Making under Uncertainty." Environment Systems & Decisions **35**(1, Sp. Iss. SI): 88-109.

Carroll, J. M. Scenarios and Design Cognition, IEEE. in "The human-computer interaction handbook" 2003 pages 1032-1050 L. Erlbaum Associates Inc.

Christensen, F. M., H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger (2010). "Nano-Silver - Feasibility and Challenges for Human Health Risk Assessment Based on Open Literature." Nanotoxicology **4**(3): 284-295.

Christensen, F. M., H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger (2011). "Nano-Tio2-Feasibility and Challenges for Human Health Risk Assessment Based on Open Literature." Nanotoxicology **5**(2): 110-124.

Cohen, Y., R. Rallo, R. Liu and H. H. Liu (2012). "In Silico Analysis of Nanomaterials Hazard and Risk." Accounts of Chemical Research **46**(3): 802–812.

Craig, D. S. (2010). "The Food and Drug Administration Risk Evaluation and Mitigation Strategy." Journal of Pain & Palliative Care Pharmacotherapy **24**(2): 145-148.

David, R. M. (2013). "Measuring Engineered Nanomaterials in the Environment: A Consortium View of How to Address the Problem." Environmental Engineering Science **30**(3): 97-100.

Dekkers, S., F. Cassee, E. Heugens, A. Baun, M. Pilou, C. Asbach, M. Dusinska, C. Nickel, M. Riediker and C. de Heer (2010). Consensus Report Risk Assessment of Nanomaterials: *in Vitro* – *in Vivo* Extrapolation consensus Report Risk Assessment of Nanomaterials: in Vitro – in Vivo Extrapolation Laussanne, Switzerland, NanoImpactNet consortium

DUPONT. (2007). "Nanorisk Framework." from http://www.nanoriskframework.org/.

Duvall, M. N. (2012). FDA Regulation of Nanotechnology, Washington (DC): Beveridge & Diamond, PG. http://www.bdlaw.com/assets/attachments/323.pdf

Eckelman, M. J., M. S. Mauter, J. A. Isaacs and M. Elimelech (2012). "New Perspectives on Nanomaterial Aquatic Ecotoxicity: Production Impacts Exceed Direct Exposure Impacts for Carbon Nanotoubes." Environmental Science & Technology **46**(5): 2902-2910.

EPA (1998). Guidelines for Ecological Risk Assessment. Washington, D.C.

EPA (2000). Risk Characterization Handbook. S. P. Council. [Washington, D.C.], EPA.

EPA (2010). Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen.

EPA. (2012). "CADDIs: The Causal Analysis/Diagnosis Decision Information System." Retrieved September 21, 2015, from http://www.epa.gov/caddis/index.html.

EPA. (2016). "Reviewing New Chemicals under the Toxic Substances Control Act (Tsca)." Retrieved March, 2017, from https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/regulatory-actions-under-tsca.

EPA, U. (2010). Benchmark Dose Software, Version 2.1.2. U.S. Washington. https://www.epa.gov/bmds

EU (2011). Commission Recommendation of 18 October 2011 on the Definition of Nanomaterial. Official Journal of the European Union.

FDA. (2016). "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology " Retrieved March 8, 2017, from https://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm.

Gajewicz, A., B. Rasulev, T. C. Dinadayalane, P. Urbaszek, T. Puzyn, D. Leszczynska and J. Leszczynski (2012). "Advancing Risk Assessment of Engineered Nanomaterials: Application of Computational Approaches." Advanced Drug Delivery Reviews **64**(15): 1663-1693.

Gavankar, S., S. Suh and A. F. Keller (2012). "Life Cycle Assessment at Nanoscale: Review and Recommendations." International Journal of Life Cycle Assessment **17**(3): 295-303.

Geraci, C., D. Heidel, C. Sayes, L. Hodson, P. Schulte, A. Eastlake and S. Brenner (2015). "Perspectives on the Design of Safer Nanomaterials and Manufacturing Processes." Journal of Nanoparticle Research **17**(9): 366.

Godet, M. and F. Roubelat (1996). "Creating the Future: The Use and Misuse of Scenarios." Long Range Planning **29**(2): 164-171.

Gottschalk, F., E. Kost and B. Nowack (2013). "Engineered Nanomaterials in Water and Soils: A Risk Quantification Based on Probabilistic Exposure and Effect Modeling." Environmental Toxicology and Chemistry **32**(6): 1278-1287.

Gottschalk, F. and B. Nowack (2013). "A Probabilistic Method for Species Sensitivity Distributions Taking into Account the Inherent Uncertainty and Variability of Effects to Estimate Environmental Risk." Integrated Environmental Assessment and Management **9**(1): 79-86.

Gottschalk, F., R. W. Scholz and B. Nowack (2010). "Probabilistic Material Flow Modeling for Assessing the Environmental Exposure to Compounds: Methodology and an Application to Engineered Nano-Tio2 Particles." Environmental Modelling & Software **25**(3): 320-332.

Grieger, K. D., A. Laurent, M. Miseljic, F. Christensen, A. Baun and S. I. Olsen (2012). "Analysis of Current Research Addressing Complementary Use of Life-Cycle Assessment and Risk Assessment for Engineered Nanomaterials: Have Lessons Been Learned from Previous Experience with Chemicals?" Journal of Nanoparticle Research **14**(7): 23.

Grieger, K. D., I. Linkov, S. F. Hansen and A. Baun (2012). "Environmental Risk Analysis for Nanomaterials: Review and Evaluation of Frameworks." Nanotoxicology **6**(2): 196-212.

Hansen, S., E. Michelson, A. Kamper, P. Borling, F. Stuer-Lauridsen and A. Baun (2008). "Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products." Ecotoxicology **17**(5): 438-447.

Hansen, S. F., K. A. Jensen and A. Baun (2014). "Nanoriskcat: A Conceptual Tool for Categorization and Communication of Exposure Potentials and Hazards of Nanomaterials in Consumer Products." Journal of Nanoparticle Research **16**(1): 1-25.

Hansen, S. F., B. H. Larsen, S. I. Olsen and A. Baun (2007). "Categorization Framework to Aid Hazard Identification of Nanomaterials." Nanotoxicology **1**(3): 243-U369.

Hansen, S. F., K. N. Nielsen, N. Knudsen, K. D. Grieger and A. Baun (2013). "Operationalization and Application of "Early Warning Signs" to Screen Nanomaterials for Harmful Properties." Environmental Science-Processes & Impacts **15**(1): 190-203.

Health, F. O. o. P. (2013). "Precautionary Matrix for Synthetic Materials." Retrieved March, 2016, from https://www.bag.admin.ch/bag/en/home/themen/mensch-gesundheit/chemikalien/nanotechnologie/sicherer-umgang-mit-nanomaterialien/vorsorgeraster-nanomaterialien-webanwendung.exturl.html/aHR0cDovL3d3dy5iYWctYW53LmFkbWluLmNoLzIwMTZfdGFnbG/FiLzIwMTZfbmFub3Jhc3Rlci9wb3J0YWxfZW4ucGhwP21vZD1h/Jmxhbmc9ZW4mU0lEPWQwOHA2Mmhna3FwbjI3dHVudjd1ZzhhbT/gy.html.

Healy, M. L., L. J. Dahlben and J. A. Isaacs (2008). "Environmental Assessment of Single-Walled Carbon Nanotube Processes." Journal of Industrial Ecology **12**(3): 376-393.

Hendren, C. O., M. Lowry, K. D. Grieger, E. S. Money, J. M. Johnston, M. R. Wiesner and S. M. Beaulieu (2013). "Modeling Approaches for Characterizing and Evaluating Environmental Exposure to Engineered Nanomaterials in Support of Risk-Based Decision Making." Environmental Science & Technology **47**(3): 1190-1205.

Hischier, R. and T. Walser (2012). "Life Cycle Assessment of Engineered Nanomaterials: State of the Art and Strategies to Overcome Existing Gaps." Science of the Total Environment **425**: 271-282.

Höck J., E. T., Furrer E., Gautschi. M, Hofmann H., Höhener K., Knauer K., Krug H., Limbach, G. P. L., Nowack B., Riediker M., Schirmer K., Schmid K., Som C., Stark W., Studer C., Ulrich A., and W. A. von Götz N., Wengert S., Wick P. (2013). Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Berne, Federal Office of Public Health and Federal Office for the Environment. **Version 3.0:** 26.

Hristozov, D. R., S. Gottardo, A. Critto and A. Marcomini (2012). "Risk Assessment of Engineered Nanomaterials: A Review of Available Data and Approaches from a Regulatory Perspective." Nanotoxicology **6**(8): 880-898.

Kuempel, E. D., V. Castranova, C. L. Geraci and P. A. Schulte (2012). "Development of Risk-Based Nanomaterial Groups for Occupational Exposure Control." Journal of Nanoparticle Research **14**(1029): 1-15.

Kuempel, E. D., C. L. Geraci and P. A. Schulte (2012). "Risk Assessment and Risk Management of Nanomaterials in the Workplace: Translating Research to Practice." Annals of Occupational Hygiene **56**(5): 491-505.

Linkov, I., M. E. Bates, B. D. Trump, T. P. Seager, M. A. Chappell and J. M. Keisler (2013). "For Nanotechnology Decisions, Use Decision Analysis." Nano Today **8**(1): 5-10.

Linkov, I., F. K. Satterstrom, J. Steevens, E. Ferguson and R. C. Pleus (2007). "Multi-Criteria Decision Analysis and Environmental Risk Assessment for Nanomaterials." Journal of Nanoparticle Research **9**(4): 543-554.

Linkov, I. and T. P. Seager (2011). "Coupling Multi-Criteria Decision Analysis, Life-Cycle Assessment, and Risk Assessment for Emerging Threats." Environmental Science & Technology **45**(12): 5068-5074.

Linkov, I., J. Steevens, M. Chappell, T. Tervonen, J. R. Figueira and M. Merad (2009). Classifying Nanomaterial Risks Using Multi-Criteria Decision Analysis. Nanomaterials: Risks and Benefits. I. Linkov and J. Steevens, Springer Netherlands**:** 179-191.

Liu, H. H. and Y. Cohen (2014). "Multimedia Environmental Distribution of Engineered Nanomaterials." Environmental Science & Technology **48**(6): 3281-3292.

Liu, S. Y., H. Q. Wang and Y. L. Li (2012). "Current Progress of Environmental Risk Assessment Research." Procedia Environmental Sciences **13**(0): 1477-1483.

Mauter, M. S. and M. Elimelech (2008). "Environmental Applications of Carbon-Based Nanomaterials." Environmental Science & Technology **42**(16): 5843-5859.

Maynard, A. D., D. B. Warheit and M. A. Philbert (2011). "The New Toxicology of Sophisticated Materials: Nanotoxicology and Beyond." Toxicological Sciences **120**: S109-S129.

Money, E. S., K. H. Reckhow and M. R. Wiesner (2012). "The Use of Bayesian Networks for Nanoparticle Risk Forecasting: Model Formulation and Baseline Evaluation." Science of the Total Environment **426**: 436-445.

Mueller, N. C. and B. Nowack (2008). "Exposure Modeling of Engineered Nanoparticles in the Environment." Environmental Science & Technology **42**(12): 4447-4453.

Mustajoki, J. and M. Marttunen (2013). Comparison of Multi-Criteria Decision Analytical Software.

National Research, C. and H. Committee on the Institutional Means for Assessment of Risks to Public (1983). "Risk Assessment in the Federal Government Managing the Process."

NRC (1994). Ranking Hazardous-Waste Sites for Remedial Action, The National Academies Press.

NRC (2009). Science and Decisions: Advancing Risk Assessment, The National Academies Press.

O'Brien, N. and E. Cummins (2010). "Ranking Initial Environmental and Human Health Risk Resulting from Environmentally Relevant Nanomaterials." Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering **45**(8): 992-1007.

O'Brien, N. J. and E. J. Cummins (2011). "A Risk Assessment Framework for Assessing Metallic Nanomaterials of Environmental Concern: Aquatic Exposure and Behavior." Risk Analysis **31**(5): 706-726.

OECD (2012). Important Issues on Risk Assessment of Manufactured Nanomaterials. Series on the Safety of Manufactured Nanomaterials.

Paik, S. Y., D. M. Zalk and P. Swuste (2008). "Application of a Pilot Control Banding Tool for Risk Level Assessment and Control of Nanoparticle Exposures." Annals of Occupational Hygiene **52**(6): 419-428.

Powers, C. M., G. Dana, P. Gillespie, M. R. Gwinn, C. O. Hendren, T. C. Long, A. Wang and J. M. Davis (2012). "Comprehensive Environmental Assessment: A Meta-Assessment Approach." Environmental Science & Technology **46**(17): 9202-9208.

Powers, C. M., K. D. Grieger, C. O. Hendren, C. A. Meacham, G. Gurevich, M. G. Lassiter, E. S. Money, J. M. Lloyd and S. M. Beaulieu (2014). "A Web-Based Tool to Engage Stakeholders in Informing Research Planning for Future Decisions on Emerging Materials." Science of the Total Environment **470**: 660-668.

Ramirez, R., M. Mukherjee, S. Vezzoli and A. M. Kramer (2015). "Scenarios as a Scholarly Methodology to Produce "Interesting Research"." Futures **71**: 70-87.

Rebitzer, G., T. Ekvall, R. Frischknecht, D. Hunkeler, G. Norris, T. Rydberg, W. Schmidt, S. Suh, B. Weidema and D. Pennington (2004). "Life Cycle Assessment Part 1: Framework, Goal and Scope Definition, Inventory Analysis, and Applications." Environment International **30**(5): 701-720.

Robichaud, C. O., D. Tanzil, U. Weilenmann and M. R. Wiesner (2005). "Relative Risk Analysis of Several Manufactured Nanomaterials: An Insurance Industry Context." Environmental Science & Technology **39**(22): 8985-8994.

Roco, M. (2011). The Long View of Nanotechnology Development: The National Nanotechnology Initiative at 10 Years. Nanotechnology Research Directions for Societal Needs in 2020, Springer Netherlands. **1:** 1-28.

Rosenbaum, R. K., T. M. Bachmann, L. S. Gold, M. A. J. Huijbregts, O. Jolliet, R. Juraske, A. Koehler, H. F. Larsen, M. MacLeod, M. Margni, T. E. McKone, J. Payet, M. Schuhmacher, D. van de Meent and M. Z. Hauschild (2008). "Usetox-the UNEP-SETAC Toxicity Model: Recommended Characterisation Factors for Human Toxicity and Freshwater Ecotoxicity in Life Cycle Impact Assessment." International Journal of Life Cycle Assessment **13**(7): 532-546.

Sass, J., L. Heine and N. Hwang (2016). "Use of a Modified Greenscreen Tool to Conduct a Screening-Level Comparative Hazard Assessment of Conventional Silver and Two Forms of Nanosilver." Environmental Health: A Global Access Science Source **15**(1): 105.

Shatkin, J. A. (2008). "Informing Environmental Decision Making by Combining Life Cycle Assessment and Risk Analysis." Journal of Industrial Ecology **12**(3): 278-281.

Shatkin, J. A. (2008). "Nanotechnology: Health and Environmental Risks." Nanotechnology: Health and Environmental Risks **2**.

Shatkin, J. A. (2013). Nanotechnology: Health and Environmental Risks, CRC Press.

Shatkin, J. A. and B. Kim (2015). "Cellulose Nanomaterials: Life Cycle Risk Assessment, and Environmental Health and Safety Roadmap." Environmental Science: Nano **2**(5): 477-499.

Som, C., M. Berges, Q. Chaudhry, M. Dusinska, T. F. Fernandes, S. I. Olsen and B. Nowack (2010). "The Importance of Life Cycle Concepts for the Development of Safe Nanoproducts." Toxicology **269**(2-3): 160-169.

Teow, Y., P. V. Asharani, M. P. Hande and S. Valiyaveettil (2011). "Health Impact and Safety of Engineered Nanomaterials." Chemical Communications (Cambridge, United Kingdom) **47**(25): 7025-7038.

Tervonen, T., R. Lahdelma, J. Almeida Dias, J. Figueira and P. Salminen (2007). Smaa-Tri. Environmental Security in Harbors and Coastal Areas: Management Using Comparative Risk Assessment and Multi-Criteria Decision Analysis. I. Linkov, G. A. Kiker and R. J. Wenning. Dordrecht, Springer Netherlands**:** 217-231.

Tervonen, T., I. Linkov, J. R. Figueira, J. Steevens, M. Chappell and M. Merad (2009). "Risk-Based Classification System of Nanomaterials." Journal of Nanoparticle Research **11**(4): 757-766.

Theis, T. L., B. R. Bakshi, D. Durham, V. M. Fthenakis, T. G. Gutowski, J. A. Isaacs, T. Seager and M. R. Wiesner (2011). "A Life Cycle Framework for the Investigation of Environmentally Benign Nanoparticles and Products." Physica Status Solidi-Rapid Research Letters **5**(9): 312-317.

Van Duuren-Stuurman, B., S. R. Vink, K. J. M. Verbist, H. G. A. Heussen, D. H. Brouwer, D. E. D. Kroese, M. F. J. Van Niftrik, E. Tielemans and W. Fransman (2012). "Stoffenmanager Nano Version 1.0: A Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects." Annals of Occupational Hygiene **56**(5): 525-541.

van Vuuren, D. P., M. T. J. Kok, B. Girod, P. L. Lucas and B. de Vries (2012). "Scenarios in Global Environmental Assessments: Key Characteristics and Lessons for Future Use." Global Environmental Change **22**(4): 884-895.

WHO (2013). Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Report of the Who Expert Meeting 10–11 December 2012. Bonn, Germany, WHO Regional Office for Europe.

Wiesner, M. R. and J.-Y. Bottero (2011). "A Risk Forecasting Process for Nanostructured Materials, and Nanomanufacturing." Comptes Rendus Physique **12**(7): 659-668.

Xia, T., N. Li and A. E. Nel (2009). "Potential Health Impact of Nanoparticles." Annual Review of Public Health **30**: 137-150.

Zalk, D. M. and D. I. Nelson (2008). "History and Evolution of Control Banding: A Review." Journal of Occupational and Environmental Hygiene **5**(5): 330-346.

Zalk, D. M., S. Y. Paik and P. Swuste (2009). "Evaluating the Control Banding Nanotool: A Qualitative Risk Assessment Method for Controlling Nanoparticle Exposures." Journal of Nanoparticle Research **11**(7): 1685-1704.

CHAPTER 3: ASSESSMENT OF INFORMATION AVAILABILITY FOR ENVIRONMENTAL IMPACT ASSESSMENT OF ENGINEERED NANOMATERIALS

(This chapter has been submitted for publication to the Journal of Nanoparticle Research)

ABSTRACT

Environmental Impact Assessments of engineered nanomaterials can be hampered by the lack of data/information and result in paralysis by analysis. To address this issue, a systematic approach (termed here "IANano") was developed and demonstrated for assessing the availability of information for EIA of engineered nanomaterials. In the proposed approach, the required information is evaluated following the typical EIA process whereby information elements for exposure and hazard potential assessments are classified based on major categories, sub-categories and attributes. Scores for the different information attributes are then assigned, based on a selected scoring scale and weights, and aggregated up to the level of exposure and hazard potential information (EPI and HPI), considering both the available and unavailable information, via the Dempster-Shafer evidential reasoning algorithm. The utility of IANano was demonstrated for several specific EIA scenarios for nano TiO_2, nano Cu-CuO and nano ZnO. For each of the three nanomaterials in each different EIA scenario, the EPI scores were lower than for the HPI scores consistent with the more abundant information available for hazard attributes. For nano TiO_2, there is significant information regarding potential exposures and correspondingly the EPI and HPI scores were more closely matched relative to nano Cu-CuO and nano ZnO. For the EIA focusing on direct release of ENMs in the aquatic environment, the EPI score for ZnO was higher than for nano Cu-CuO; reflecting greater research efforts to date on the environmental impacts of nano

ZnO. Results of the present study suggests that information screening, as illustrated via IANano, can be valuable for ranking the adequacy of the available information for conducting specific EIAs and for identifying information needs.

INTRODUCTION

Engineered nanomaterials (ENMs) are rapidly evolving and being used in a myriad of applications (Hansen et al. 2014). At the same time, concerns regarding the potential environmental and health impacts of ENMs are growing and so is the need for suitable frameworks to conduct environmental impact assessment (EIA) of these materials (Hristozov et al. 2012, Romero-Franco et al. 2017). EIA frameworks have been reviewed to assess if traditional risk assessment methodologies can be applied to ENMs (SCENIHR 2005, SCENIHR 2007, SCENIHR 2009). Other analyses have focused on how EIA frameworks can be modified for ENMs (Hristozov et al. 2012, Hristozov et al. 2016, Oomen et al. 2017, Romero-Franco et al. 2017).

Various EIA frameworks are suitable for ENMs through their lifecycle stages, depending on the EIA objectives. For example, to identify or assess hazards posed by ENMs, available frameworks include the Swiss Precautionary Matrix (SPM) (Höck J. et al. 2013) and the Risk Classification System based on Multi Criteria Decision Analysis (MCDA risk classification) (Linkov et al. 2007, Linkov et al. 2009, Tervonen et al. 2009). To conduct ecological EIA, available frameworks include the Engineered Nanoparticles - Review of Health and Environmental Safety: Human Health and Ecological Risk Assessment (ENRHES RA) (Aschberger et al. 2011) and the Risk Assessment Framework for Metallic Nanomaterials of Environmental Concern (NanoHAZ) (O'Brien and Cummins 2011). Available frameworks for conducting EIA in occupational settings include the Risk Based Classification for Occupational Exposure Control (Risk based OEL)

(Kuempel et al. 2012) and Risk Classification based on an Industry Insurance Protocol (RCIP) (Robichaud et al. 2005)). Specific case studies of the application of currently available EIA frameworks include: the application of a risk assessment methodology (ENRHES-RA) to assess environmental and human health risks of four different ENMs (nano TiO_2, nano ZnO, fullerenes and Carbon Nanotubes (CNTs)) based on publicly available literature under methodology guidelines established by the European REACH (Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals) program (Aschberger et al. 2011); the application of NanoHAZ to assess ecological and human health risks of Metallic ENMs (nano TiO_2, nano Ag and nano CeO_2) in aquatic environments (O'Brien and Cummins 2011); and the application of a Risk based OEL to assess occupational risks posed by nano TiO_2, Fullerene and CNTs (Kuempel et al. 2012).

Despite the availability of various EIA frameworks for different scenarios and ENMs, their utilization is often hampered by the lack of data/information. Moreover, in the typical EIA approach, the specific information/data that is missing and its impact on the analyses is generally identified after the assessment has been completed. For instance, in the application of the ENRHES-RA framework for a case study involving nano TiO_2, nano ZnO, fullerenes and Carbon Nanotubes (CNTs), information gaps (i.e., use, exposure and risk management measures in place) limited the study's conclusions regarding potential risks (Aschberger et al. 2011). As reported by the authors, the nature of available quantitative information of exposure limited the assessment to qualitative findings (e.g., the main environmental risk, specifically for algae and *Daphnia*, was likely to be due to nano ZnO release, and the main risk to human health was related to chronic inhalation exposure to nano TiO_2). The above study noted that these qualitative results should not be used for regulatory decision making (Aschberger et al. 2011). In the case of the application of

nano HAZ, limited information regarding model inputs (e.g., quantitative data of ENM release to the environment from consumer products) used for estimating exposure values meant that the authors needed to rely on expert knowledge. The resulting estimations did not allow for a quantitative risk assessment to be made (O'Brien and Cummins 2011). Instead, the results were casted into qualitative categories of exposure concern (ranging from very low, low, medium, high to very high). In the application of Risk Based OEL (Kuempel et al. 2012), lack of information regarding sub-chronic toxic effect of nano TiO_2 led the authors to assume that data for ultrafine particles can be used as equivalent for ENMs. The authors suggested that while cautionary occupational exposure levels can be derived in this approach, they should be considered an initial step in the EIA until more precise data can be obtained (Kuempel et al. 2012).

The lack of data/information affecting quantitative EIA can result in "paralysis by analysis" (Maynard and Aitken 2016) and hence slow progress in establishing suitable regulations (Hansen and Baun 2012). EIAs for specific ENMs have been limited by various factors including: conflicting conclusions regarding toxicity (e.g., studies performed without standardized protocols) (Hristozov et al. 2014); studies that provide only partial information regarding study conditions, ENM characterization (Boverhof and David 2010), and lacking or highly approximate data and estimates of ENM releases (Nowack et al. 2015); and exposure values for various routes of exposure in human health risk assessment (Aschberger et al. 2011). These data gaps can introduce uncertainties and thus presents a major challenge to conducting a quantitative EIA.

The above examples suggest that EIA analysts would benefit from being able to evaluate the adequacy of the body of information pertinent to conducting an EIA, during the problem formulation stage. It is noted that the problem formulation stage is critical to the success of an EIA, given that, if conducted poorly, it may lead to insufficient clarity regarding the purpose and the

use of data being collected (EPA 2004). The problem formulation stage of an EIA typically entails identifying relevant exposure scenarios for each step of the ENM life cycle and identifying potential data gaps related to the EIA objectives. This is usually accomplished via an iterative process in which one determines what data are relevant to the exposure scenario of interest (Bos et al. 2015). A more efficient alternative would be to assess the adequacy of the available information for carrying out the EIA at the beginning of the problem formulation stage with the aim of also determining the potential influence of the lack of information on the desired EIA objectives. A major distinction to be made here is that assessing the body of information at the problem formulation stage is different from applying a scoring method to assess the weight of evidence (WOE) (Hristozov et al. 2014). It is noted that the WOE scoring method provides a weighted average score given to hazard studies to draw a conclusion on potential effects of ENMs (Hristozov et al. 2014) as an approximate approach to estimate potential risks of ENMs.

Furthermore, assessing the body of information needed for EIA of ENMs (nano-EIA), in the early phase of problem formulation, could be greatly facilitated by the development of approaches deployed as Decision Support Tools (DSTs) (i.e., computer-based information system that utilize decision rules and models). In the area of ENMs, a theoretical decision support system has been proposed as a computer system consisting of data sources, knowledge rules, vocabularies and a user interface (Marvin et al. 2013). In this system, it is envisioned that input information could be extracted via text mining and manual data curation from international databases (e.g., Woodrow Wilson, OECD, NanoHub) and academic literature. Such information (e.g., ENM properties and toxicological data (in vitro, in vivo)), can be processed by knowledge rules (established by expert judgment) and logic reasoning to rank ENMs of concern and identify research priorities (Marvin

et al. 2013). Case studies based on the above decision support system are yet unavailable, but the utility of the approach could prove useful once casted as a software application for rapid analysis.

Given the lack of specific approaches for early assessment of the body of information for nano-EIA this work presents an approach, which can be deployed as a DST, that provides the following functions: (i) organization of EIA relevant information into categories and sub-categories in a decision tree pattern suitable for EIA, (ii) representation of the adequacy of information in terms of scores that quantify the extent of both available and missing information, and (iii) aggregation of the information scores to arrive at an assessment of the adequacy of the available information for conducting EIA for ENMs. This approach follows a structured process that mirrors that of quantitative risk assessment to guide the evaluation of the adequacy of the available body of information for conducting various scenario specific EIAs. The approach of information assessment for nanomaterials ("IANano") is presented and demonstrated for three ENMs (nano Cu-CuO, nano ZnO and nano TiO_2). IANano is powered by the Dempster-Schafer evidential reasoning algorithm that serves to arrive at an information score that quantifies the adequacy of the information/data available in the published literature for conducting an EIA.

METHODS

Methods Section 1. The IANano Approach

The overall methodology for assessing the adequacy of available information for conducting EIA for ENMs (IANano) was developed and demonstrated following the workflow shown in **Figure 3.1**. In this approach an EIA framework was first selected as a guide to determine the basic information elements required for the EIA. Accordingly, the information is assessed with respect

to both hazard and exposure information as per various categories, sub-categories and attributes (**Figure 3.2**, **Methods Sections 2** and **3** and **Tables 3.1** and **3.2**). Once the EIA scenario of interest is established, weights are assigned to the different information elements. The scores are then aggregated, using the Dempster-Shafer algorithm (Shafer 1992, Yang and Xu 2002) **(Methods Section 3)** which considers the influence of both available and unavailable information, to arrive at the resulting overall scores for exposure and hazard potentials.

To illustrate the IANano approach, a baseline general scenario (Scenario I) was first evaluated for a number of ENMs (nano Cu-CuO, nano TiO_2 and nano ZnO) (Note: the scenarios used in this chapter do not correspond to those presented in **Chapter 2**). In Scenario I equally distributed weights (e.g., significance) were considered for all categories, sub-categories and attributes within their respective groups. This scenario considers the totality of the available information for these ENMs without discerning the adequacy of information (e.g., target receptors, regions, exposure in specific media, etc.) for any particular scenario. As a result, this scenario could provide an overly optimistic assessment of the availability of information since it is not rooted in a specific context. Subsequently, the adequacy of information was evaluated for a number of specific EIA scenarios (for the same three ENMs) and then compared with the general scenario.

Methods Section 2. Framework for Compilation of Information for EIA

EIAs often require the extraction of information/data from complex and heterogeneous datasets. As a first step, the available body of information must be organized into a structured format that mimics the EIA process. In this regard, existing EIA frameworks (Romero-Franco et al. 2017) and the general schemes reported by the OECD Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) (SCENIHR 2005, SCENIHR 2009) were selected as the foundation for establishing the EIA elements as described graphically in **Figure 3.2** with

additional details provided in **Tables 3.1** and **3.2**. The resulting information tree (**Figure 3.2**), which was constructed based on domain/expert knowledge, provides a convenient graphical representation of the information hierarchy that follows the EIA process. In this approach, the first EIA step/element for a selected ENM involves screening for *exposure potential information*, followed by *hazard potential information,* and ultimately arriving at a suitable measure (or metric) of environmental impacts (Hristozov et al. 2012, Oomen et al. 2017, Romero-Franco et al. 2017). For a given ENM, the information required for ENM identification, exposure and hazard potential information, depicted in **Figure 3.2** *(a), (b)* and *(c)*, is subdivided into multiple categories (designated by the several dash containers in **Figure 3.2** as provided in **Tables 3.1** and **3.2**.

The analysts begin by identifying the ENM for which they wish to assess the adequacy of information. Next, the information availability for exposure potential (items under group *b* in **Figure 3.2**) is assessed by considering the following information categories:

(i) *ENM characteristics* (e.g., *state of the ENM in the matrix/product* and the *state in which the ENM is released from the matrix/product*);

(ii) *Fate and Transport* (e.g., *life cycle stage*, where the release occurs, *environmental compartment* where release occurs or for which ENM concentration measures are available, and information regarding *geography* and *meteorology* for a location of interest); and

(iii) *Exposure scenarios* (e.g., *exposure conditions* and *exposure receptors*). The category of *ENM characteristics* includes information about the *physical state of the ENM in the matrix/product* and the *form of the released ENM*.

The *Fate and Transport* category includes information sub-categories regarding the *ENM life cycle stage* of the release and the *environmental media compartment* for the ENM release that is

pertinent for quantifying the ENM concentrations in the media of interest. The *exposure conditions* category includes sub-categories of *exposure scenarios* and *potential receptors* of concern. The information for the categories under the *hazard potential* (group *c* in **Figure 3.2**) are:

(i) *in vivo toxicity;*

(ii) *in vitro toxicity;* and

(iii) *in silico toxicity*.

The *in vivo toxicity* sub-categories refer to information related to *human health* and *ecological outcomes*. The *in vitro toxicity* sub-categories include information on *human health* and *other outcomes* (e.g., bactericidal effects), while the *in silico toxicity* category includes *modeling/quantitative structure activity relationships QSARs, toxicokinetics and information from domain knowledge/expert elicitation*.

In the present approach, a category and its sub-categories consist of units of data/information (e.g., documented publications/peer review publications), supporting specific constituents of hazard or exposure potential for the considered ENMs. The sub-categories in turn contain attributes that identify the type of data/information needed under each of the sub-categories. Additional details regarding the attributes of the different sub-categories of the information relevant for exposure and hazard potentials are provided in **Tables 3.1** and **3.2.** It is emphasized that the categories, sub-categories and their attributes suggested herein could be further refined and/or expanded as additional information becomes available regarding exposure and hazard quantification/assessment and/or depending upon the analyst's specific assessment goals.

Methods Section 3. Scoring Methodology for Quantifying the Adequacy of Available Information for EIA

The path of information compilation and assessment, as arranged into a layered tree structure (**Figure 3.2**), consists of *categories* (high-level abstract information), *sub-categories* and *attributes* (i.e., parameters with quantifiable metrics). Attributes are under the different sub-categories (**Tables 3.1** and **2**) at the bottom layer (not shown in **Figure 3.2** for clarity of presentation). The process of quantifying the adequacy of information follows the above information path, where scores are first assigned to attributes based on the level of information availability. Each attribute *i*, **(Tables 3.1** and **3.2)** is assigned a score ($\beta_{n,i}$) to represent the level of information availability grade designated by the information grade index *n.* It is note that in the present work only the grades of information availability (n=1) and unavailability (n=2) were utilized in the presented the example EIA scenarios. The scores are aggregated for each sub-category and these are further aggregated under each of the (parent) categories as per the Dempster-Shafer (DS) algorithm (Yang and Xu 2002).

It is important to note that a variety of methods for score aggregation (e.g., linear and non-linear addition, weighted average (Hristozov et al. 2014)) only account for the available. Aggregation of scores should consider the impact of missing information (which itself introduces uncertainty) to allow for proper compensation among attributes so that an attribute can play the appropriate role relative to its weight (Yang and Xu 2002). Aggregation of the attribute scores via the Dempster-Shafer (DS) algorithm fulfills the above objective. The DS evidential reasoning algorithm follows a hierarchical tree structure for the various EIA information elements, whereby the impacts of both the available and unavailable information are considered in the determination of the aggregated scores (**Section C1** of **Appendix C**). It is also noted that DS reduces complexity

by grouping together only those mutually exclusive attributes that have intrinsic relationships/grouped under the same branch (i.e., breaking of a complex problem into smaller constituent parts and subsequently guiding the decision analyst through a series of pairwise aggregation of judgements) (Yang and Xu 2002).

Once the scores are assigned, the Dempster-Shafer (DS) algorithm (**Section C1** of **Appendix C**) was used to arrive at an aggregated score making use of the metrics that represent the availability and unavailability of information (i.e., $m_{n,i} = \beta_{n,i} \times \omega_i$ and $1 - m_{n,i}$ respectively, where ω_i and $m_{n,i}$ are the weight and metric representing the availability of information for attribute i, respectively). In a similar fashion, updated scores are obtained sequentially at the higher levels of sub-categories and then the categories and ultimately for the overall score of information availability for exposure and hazard potentials, namely the exposure potential information (EPI) and hazard potential information (HPI) scores.

In principle, the scoring scale for $\beta_{n,i}$ can be established by the analyst to emphasize the importance of the information elements (**Figure 3.2**) consistent with the specific goals of the EIA scenario. In the present work, as an illustration of the overall approach two different scoring scales are presented both having scores ($\beta_{n,i}$) in the range of [0,1]. The first scale was a simple scoring rule whereby $\beta_{n,i}$ scores of 0.25, 05, 0.75 and 1 were assigned for a given information attribute when the documented literature consisted of only one publication, at least two but less than five publications, five or less than ten publications, and more than ten publications, respectively. The second scoring approach was based on a logarithmic scale (**Section C2** of **Appendix C**), whereby $\beta_{n,i}$ scores of 0.2, 0.3, 0.6, 0.7, 0.85, 0.95 and 1 were assigned for a given information attribute that was supported by one publication, two to three publications, four to five publications, between six and nine publications, more than ten and less than fifteen publications, sixteen to twenty

publications, and more than twenty one publications, respectively. It is noted that the log scoring scale places greater significance on the need for a larger number of papers to attain a score of unity (i.e., complete adequacy of information availability). In contrast, in the linear scoring scale totally adequate information is attained at or above 10 publications. The differences in the EPI and HPI scores attained by the above scoring scales are presented for different EIA Scenarios in a number of EIA scenarios see **Methods Section 4** and **Results and Discussion**.

Once the information is compiled and scores are assigned to the different attributes, aggregation of scores is accomplished using the DS algorithm with the weight factors (ω_i) assigned to the different information nodes (i.e., attributes, sub-categories and categories. In principle, the weights can be assigned by the analyst to reflect the importance of certain elements aim (e.g., the significance of the body of evidence from toxicity assays being in the decreasing order of *in vivo*>*in vitro* > *in silico* (Council 2009, Krewski et al. 2010)) and/or the specific EIA scenario and its aim. It is noted that the process presented here for scoring peer-review publications, as well as the steps taken to assess the quality of such publications, can serve as a first step/semi-quantitative rapid screening to assess the availability of information for specific ENMs. This approach should not be considered as an assessment of the ENMs quantitative datasets or the potential exposure/hazards.

Methods Section 4. IANano Case studies

Six EIA scenarios were developed to illustrate the IANano approach for nano Cu-CuO, nano ZnO and nano TiO_2 ENMs. These ENMs were selected given their widespread applications in commercial products and various technology applications, and their estimated high global levels of production (78,000 metric tons (nano TiO_2), 34,000 metric tons (nano ZnO), and 200 metric tons (nano Cu-CuO) were produced in 2010 (Keller et al. 2013; Keller et al. 2014)). Scenario I

(detailed in **Methods Section 1**) was set as a general EIA with equal weights for the information elements under each branch of the information compilation tree (**Figure 3.2**). The five additional EIA scenarios were for the following specific conditions: (II) release of nano Cu-CuO into aquatic environments; (III) release of nano ZnO to the aquatic environment; (IV) release of nano Cu-CuO from wastewater treatment plants to soil and water; (V) human inhalation exposure associated with release of nano TiO_2 into air; and (VI) human exposure to nano TiO_2 from consumer products use. For the above scenarios, the relevant number of publications for each of the sub-categories under the exposure and hazard potential information categories provided in **Figure 3.3**. The assigned scores (following the scoring scales described in **Methods Section 3**) for the various scenario attributes are listed in **Tables C15-18** in **Appendix C**.

Compilation of the available information for conducting EIA for the above ENMs followed a literature search of peer-reviewed publications via available search engines (e.g., PubMed, Web of Science, and Google Scholar). For the present study, the published literature was assessed for the period up to and including 2016, but the methodology presented is applicable to any period without a loss of generality. The collected publications were screened following general criteria adapted from Oh et al (Oh et al. 2016) as detiled in **Table C2** and **Figure C2** in **Appendix C**. Briefly, each publication was reviewed with respect to the availability and adequacy of information and data provided regarding the following elements:

(a) ENM's physicochemical properties (i.e., physicochemical characterization);

(b) Experimental conditions (e.g., experiment type such as in vitro/in vivo and target organism/animal model and/or modeling approach);

(c) Exposure concentrations, dosage or ENM delivery information; and

(d) Quantifiable metrics of ENM hazard/toxicity.

Subsequent to the above analysis, the identified publications and number of information sources (publications) for each attribute were recorded for use in the scoring process (**Methods Section 2**). Following the above criteria, 256 publications (for the three ENMs) were identified as suitable for the selected EIA scenarios. It is noted that for nano Cu-CuO, 28 articles were identified as relevant for exposure potential information, and 70 articles were identified for hazard potential information. For nano TiO_2, 48 and 62 articles were found to contain suitable information for exposure and hazard potentials, respectively. Finally, for nano ZnO, 27 and 70 publications were identified to contain information for assessment of exposure and hazard potentials, respectively. Details regarding each of the identified publications and their classifications according to the information attributes needed for EIA are provided in **Tables C19-C24** (**Appendix C**).

RESULTS AND DISCUSSION

General EIA (Scenario I)

Evaluation of the adequacy of the available information for conducting EIA (**Methods Section 1**) was first performed for the general (baseline) Scenario I for three ENMs (nano Cu-CuO, nano TiO_2 and nano ZnO) (**Figure 3.4**). In this scenario, all weight factors were set to be equal (for categories and sub-categories belonging to a branch unit). The resulting (aggregate) scores for hazard and exposure potential information (HPI and EPI), based on both the log and (simple linear) scoring scale(s) (**Methods Section 3**), were in the range of 0.42-0.56 (0.55-0.64) and 0.15-0.54 (0.26-0.64), respectively for the three ENMs (**Figure 3.4**, **Table 3.4**). The hazard and exposure potential scores were approximately 13-24% and 35-15% lower, respectively, when determined based on the log scoring scale compared to the linear scoring scale. While the linear scoring scale assigns a measurable information score even for a small number of publications (e.g., 0.25 and 0.5

for one and two publications, respectively), the log scoring scale is more conservative, as it assigns a lower score for attributes with small number of publications (e.g., 0.17 and 0.3 for the cases of one and two publications, respectively). The simple linear scoring scale, which establishes a score of unity for attributes supported by 10 or more publications, is less conservative than the log scoring scale which was also set with the requirement of 25 publications to reach a score of unity.

For all three ENMs the HPI scores for Scenario I (0.42-0.56 and 0.55-0.64 based on the log and the linear scoring scales, respectively) were higher than the corresponding exposure potential information score. The higher level of information availability for the hazard potential of these materials is not surprising given that over the last decade, significant effort has been devoted to hazard assessment of ENMs (Cohen et al. 2012, Xia et al. 2013, Holden et al. 2016, Maynard and Aitken 2016) supported by funding from federal agencies such as the U.S. EPA and the National Science and Technology Council (NSTC) (NRC 2003, NSET 2006, Xia et al. 2013). In addition, the United States (e.g., through the 21st Century Nanotechnology Research and Development Act (U.S.C. 2003)) has explicitly encouraged research on the potential environmental and health impacts of ENMs. In Europe, funding allocated to environmental health and safety associated with ENMs (i.e., Nano EH&S) has also increased by about 25% over the period of time from 2006 through 2016 (Horizon 2020) (Maynard and Aitken 2016).

The very low exposure potential information (EPI) scores for nano ZnO and nano Cu-CuO (0.19 and 0.15, respectively) compared to nano TiO_2 (0.54) reflect the relative importance of workplace exposures for nano TiO_2. Studies on nano TiO_2 workplace exposure have been driven, in part, by efforts from regulatory agencies to establish safe occupational exposure limits (Schulte et al. 2016). The above has been, in part, due to evidence of adverse health effects in workers triggered by inhalation exposure to particulate matter (ultrafine particles) (Kuempel et al. 2012,

Kuempel et al. 2012, Schulte et al. 2016). Occupational studies are by their nature on a smaller geographical scale relative to environmental exposure studies. The latter can be of a significantly higher cost given the need for quantifying ENM releases from all pertinent sources, and acquire provide multimedia exposure data (Cohen et al. 2012, Hendren et al. 2013, Liu and Cohen 2014, Koivisto et al. 2017, Kuhlbusch et al. 2017). At present there is a limited capability to quantify the environmental concentrations of ENMs that is distinguishable from the levels of naturally occurring nanoparticles (Liu and Cohen 2014, Nowack et al. 2015). Moreover, exposure modeling studies have been limited, in part, due to lack of specific regulatory demand (Nowack 2017) as well as lack of field data for model validation (Liu and Cohen 2014, Liu et al. 2015, Nowack et al. 2015, Nowack 2017).

For the general EIA Scenario (I), the ENMs' EPI score in the range of 0.54-0.64 for nano TiO_2 (considering both the log and simple linear scoring scales) was greater by factors of 2.8-2.0 and 3.6-2.5 than for nano ZnO (0.19 and 0.34) and nano Cu-CuO (0.15 and 0.26), respectively. Although all of the above ENMs are used in numerous applications (Nowack et al. 2013), it is likely that the higher exposure potential information for nano TiO_2 may be, in part, due to the large global scale production of TiO_2 which has been reported to be about ~40,000 tons/yr compared to a significantly lower production level of ~5,000 and ~200 tons/yr for nano ZnO and nano Cu-CuO respectively (Keller et al. 2013). It is emphasized that exposure studies pertaining to nano TiO_2 ENMs have had a higher focus on workplace exposure given that (a) inhalation is a primary exposure pathway for particulate matter in industrial facilities and to potential pulmonary effects (NIOSH 2011, Shi et al. 2013), and (b) that there are existing regulations regarding occupational exposure to ultrafine particles (Kuempel et al. 2012). In contrast, exposure studies for nano Cu-CuO and nano ZnO ENMs, have focused on model estimation of exposure concentrations in

various environmental media (Nowack 2017), with a greater focus on the aquatic environment, given their higher aqueous solubility (and possibly greater potential release (or entry) to aquatic bodies) relative to nano TiO_2 (Girigoswami 2018).

For the general EIA (i.e., Scenario I), the HPI and EPI scores do not reflect the adequacy of information for conducting a specific EIA scenario where there may be an interest to consider specific exposure conditions or exposure targets. Specific EIA scenarios require information for the relevant attributes that are specific to the intended EIA scenario. Therefore, when evaluating the adequacy of information for specific EIA scenarios (e.g., Scenarios II-VI, **Methods Section 4**) only the relevant attributes and/or sub-categories for these scenarios should be considered. In other words, attributes that are not needed for the target EIA scenario are excluded from the analysis (i.e., assigned a weight of 0) (**Tables C3-C12** in the **Appendix C**).

Scenarios II.A and II.B: Environmental release of nano Cu-CuO into aquatic environments

The release of nano ZnO and nano Cu-CuO into water has been reported to be a considerable fraction of the global production of these ENMs (Keller and Lazareva 2013, Keller et al. 2013). Both nano Cu-CuO and nano ZnO are used in various commercial applications (Wang et al. 2015). Once released to aquatic environments these ENMs are may adversely affect various aquatic species (Garner and Keller 2014, Adam et al. 2015, Amde et al. 2017). Thus, Scenario II.A focuses on the cumulative direct release of nano Cu from all commercial products and applications into the water compartment and the associated potential impact on ecological receptors. Accordingly, only the relevant categories/subcategories and attributes relevant to this scenario's conditions of exposure were considered, while those not required for this scenario were excluded (**Tables C5** and **C6** in **Appendix C**). For example, since information regarding exposure in air and soil is not

required for Scenario II, the attributes pertaining to release to *air* and *soil* (under the sub-category of *compartment where release occurs* were each assigned a weight of zero (i.e. $\omega = 0$).

Following the assignment of weights and information scores for Scenario II.A (**Tables C5** and **C6** in **Appendix C**), the EPI scores (shown in **Figure 3.5)**, derived based on both the log and simple linear scoring scales, were found to be greater by a factor of ~1.5, than for the general EIA (Scenario I). This observation is not surprising given that for this scenario there is a greater likelihood that nano sized Cu will be released to the aquatic environment than to the other environmental compartments. The release of nano Cu to the aquatic environment is due to its commercial applications, which include, marine antifouling paints and agriculture (pesticides) and personal care products (Adeleye et al. 2016); thus, environmental concerns about impacts to aquatic (freshwater and marine) ecosystems have driven exposure studies on these media (Bondarenko et al. 2013, Keller et al. 2017).

It is noted that for nano Cu-CuO the HPI score in Scenario II.A was a factor of ~2 times greater than for its EPI score. The above reflects the larger body of information available regarding hazard (36 publications) than for exposure (20 publications). Information for the attributes nested under the sub-categories *in vivo toxicity for ecological outcomes* and *in vitro toxicity other outcomes* (**Table C14** in **Appendix C**) for Scenario II.A for nano Cu-CuO was available from 24 and 12 publications, respectively. The higher level of information availability regarding hazard relevant to the aquatic environment likely reflects a greater emphasis on aquatic species (relative to terrestrial species) by the nanoecotoxicology research community (Kahru and Ivask 2013) and regulatory agencies (Koelmans et al. 2015). This in turn reflects EU REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) requirements for testing of chemicals and ENMs in aquatic model organisms (freshwater and marine) (Kahru and Ivask 2013, Koelmans

et al. 2015). Also, in both the U.S. and EU, there are regulatory measures that establish acceptable/maximum contaminant concentrations, for protecting aquatic ecosystems and their functions (Kahru and Ivask 2013, Koelmans et al. 2015).

Exploration of a subset of Scenario II (Scenario II.B), focusing on nano Cu associated with antifouling paints, revealed an EPI score of 0.13-0.24 (for the log and simple linear scoring scales) that was lower by ~ 7-13% relative to the general Scenario I. For the simple linear scoring scale, the EPI score in Scenario II.B is the same as for Scenario I because the number of publications (2) regarding the release of nano Cu from antifouling paints into water is assigned the same score as the total for all sources/applications. The relatively small number of studies pertaining to nano Cu used in antifouling paints is surprising given that the annual global market for marine antifouling paints is ~0.5 million metric tons per year (Keller et al. 2017) and where the active ingredient can contain 20-76% Cu (Schiff et al. 2004). The HPI score for nano Cu in Scenario II.B, although higher than the exposure potential information score, was lower by about ~35% (based on both scoring scales) relative to the case of nano Cu release to the aquatic environment from all sources (Scenario II.A). Here we note that studies have reported increased toxicity with decreased copper particle size (Kiaune and Singhasemanon 2011), which has been attributed, part, to the associated increased rate of dissolution and consequently greater bioavailability (Kiaune and Singhasemanon 2011), hence the low number of studies available for the attributes under the sub-category *ENM state as released from product/application*. Furthermore, while there is a greater level of information regarding exposure and toxicity of Cu associated with antifouling paints, it is unclear if and how such information could be adopted for assessing exposure and hazard associated with nano sized Cu. It is noted that the U.S. EPA regulates the application of copper and copper compounds in antifouling paints within the pesticide regulatory framework in accordance with the

regulatory definition (U.S.C. 2011) that "…any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insect, rodent, nematode, fungus, weed, or any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism…". In fact, the U.S. EPA has the authority under the Federal Insecticide Fungicide, and Rodenticide Act (FIFRA) to require risk assessment for antifouling paints containing nano Cu for registration review (EPA 2017). It is conceivable that the increased commercial interest in antifouling paints and the regulatory mandate given to the U.S. EPA will drive expansion of the knowledgebase regarding exposure and hazard associated with nano Cu in antifouling paints.

Scenario III: Release of nano ZnO to the Aquatic Environment

Scenario III in which nano ZnO is released, from all products and applications, into the aquatic environment had an HPI score (0.31-0.41) lower than for overall Scenario I (0.52-0.70) based on both scoring scales. The lower HPI score for Scenario III reflects the emphasis in the research community on ecological hazard (22 publications) while EIA under Scenario I includes both human and ecological hazards (81 publications). We note, however, that over the past decade, there have been a growing number of studies that focus on establishing quantitative relationships between the physicochemical characteristics and toxicity of ZnO ENMs to organisms in seawater (Yung et al. 2014, Minetto et al. 2016, Hou et al. 2018) and freshwater (He et al. 2014, Vale et al. 2016, Hou et al. 2018).

In contrast to the HPI score, the EPI score (based on both scoring scales) for Scenario III (0.22-0.39) was somewhat higher than for Scenario I (0.18-0.30). The above results are attributed to the exclusion from scenario III of *soil* and *air* attributes from the *environmental compartments where release occurs* sub-category and greater emphasis on the *water* attribute. It appears that, relative

to the soil and air media, there has been greater research emphasis on the release from industrial and wastewater (Gottschalk et al. 2009, Liu and Cohen 2014, Beegam et al. 2016)) and fate and transport of ZnO ENMs in the aquatic environment (Gottschalk et al. 2009, Liu and Cohen 2014). The above disparity could be, in part, due to the concern regarding the greater mobility of zinc ions upon dissolution of nano ZnO (Liu and Cohen 2014).

Scenario IV: Environmental release of nano Cu-CuO from wastewater treatment plants to soil and water

The focus of Scenario IV (**Tables C6** and **C7** in **Appendix C**) is on the release of nano Cu-CuO from wastewater treatment plants (WWTP), given that these facilities can be a relevant pathway for the entry (and thus potential exposures) of nano Cu-CuO to terrestrial and aquatic ecosystems (Brar et al. 2010, Lazareva and Keller 2014, Liu and Cohen 2014). ENMs released from different household and industrial applications are expected to be transported to WWTP and thus are likely to be found in wastewater sludge that may in certain cases be applied to soil (Brar et al. 2010). In the present example scenario, only direct release of nano Cu to environmental water and soil compartments associated with product use was considered (i.e., life cycle stage where release occurs). For Scenario IV, the log and simple linear scoring scales led to EPI scores (for nano Cu-CuO) in the range of 0.19-0.31), which were 21-16% higher compared to Scenario I (0.15-0.26). Similarly, the HPI scores based on the log and simple linear scoring scales were in the range of 0.44-0.60, which was about 5-9% higher than those of Scenario I. The higher HPI and EPI cores relative to Scenario I are consistent with the fact that only information for attributes relevant to the specific scenario were required and that a measurable level of such specific information was available for Scenario IV. For example, estimates nano Cu release via sludge,

wastewater and estimates of exposure concentrations have been provided in various studies (Gottschalk and Nowack 2011, Liu and Cohen 2014, Lazareva and Keller 2014). Also, hazard information has been provided in various studies regarding toxicity of nano Cu relevant to the aquatic (Wang et al. 2016) and soil (Zuverza-Mena et al. 2017) environments.

Scenarios V.A and V.B: Human Inhalation Exposure associated with release of nano TiO_2 into air

Scenarios V.A and V.B were selected as examples that focus on human health risks related to release of nano TiO_2 into air and potential resulting exposures. The focus of Scenario V.A is on environmental (outdoor) exposures while that of Scenario V.B is on occupational settings. Nano TiO_2 was selected as the ENM of interest because of its high-volume production (Gottschalk and Nowack 2011, Keller and Lazareva 2013, Keller et al. 2013, Sun et al. 2014) and given the potential inhalation toxicity. In Scenario V.A (**Tables C8** and **C9** in **Appendix C**), the ENM release was considered to occur only during product use into air and where the ENM was released as airborne material. Scenario V.A considers exposure solely due to inhalation; thus, only studies that reported inhalation as a pathway of exposure were considered. In Scenario V.A, the EPI score for nano TiO_2 (0.33-0.45) was lower relative to Scenario I (0.54-0.64) when compared based on both the log and simple linear scoring scales (**Figure 3.6**). The hazard potential information score was also lower (0.53-0.53) than for Scenario I (0.56-0.58). It is noted that for the analysis of Scenario V.A, only studies with inhalation as a primary route of exposure were selected. There was a relatively small the number of publications (only 6 of 21 studies involved inhalation exposure of a model organism) for assigning attribute scores under the sub-category *in vivo toxicity for human health outcomes.*

The focus of Scenario V.B is on human health risk assessment related to occupational (worker) exposure to nano TiO_2 in enclosed manufacturing facilities, given that it is critical to protect worker's health in ENMs manufacturing activities (Lee et al. 2011, Vaquero et al. 2015, Spinazzè et al. 2016). Scenario V.B considers only direct release of nano TiO_2 into air as airborne particulate material from manufacturing activities. The EPI score for nano TiO_2 in Scenario V.B (0.50-0.66) was higher by ~4% and ~34-32% relative to Scenarios I and V.A, respectively. Inspection of the availability of information (**Figure 3.3**) for the different attributes revealed that the majority of available information regarding human exposure (as of 2016) was primarily for occupational exposures (12 publications) with fewer studies on population (outdoor) exposures (8 publications). The attributes with the lowest number of available publications (≤ 2) (**Figure 3.3)** were in the sub-category of *ENM characteristics*: *ENM state as released from a matrix/product* as *heteroaggregates* and *ENM state as applied a matrix/product* as *airborne*.

Scenario VI: Human exposure to nano TiO_2 from consumer products use

Consumers' exposure to nano-containing products could occur as the result of product use/misuse and/or disposal (Gottschalk and Nowack 2011, Mackevica and Foss Hansen 2016). Upon release of nano TiO_2 from a consumer product, exposures may be via inhalation (Tsuji et al. 2009, Nazarenko et al. 2011), dermal contact, oral intake, ingestion or mouthing (primarily for children). In this scenario, only exposure due to direct use of consumer products was considered (i.e., the attributes related to environmental exposures in air, water and soil were not included) as described in **Tables C10** and **C11** in **Appendix C**. The EPI and HPI scores (considering both the log and simple linear scoring scales) were in the range of 0.58-0.72 and 0.74-0.77, respectively, which was greater by about 7-12% and ~25% relative to Scenario I. Clearly, when the EIA scenario

is more specific, more information is required for particular attributes and if such information is unavailable there is a greater impact on the information score (**Figure 3.3**).

The higher information scores for the above specific EIA scenario are largely due to the body of information available reporting the application of nano TiO_2 in consumer products (e.g., suites of analytical methods and tools to monitor TiO_2 ENMs released from a variety of products, including printing toners (Pirela et al. 2015), paints (Al-Kattan et al. 2013), building materials (Shandilya et al. 2015), and food and personal care products (Weir et al. 2012)), as well as studies focusing on human exposure (e.g., modelled potential exposure related to product use (Lorenz et al. 2011)) (**Figure 3.3)**. For example, inspection of the sub-categories *ENM characteristics, lifecycle stage where release occurs* and *exposure conditions (consumer product)* indicate that there is more information for nano TiO_2 (total of 12 publications) compared to nano Cu-CuO and nano ZnO (4 and 7 publications, respectively).

Acceptance or rejection of aggregate scores

It is emphasized that while the approach of aggregating the scores of attributes and sub-categories accounts for both the available and missing (or unavailable) information (**Methods Section 3**), the assignment of weights and scoring scales has to be established by the analyst consistent with the aim of the target EIA and its purpose. For the case studies presented herein, the weights of sub-categories under a given category and attributes under a given sub-category were equally distributed. For the specific scenarios, categories and attributes that were not relevant to the scenario were assigned a score of zero. Scores for specific attributes were then assigned based on scales based on the number of publications that information regarding the scenario specific attributes.

In this regard, the EIA analyst has to recognize that thresholds for exposure and hazard information scores set for acceptance of the information availability for conducting an EIA will be governed by both the attributes for which information is available and their scores (e.g., related to the number of publications). As an example, it is instructive to consider the following cases based on the log scoring scale: (a) information for the attributes under each sub-category is available for all attributes, and (b) unavailable for one attribute or for two attributes. The latter scenario implies that no attribute information is available under a subcategory that has two or less attributes. For this illustrative example, the aggregate scores were determined as a function of the number of available publications per attribute but assuming these to be identical for all attributes for which information was available. As shown in **Figure 3.7 (a)** for the analysis of the general EIA (Scenario I), the EPI score increases as information becomes available for more attributes. Also, a drive toward a higher EPI for acceptance of the body of information for conducting the EIA will arise when the analyst establishes a higher threshold for the number of publications that would be accepted as necessary to establish a reasonable weight of evidence for the individual attributes. For example, when the required minimum body of evidence is set to 4-5 publications, the EPI scores for the cases of two, one and no missing attributes (for each sub-category) are 0.06, 0.27 and 0.67, respectively. As one demands a higher weight of evidence by requiring at least 10-15 studies per attribute, the EPI scores increase correspondingly to 0.1, 0.55 and 0.94. Clearly as more information becomes available, there will be greater confidence in the ability to conduct a meaningful EIA. However, one could argue that reasonable EIA could be conducted even with partial information (e.g., EPI~0.5) depending on the level or implications of the decisions that are to be derived from such analysis.

It should be recognized that unavailability of information is not equally distributed among the various EIA categories (**Tables 3.1** and **3.2** and **Figure 3.2**) and one may be confronted with an entire block of missing information. As an illustration, the EPI scores are shown in **Figure 3.7 (b)** for the following cases were information was considered missing for the last 9 or 4 attributes (~47% and ~21%, respectively, of attribute information availability) and when information is available for all attributes. The EPI scores when information is unavailable primarily from the fate and transport and exposure blocks (9 missing attributes) and exclusively from the exposure block (4 missing attributes) were higher (10%-75%) compared to the case of equally distributed level of missing information (across sub-categories) shown in **Figure 3.7**. Of course, when information is available for all attributes, the EPI scores remain essentially the same for a similar level of available information (i.e., publications) per attribute.

Compilation and evaluation of the information/data needed for conducting EIA is a necessary and tedious endeavor. In this regard, the present study provides a systematic approach that guides the EIA analyst through the process of identifying the categories of information to be compiled and quantifying the adequacy of the available information for conducting the target EIA scenario. The level of available information is quantified via attribute scoring scale (e.g., based on the number of available studies) and weights, both which can be established by the analyst based on the aim of the EIA and utilization of its outcome. While establishing aggregate scores for information availability for exposure and hazard potentials is useful for quantifying the overall status of information availability for exposure and hazard, the ability to trace the path leading to these scores provides more specific information regarding the categories for which there is missing attribute information. Admittedly, the EIA analyst will be confronted with having to make choices regarding the suitability of the available information (based on the established scoring scale) with

respect to attribute importance (i.e., via assigned weights), weight of the body of evidence (e.g., via a selected scoring scale), and the threshold aggregate or even category scores for acceptability of the body of information for conducting EIA and clearly also for indicating a critical need for additional information. In this regard, the analyst may wish to eliminate or discard the expert elicitation information attribute within the *in silico and expert judgment* sub-category based on their decision-context; particularly, given that there is a limited availability of legally binding guidance to incorporate *in silico* studies into regulatory decisions related to ENMs (Knudsen et al. 2015). While guidance documents exist for the use and report of QSARs for chemicals within REACH (ECHA 2016), other approaches such as read-across and expert elicitation are not at present included in regulatory frameworks for ENMs (OECD 2016).

CONCLUSIONS

An approach was developed to assess the adequacy of information as part of the problem formulation stage of EIA for engineered nanomaterials (ENMs). This approach (IANano) for assessing the level of information availability followed the typical EIA process whereby information elements for exposure and hazard potential assessment were classified based on major categories, sub-categories and attributes. The level of information availability for each attribute was then quantified using a selected scoring scale (e.g., based on the number of published studies), in addition to specifying an attribute weight factor. The scores for the different categories and for exposure and hazard potential information (EPI and HPI) were determined (i.e., aggregated), considering both the available and unavailable information, via the Dempster-Shafer evidential reasoning algorithm. The application of IANano was demonstrated for a number of specific EIA scenarios for the ENMs nano TiO_2, nano Cu-CuO and nano ZnO. For all three ENMs and for all

EIA scenarios, the scores for EPI were lower than for HPI given that more information was available for the hazard attributes. It is noted, however, that for TiO_2 there is significant body of information regarding exposure and thus the closer EPI to the HPI scores. For the case of EIA focusing on direct release of ENMs into the aquatic environments, the EPI scores for nano ZnO were in proximity but higher than for nano Cu-CuO, whereas the HPI scores were higher for nano Cu-CuO, which is indicative of greater emphasis by the research community on the environmental impacts of nano Cu-CuO.

The present approach, which can be tailored via the selected scoring scale and assigned attribute weights, can serve as a tool for ranking the suitability of the body of available information for conducting specific EIAs and for requiring additional modeling and/or testing to acquire the missing information. It is acknowledged that IANano is just a preliminary step in the EIA process. Subsequent steps would consist of quantitative analysis of data/information reliability/uncertainty, integration of both continuous and categorical information via suitable meta-analysis (i.e., including data curation, mining and knowledge extraction) and finally a quantitative EIA.

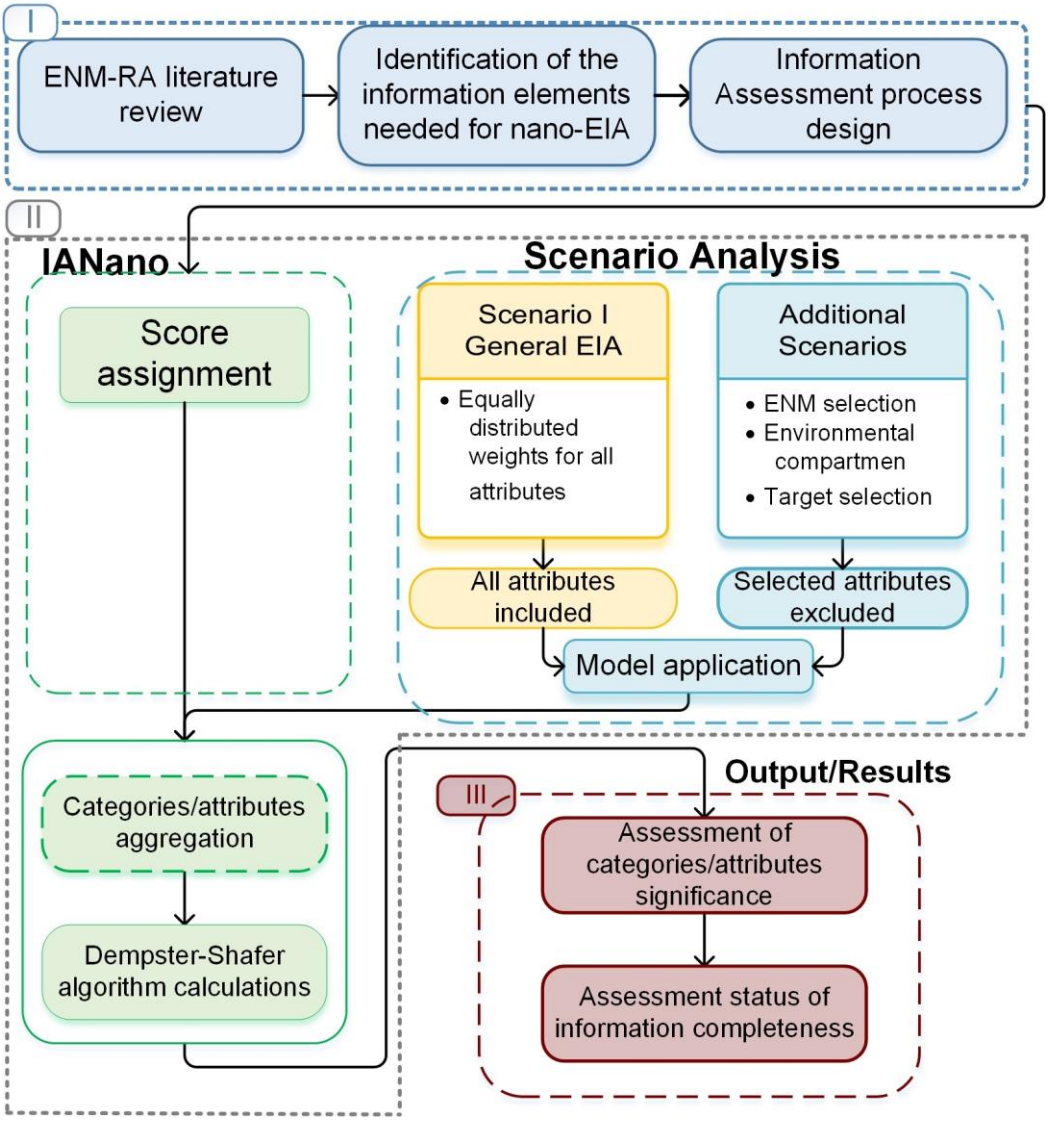


Figure 3.1 Workflow diagram of the development and demonstration of the IANano approach for assessing the adequacy of the available information for conducting EIA for ENMs.

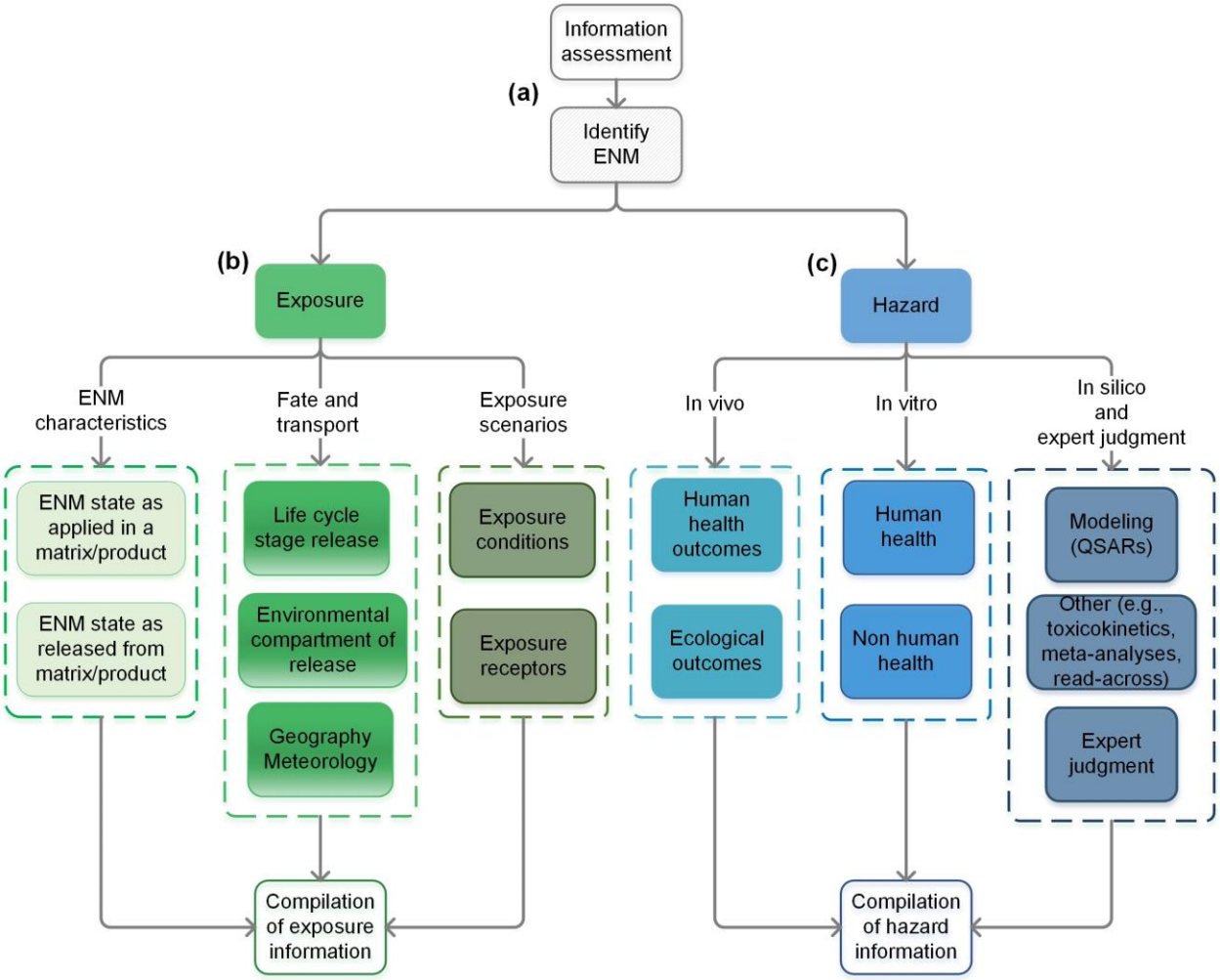


Figure 3.2 Overview of the information assessment process nodes.
At each node, the analyst has to provide responses regarding information/data availability. Response options for different nodes are provided in the supporting information, along with default scores for each response option. Scores for the nodes are aggregated as described in **Methods Section 3**

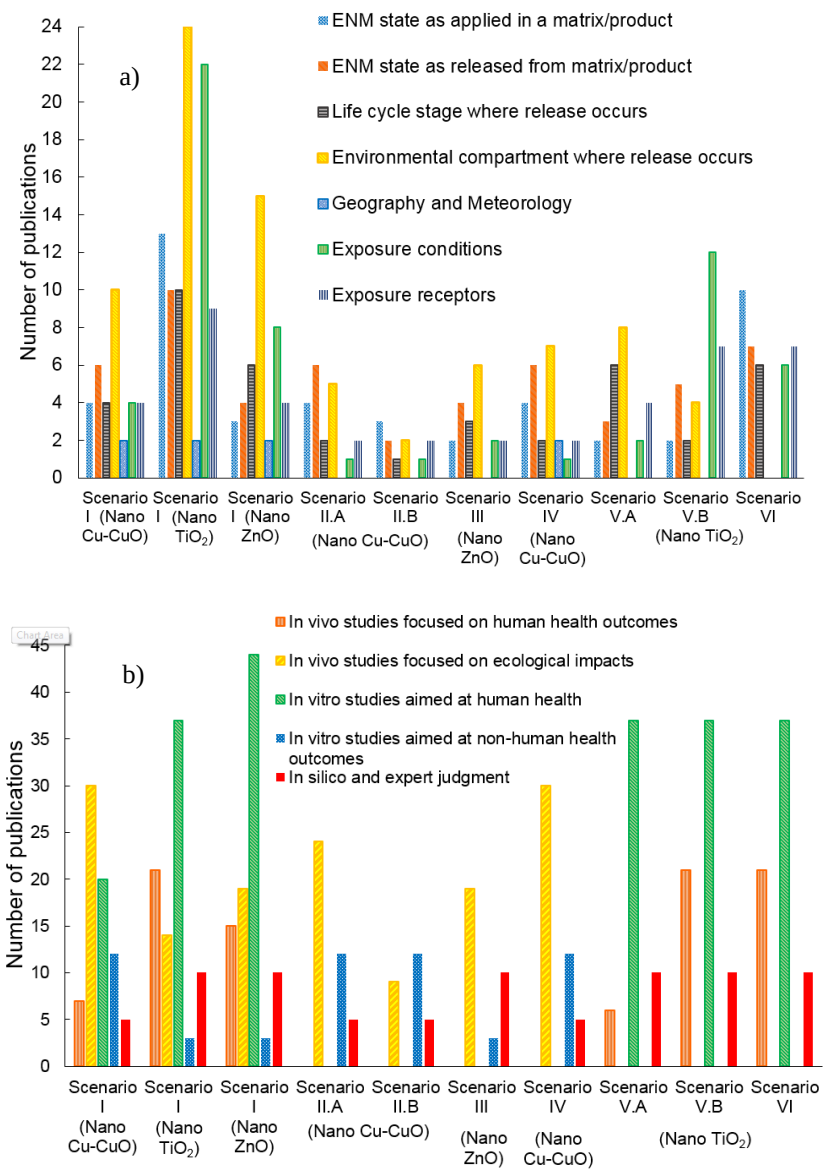


Figure 3.3 Number of publications regarding (a) exposure potential information, and (b) hazard potential information, for the different example EIA scenarios described in Methods Section 4.

The large number of publications for Scenario I is expected given that this general EIA scenario incorporates all possible exposure scenarios. This all-encompassing scenario is used for comparison purposes only and should not be taken to represent a practical EIA scenario

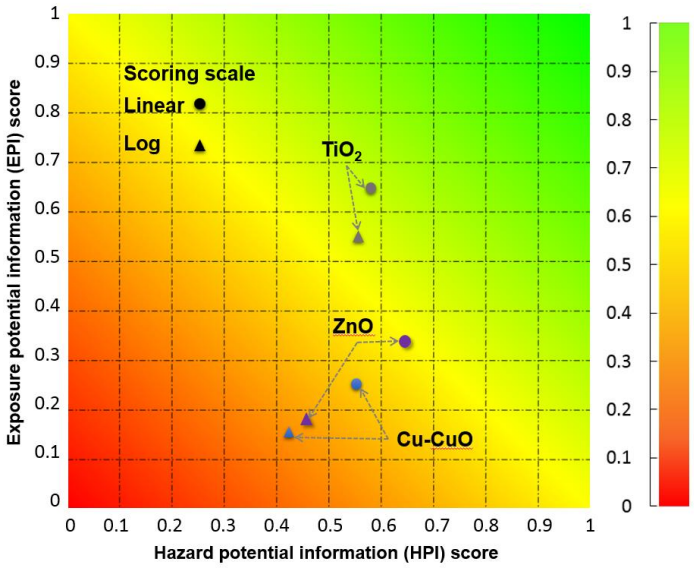


Figure 3.4 Exposure and Hazard potential information scores for nano Cu-CuO, nano TiO_2 and nano ZnO for the general EIA scenario (I).
Data points indicated by the triangles denote the aggregate scores calculated via the logarithmic scoring scale for the individual attributes, whereas the circles refer to the simple linear scoring scale.

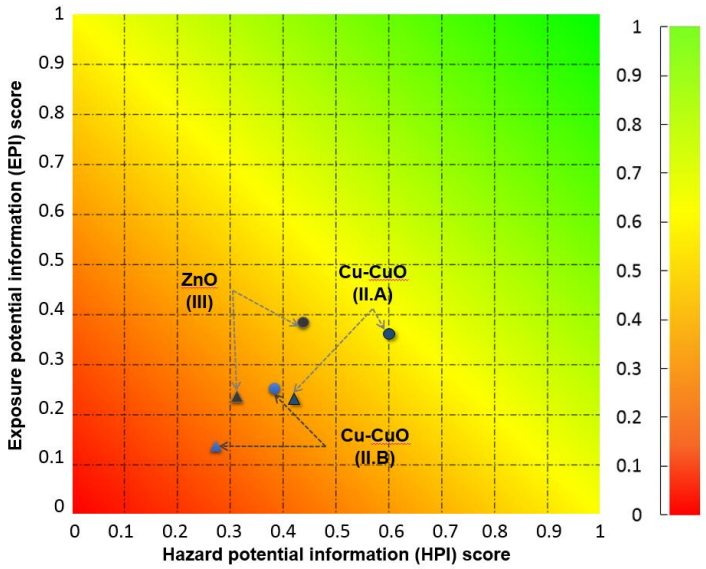


Figure 3.5 Exposure and Hazard Potential Information scores for release of ENMs into aquatic environments.

(a) nano Cu-CuO release into aquatic environments from all sources and applications (Scenario II.A); (b) nano Cu-CuO release into aquatic environments from antifouling paints (Scenario II.B); and (c) nano ZnO release into aquatic environments (Scenario III).

Data points indicated by the triangles denote the aggregate scores calculated via the logarithmic scoring scale for the individual attributes, whereas the circles refer to the simple linear scoring scale.

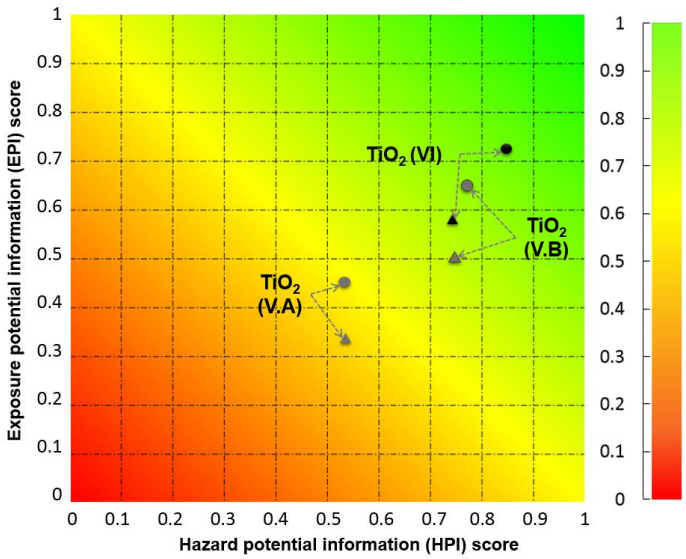


Figure 3.6 Exposure and Hazard Potential Information scores for human exposure to nano TiO_2.

(a) ENM release into air from all sources and applications (Scenario V.A), (b) Exposure to the ENM in a manufacturing facility where the release occurs or other occupational exposure (Scenario V.B), and (c) Exposure to the ENM associated with consumer product use (Scenario VI). Data points indicated by the triangles denote the aggregate scores calculated via the logarithmic scoring scale for the individual attributes, whereas the circles refer to the simple linear scoring scale

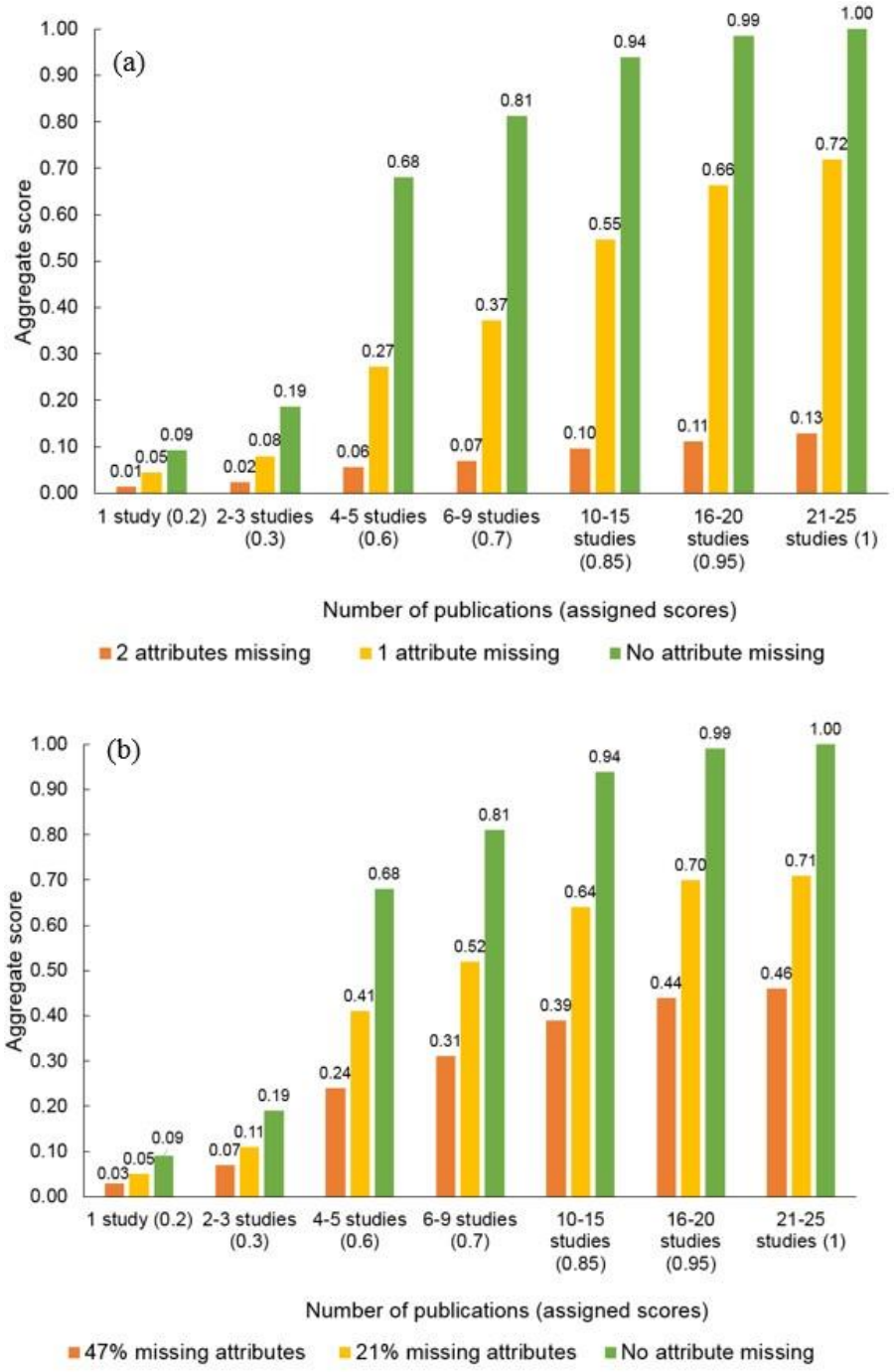


Figure 3.7 Exposure potential information scores for different examples of information availability and support as quantified by the number of available papers per attribute.

(a) Information is missing for one or two attributes per sub-category or available for all attributes, and (b) information is missing for 47% and 21% of the attributes in the fate and transport and exposure scenarios sub-categories and solely from the exposure scenarios sub-category, respectively.

Table 3.1 Exposure information categories, sub-categories and attributes used in IANano.

Categories	Description		
ENM Characteristics	Assessment of potential exposure requires information regarding ENM characteristics including the specific form/state of the ENM in the ENM material or product and the state/form of release at the point of exposure (Council 2012).		
	Sub-categories	**Attributes**	**Description/relevance of the attributes to a risk assessment process**
	ENM state as applied in a matrix/product	• Airborne • Suspended in liquid • Embedded in a solid matrix (solid article)	Physical form of the ENM within the specific product or matrix. For example, greater exposure may be associated with ENMs present in aerosols, powders, and suspensions (Abbott and Maynard 2010), compared to ENMs embedded in solid matrices (where wear and tear are the main factors responsible for the ENM release) (Hansen et al. 2008, Hansen et al. 2014).
	ENM state as released from matrix/product	• Free nanoparticle Free ENM • Homoaggregates • Heteroaggregates (NPs attached to other/larger particles)	The form in which the ENM is released from products (i.e., free ENMs, ENM agglomerates, and ENMs within or attached to larger particles) is relevant to fate and transport analysis as well as exposure assessment of ENMs (Nowack 2014). Subsequent transformations impact not only the ENM's exposure potential but also may affect associated hazards (Nazarenko et al. 2012).
Fate & Transport	Analysis of the fate and transport (F&T) of ENMs (for the purpose of arriving at exposure assessment) requires information regarding their source and applications (industrial, commercial, or consumer) and potential for releases over a product's life cycle (Council 2012). F&T analysis requires information regarding the environmental compartments where the release occurs, the release rates and geographical and meteorological conditions affecting the fate and distribution of the ENM in the environment (Abbott and Maynard 2010, Cohen et al. 2012, Liu and Cohen 2014).		
	Sub-categories	**Attributes**	**Description/relevance of the attributes to a risk assessment process**
	Life cycle stage where release occurs	• Manufacture • Use • Disposal	The life cycle stage where release or exposure of ENMs might occur is considered here due to role of this parameter in the estimation of environmental concentrations across environmental compartments (Liu et al. 2015, Caballero-Guzman and Nowack 2016).
	Environmental compartment where release occurs	• Water • Soil • Air	Exposure of human and ecological receptors is impacted by the direct release to water, soil and air (Cohen et al. 2012, Nowack et al. 2012, Liu and Cohen 2014, Hristozov et al. 2016).
	Geography Meteorology	• Geographical information • Meteorological information	Geographical characteristics (e.g., area surface/size, population size) and meteorological conditions (e.g., wind, rain) at specific locations (areas) that affect the F&T of nanomaterials and thus their potential exposure concentrations (Liu and Cohen 2014, Bilal et al. 2017, Giese et al. 2018).
Exposure Scenarios	Exposure assessment requires clear description of the specific exposure conditions (e.g., occupational, environmental or through produce use) and specification of the exposure receptors (SCENIHR 2005, Hristozov et al. 2016).		

Categories	Description		
	Sub-categories	**Attributes**	**Description/relevance of the attributes to a risk assessment process**
	Exposure conditions	• Occupational • Environmental • Consumer product use	Exposure to ENMs can be due to use of consumer products (Colvin 2003, Alvarez et al. 2009) if ENMs are released/freed from the product matrix during use or disposal (Abbott and Maynard 2010). Additionally, information regarding exposure under occupational and environmental settings is needed in in order to conduct risk assessment for such scenarios and for establishing risk management strategies (Kuempel et al. 2012, Powers et al. 2012, Schulte et al. 2013).
	Exposure receptors	• Human • Ecological	Human and ecological receptors are the relevant target receptors for risk analysis associated with ENMs (Council 2009, SCENIHR 2009).

Table 3.2 Hazard information categories, sub-categories and attributes used in IANano.

Categories	Description		
In vivo	According to recommendations by the European Union's Registration, Evaluation, Authorization and Restriction of Chemicals Program (REACH) (ECHA 2011), and the OECD (Crane et al. 2008, UNECE 2015) in vivo and in vitro studies are required to identify potential biological effects associated with exposure to ENMs as with any other man-made materials.		
	Sub-categories	**Attributes**	**Description/ relevance of the attributes to a risk assessment process**
	In vivo studies focused on human health outcomes	• Carcinogenicity, Mutagenicity, Developmental, Reproductive, and Acute toxicity • Systemic toxicity, Neuro-toxicity, Skin/eye irritation • Chronic toxicity	This category includes toxicity endpoints described by the Global Harmonized System (GHS) of the OECD (UNECE 2015).
	In vivo studies focused on ecological impacts	• Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence • Species Sensitivity Distribution (SSD) • Toxicity in environmentally relevant species	Grouping of these attributes follows the approach recommended by the European Union's Registration, Evaluation, Authorization and Restriction of Chemicals Program (REACH) (ECHA 2011), OECD (Crane et al. 2008, UNECE 2015), and other sources that describe approaches of hazard assessment following tiered testing of acute and chronic endpoints (Koelmans et al. 2015, Hund-Rinke et al. 2016, Hjorth et al. 2017).
In vitro	In vitro toxicity tests can provide a rapid and relatively inexpensive way to assess the potential toxicity of large numbers of ENMs (Lai and Warheit 2015).		
	Sub-categories	**Attributes**	**Description/ relevance of the attributes to a risk assessment process**
	In vitro studies aimed at human health	• Mutagenicity/Genotoxicity Oxidative stress, Inflammation • Cytotoxicity (cell viability)	Information is needed regarding in vitro studies for human health outcomes (based on various endpoints) as per different experimental designs involving human cells and or tissue cultures.
	In vitro studies aimed at non-human health outcomes	• Cell viability, Cell lethality • ENM transport across membranes	In vitro studies of non-human health related outcomes in cases where information/data regarding microbial activity or translocation of ENMs across membranes (Baeza-Squiban et al. 2011) is available but the experimental design does not explicitly indicate a relationship with human health.
In silico and domain knowledge	The category *in silico refers to* information based on studies that provide analytical or computational models regarding biological outcomes in response to exposure to nanomaterials (Raunio 2011). For example, the *in silico* category may encompass information extracted from toxicokinetic models (e.g., physiologically based pharmacokinetic models "PBPK"), meta-analyses, read-across classification (Raies and Bajic 2016) and expert judgment.		

Categories	Description		
	Attributes	**Description/ relevance of the attributes to a risk assessment process**	**Sub-categories**
	QSARs (quantitative-structure-activity relations)	• QSARs indicating genotoxicity and or mutagenicity • QSARs indicating cytotoxicity	Quantitative Structure Activity Relationships (QSARs) are considered acceptable methods under certain conditions for filling in knowledge gaps for untested chemicals (Burello 2014, ECHA 2016). This methodology has expanded to ENMs with the purpose of advancing risk assessment (Cohen et al. 2012, Gajewicz et al. 2012, Gajewicz et al. 2017).
	Other approaches	• Toxicokinetic and pharmacokinetic models • Meta-analyses • ENM classified as toxic from read-across	Toxicokinetic and pharmacokinetic models can be useful to elucidate the temporal evolution of ENM concentrations in different parts of the body (Baeza-Squiban et al. 2011). Read-across has also been proposed as method for hazard assessment (Arts et al. 2015, Gajewicz et al. 2015). Various meta-analysis approaches have been proposed to analyze the body of published information/data to identify consistent patterns regarding the bioactivity of ENMs (Chang et al. 2013, Oh et al. 2016).
	Expert judgment	• Multiple sources (structured surveys) • At least two sources • Single expert	Expert judgment can be important as input to decision analysis, particularly when quantitative experimental data/information, predictive theoretical or computational models are unavailable.

Table 3.3 Exposure potential information (EPI) and hazard potential information (HPI) scores for all EIA Scenarios.
Score values in parentheses represent the aggregate scores based on the simple linear scoring scale.

Scenario name	ENM released and source	Exposure Receptor	Detailed description of the scenario	EPI Score	HPI Score
I ENM release into all media	Nano Cu-CuO	Human and ecological	Designed to include ENM release to all media and for both human and ecological receptors	0.15 (0.26)	0.42 (0.55)
	Nano TiO_2			0.54 (0.64)	0.56 (0.58)
	Nano ZnO			0.19 (0.34)	0.46 (0.64)
II.A ENM release into water	Nano Cu-CuO (all sources)	Ecological	EIA related to environmental release of ENMs into aquatic environments	0.22 (0.36)	0.42 (0.60)
II.B Nano Cu in antifouling paint	Nano Cu (antifouling paint)	Ecological	EIA related to environmental release of nano Cu into aquatic environments from antifouling paints	0.13 (0.24)	0.27 (0.39)
III Nano ZnO release into water	Nano ZnO (all sources)	Ecological	EIA related to environmental release of nano ZnO into aquatic environments from personal care and consumer products	0.22 (0.39)	0.31 (0.44)
IV ENM release into soil and water from WWTP	Nano Cu-CuO	Ecological	EIA related to environmental release of nano Cu-CuO into water and soil from wastewater treatment facilities	0.19 (0.31)	0.44 (0.60)
V.A ENM release into air	Nano TiO_2	Human	EIA related to population inhalation exposure to airborne nano TiO_2	0.33 (0.45)	0.53 (0.53)
V.B ENM release into air (enclosed manufacturing facilities)	Nano TiO_2	Human	EIA related to worker exposure to inhaled nano TiO_2 during manufacturing	0.50 (0.66)	0.74 (0.77)
VI ENM direct exposure from product use	Nano TiO_2	Human	EIA related to human exposure to nano TiO_2 from consumer products	0.58 (0.72)	0.74 (0.77)

REFERENCES FOR CHAPTER 3

Abbott, L. C. and A. D. Maynard (2010). "Exposure Assessment Approaches for Engineered Nanomaterials." Risk Analysis **30**(11): 1634-1644.

Adam, N., C. Schmitt, L. De Bruyn, D. Knapen and R. Blust (2015). "Aquatic Acute Species Sensitivity Distributions of ZnO and CuO Nanoparticles." Science of the Total Environment **526**: 233-242.

Adeleye, A. S., E. A. Oranu, M. Tao and A. A. Keller (2016). "Release and Detection of Nanosized Copper from a Commercial Antifouling Paint." Water Research **102**: 374-382.

Al-Kattan, A., A. Wichser, R. Vonbank, S. Brunner, A. Ulrich, S. Zuin and B. Nowack (2013). "Release of Tio2 from Paints Containing Pigment-TiO2 or Nano TiO2 by Weathering." Environ Sci Process Impacts **15**(12): 2186-2193.

Alvarez, P. J., V. Colvin, J. Lead and V. Stone (2009). "Research Priorities to Advance Eco-Responsible Nanotechnology." ACS Nano **3**(7): 1616-1619.

Amde, M., J. F. Liu, Z. Q. Tan and D. Bekana (2017). "Transformation and Bioavailability of Metal Oxide Nanoparticles in Aquatic and Terrestrial Environments. A Review." Environmental Pollution (Barking, Essex: 1987) **230**: 250-267.

Arts, J. H. E., M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, W. Wohlleben and R. Landsiedel (2015). "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (Df4nanogrouping)." Regulatory Toxicology and Pharmacology **71**(2, Supplement): S1-S27.

Aschberger, K., C. Micheletti, B. Sokull-Kluttgen and F. M. Christensen (2011). "Analysis of Currently Available Data for Characterising the Risk of Engineered Nanomaterials to the Environment and Human Health - Lessons Learned from Four Case Studies." Environment International **37**(6): 1143-1156.

Baeza-Squiban, A., G. Lacroix and F. Y. Bois (2011). Experimental Models in Nanotoxicology. Nanoethics and Nanotoxicology. P. Houdy, M. Lahmani and F. Marano, Springer**:** 63-86.

Beegam, A., P. Prasad, J. Jose, M. Oliveira, F. G. Costa, A. M. V. M. Soares, P. P. Gonçalves, T. Trindade, N. Kalarikkal, S. Thomas and M. d. L. Pereira (2016). Environmental Fate of Zinc Oxide Nanoparticles: Risks and Benefits. Toxicology - New Aspects to This Scientific Conundrum. M. L. Larramendy and S. Soloneski, InTech.

Bilal, M., H. Liu, R. Liu and Y. Cohen (2017). "Bayesian Network as a Support Tool for Rapid Query of the Environmental Multimedia Distribution of Nanomaterials." Nanoscale **9**(12): 4162-4174.

Bondarenko, O., K. Juganson, A. Ivask, K. Kasemets, M. Mortimer and A. Kahru (2013). "Toxicity of Ag, CuO and ZnO Nanoparticles to Selected Environmentally Relevant Test

Organisms and Mammalian Cells in Vitro: A Critical Review." Archives of Toxicology **87**(7): 1181-1200.

Bos, P. M. J., S. Gottardo, J. J. Scott-Fordsmand, M. van Tongeren, E. Semenzin, T. F. Fernandes, D. Hristozov, K. Hund-Rinke, N. Hunt and M.-A. Irfan (2015). "The Marina Risk Assessment Strategy: A Flexible Strategy for Efficient Information Collection and Risk Assessment of Nanomaterials." International Journal of Environmental Research and Public Health **12**(12): 15007-15021.

Boverhof, D. R. and R. M. David (2010). "Nanomaterial Characterization: Considerations and Needs for Hazard Assessment and Safety Evaluation." Analytical and Bioanalytical Chemistry **396**(3): 953-961.

Brar, S. K., M. Verma, R. D. Tyagi and R. Y. Surampalli (2010). "Engineered Nanoparticles in Wastewater and Wastewater Sludge--Evidence and Impacts." Waste Manag **30**(3): 504-520.

Burello, E. (2014). Predictive Nanotoxicology: In Silico Approaches. Handbook of Safety Assessment of Nanomaterials. B. Fadeel, Pan Stanford**:** 221-242.

Caballero-Guzman, A. and B. Nowack (2016). "A Critical Review of Engineered Nanomaterial Release Data: Are Current Data Useful for Material Flow Modeling?" Environmental Pollution **213**: 502-517.

Chang, X. H., Y. Zhang, M. Tang and B. Wang (2013). "Health Effects of Exposure to Nano Tio2: A Meta-Analysis of Experimental Studies." Nanoscale Research Letters **8**: 1-10.

Cohen, Y., R. Rallo, R. Liu and H. H. Liu (2012). "In Silico Analysis of Nanomaterials Hazard and Risk." Accounts of Chemical Research **46**(3): 802–812.

Colvin, V. L. (2003). "The Potential Environmental Impact of Engineered Nanomaterials." Nature Biotechnology **21**(10): 1166-1170.

Council, N. R. (2009). Science and Decisions: Advancing Risk Assessment. Washington, DC, The National Academies Press.

Council, N. R. (2012). Critical Questions for Understanding Human and Environmental Effects of Engineered Nanomaterials A Research Strategy for Environmental, Health, and Safety Aspects of Engineered Nanomaterials. H. Committee to Develop a Research Strategy for Environmental, and Safety Aspects of Engineered Nanomaterials. Washington, DC, National Academies Press

Crane, M., R. D. Handy, J. Garrod and R. Owen (2008). "Ecotoxicity Test Methods and Environmental Hazard Assessment for Engineered Nanoparticles." Ecotoxicology **17**(5): 421-437.

ECHA (2011). Guidance on Information Requirements and Chemical Safety Assessment. Part B: Hazard Assessment. Finland, European Chemicals Agency.

ECHA (2016). Practical Guide – How to Use and Report (Q)SARs. Finland. **1:** 36.

EPA (2004). Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. R. Jones, J. Leahy, M. Mahoney et al.**:** 92.

EPA (2017). Registration Review Proposed Interim Decisions for Several Pesticides; Notice of Availability. 82 FR 44405**:** 44405-44406.

Gajewicz, A., M. T. Cronin, B. Rasulev, J. Leszczynski and T. Puzyn (2015). "Novel Approach for Efficient Predictions Properties of Large Pool of Nanomaterials Based on Limited Set of Species: Nano-Read-Across." Nanotechnology **26**(1): 015701.

Gajewicz, A., K. Jagiello, M. T. D. Cronin, J. Leszczynski and T. Puzyn (2017). "Addressing a Bottle Neck for Regulation of Nanomaterials: Quantitative Read-Across (Nano-QRA) Algorithm for Cases When Only Limited Data Is Available." Environmental Science: Nano.

Gajewicz, A., B. Rasulev, T. C. Dinadayalane, P. Urbaszek, T. Puzyn, D. Leszczynska and J. Leszczynski (2012). "Advancing Risk Assessment of Engineered Nanomaterials: Application of Computational Approaches." Advanced Drug Delivery Reviews **64**(15): 1663-1693.

Garner, K. L. and A. A. Keller (2014). "Emerging Patterns for Engineered Nanomaterials in the Environment: A Review of Fate and Toxicity Studies." Journal of Nanoparticle Research **16**(8).

Giese, B., F. Klaessig, B. Park, R. Kaegi, M. Steinfeldt, H. Wigger, A. von Gleich and F. Gottschalk (2018). "Risks, Release and Concentrations of Engineered Nanomaterials in the Environment." Scientific Reports **8**(1): 1565.

Girigoswami, K. (2018). "Toxicity of Metal Oxide Nanoparticles." Advances in Experimental Medicine and Biology **1048**: 99-122.

Gottschalk, F. and B. Nowack (2011). "The Release of Engineered Nanomaterials to the Environment." Journal of Environmental Monitoring **13**(5): 1145-1155.

Gottschalk, F., T. Sonderer, R. W. Scholz and B. Nowack (2009). "Modeled Environmental Concentrations of Engineered Nanomaterials (Tio2, Zno, Ag, Cnt, Fullerenes) for Different Regions." Environmental Science & Technology **43**(24): 9216-9222.

Hansen, S., E. Michelson, A. Kamper, P. Borling, F. Stuer-Lauridsen and A. Baun (2008). "Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products." Ecotoxicology **17**(5): 438-447.

Hansen, S. F. and A. Baun (2012). "When Enough Is Enough." Nature Nanotechnology **7**(7): 409-411.

Hansen, S. F., K. A. Jensen and A. Baun (2014). "Nanoriskcat: A Conceptual Tool for Categorization and Communication of Exposure Potentials and Hazards of Nanomaterials in Consumer Products." Journal of Nanoparticle Research **16**(1): 1-25.

He, X., W. G. Aker, J. Leszczynski and H.-M. Hwang (2014). "Using a Holistic Approach to Assess the Impact of Engineered Nanomaterials Inducing Toxicity in Aquatic Systems." Journal of Food and Drug Analysis **22**(1): 128-146.

Hendren, C. O., M. Lowry, K. D. Grieger, E. S. Money, J. M. Johnston, M. R. Wiesner and S. M. Beaulieu (2013). "Modeling Approaches for Characterizing and Evaluating Environmental Exposure to Engineered Nanomaterials in Support of Risk-Based Decision Making." Environmental Science & Technology **47**(3): 1190-1205.

Hjorth, R., L. M. Skjolding, S. N. Sørensen and A. Baun (2017). "Regulatory Adequacy of Aquatic Ecotoxicity Testing of Nanomaterials." NanoImpact **8**: 28-37.

Höck J., E. T., Furrer E., Gautschi. M, Hofmann H., Höhener K., Knauer K., Krug H., Limbach, G. P. L., Nowack B., Riediker M., Schirmer K., Schmid K., Som C., Stark W., Studer C., Ulrich A., and W. A. von Götz N., Wengert S., Wick P. (2013). Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Berne, Federal Office of Public Health and Federal Office for the Environment. **Version 3.0:** 26.

Holden, P. A., J. L. Gardea-Torresdey, F. Klaessig, R. F. Turco, M. Mortimer, K. Hund-Rinke, E. A. Cohen Hubal, D. Avery, D. Barcelo, R. Behra, Y. Cohen, L. Deydier-Stephan, P. L. Ferguson, T. F. Fernandes, B. Herr Harthorn, W. M. Henderson, R. A. Hoke, D. Hristozov, J. M. Johnston, A. B. Kane, L. Kapustka, A. A. Keller, H. S. Lenihan, W. Lovell, C. J. Murphy, R. M. Nisbet, E. J. Petersen, E. R. Salinas, M. Scheringer, M. Sharma, D. E. Speed, Y. Sultan, P. Westerhoff, J. C. White, M. R. Wiesner, E. M. Wong, B. Xing, M. Steele Horan, H. A. Godwin and A. E. Nel (2016). "Considerations of Environmentally Relevant Test Conditions for Improved Evaluation of Ecological Hazards of Engineered Nanomaterials." Environmental Science & Technology **50**(12): 6124-6145.

Hou, J., Y. Wu, X. Li, B. Wei, S. Li and X. Wang (2018). "Toxic Effects of Different Types of Zinc Oxide Nanoparticles on Algae, Plants, Invertebrates, Vertebrates and Microorganisms." Chemosphere **193**: 852-860.

Hristozov, D., S. Gottardo, E. Semenzin, A. Oomen, P. Bos, W. Peijnenburg, M. van Tongeren, B. Nowack, N. Hunt, A. Brunelli, J. J. Scott-Fordsmand, L. Tran and A. Marcomini (2016). "Frameworks and Tools for Risk Assessment of Manufactured Nanomaterials." Environment International **95**(Supplement C): 36-53.

Hristozov, D. R., S. Gottardo, A. Critto and A. Marcomini (2012). "Risk Assessment of Engineered Nanomaterials: A Review of Available Data and Approaches from a Regulatory Perspective." Nanotoxicology **6**(8): 880-898.

Hristozov, D. R., A. Zabeo, C. Foran, P. Isigonis, A. Critto, A. Marcomini and I. Linkov (2014). "A Weight of Evidence Approach for Hazard Screening of Engineered Nanomaterials." Nanotoxicology **8**(1): 72-87.

Hund-Rinke, K., A. Baun, D. Cupi, T. F. Fernandes, R. Handy, J. H. Kinross, J. M. Navas, W. Peijnenburg, K. Schlich, B. J. Shaw and J. J. Scott-Fordsmand (2016). "Regulatory Ecotoxicity

Testing of Nanomaterials - Proposed Modifications of Oecd Test Guidelines Based on Laboratory Experience with Silver and Titanium Dioxide Nanoparticles." Nanotoxicology **10**(10): 1442-1447.

Kahru, A. and A. Ivask (2013). "Mapping the Dawn of Nanoecotoxicological Research." Accounts of Chemical Research **46**(3): 823-833.

Keller, A. A., A. S. Adeleye, J. R. Conway, K. L. Garner, L. Zhao, G. N. Cherr, J. Hong, J. L. Gardea-Torresdey, H. A. Godwin and S. Hanna (2017). "Comparative Environmental Fate and Toxicity of Copper Nanomaterials." NanoImpact **7**: 28-40.

Keller, A. A. and A. Lazareva (2013). "Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local." Environ Sci Technol Lett **1**(1): 65-70.

Keller, A. A., S. McFerran, A. Lazareva and S. Suh (2013). "Global Life Cycle Releases of Engineered Nanomaterials." Journal of Nanoparticle Research **15**(6).

Kiaune, L. and N. Singhasemanon (2011). Pesticidal Copper (I) Oxide: Environmental Fate and Aquatic Toxicity. Reviews of Environmental Contamination and Toxicology Volume 213. D. M. Whitacre. New York, NY, Springer New York**:** 1-26.

Knudsen, T. B., D. A. Keller, M. Sander, E. W. Carney, N. G. Doerrer, D. L. Eaton, S. C. Fitzpatrick, K. L. Hastings, D. L. Mendrick, R. R. Tice, P. B. Watkins and M. Whelan (2015). "Futuretox Ii: In Vitro Data and in Silico Models for Predictive Toxicology." Toxicological Sciences **143**(2): 256-267.

Koelmans, A. A., N. J. Diepens, I. Velzeboer, E. Besseling, J. T. Quik and D. van de Meent (2015). "Guidance for the Prognostic Risk Assessment of Nanomaterials in Aquatic Ecosystems." Science of the Total Environment **535**: 141-149.

Koivisto, A. J., A. C. Ø. Jensen, K. I. Kling, A. Nørgaard, A. Brinch, F. Christensen and K. A. Jensen (2017). "Quantitative Material Releases from Products and Articles Containing Manufactured Nanomaterials: Towards a Release Library." NanoImpact **5**: 119-132.

Krewski, D., D. Acosta, M. Andersen, H. Anderson, J. C. Bailar, K. Boekelheide, R. Brent, G. Charnley, V. G. Cheung, S. Green, K. T. Kelsey, N. I. Kerkvliet, A. A. Li, L. McCray, O. Meyer, R. D. Patterson, W. Pennie, R. A. Scala, G. M. Solomon, M. Stephens, J. Yager and L. Zeise (2010). "Toxicity Testing in the 21st Century: A Vision and a Strategy." J. Toxicol. Environ. Health, Part B **13**(2-4): 51-138.

Kuempel, E. D., V. Castranova, C. L. Geraci and P. A. Schulte (2012). "Development of Risk-Based Nanomaterial Groups for Occupational Exposure Control." Journal of Nanoparticle Research **14**(1029): 1-15.

Kuempel, E. D., C. L. Geraci and P. A. Schulte (2012). "Risk Assessment and Risk Management of Nanomaterials in the Workplace: Translating Research to Practice." Annals of Occupational Hygiene **56**(5): 491-505.

Kuhlbusch, T. A. J., S. W. P. Wijnhoven and A. Haase (2017). "Nanomaterial Exposures for Worker, Consumer and the General Public." NanoImpact **10**: 11-25.

Lai, D. and D. Warheit (2015). Nanotoxicology: The Case for in Vivo Studies. Handbook of Safety Assessment of Nanomaterials : From Toxicological Testing to Personalized Medicine. B. Fadeel, Pan Stanford**:** 153-219.

Lazareva, A. and A. A. Keller (2014). "Estimating Potential Life Cycle Releases of Engineered Nanomaterials from Wastewater Treatment Plants." ACS Sustainable Chemistry & Engineering **2**(7): 1656-1665.

Lee, J. H., M. Kwon, J. H. Ji, C. S. Kang, K. H. Ahn, J. H. Han and I. J. Yu (2011). "Exposure Assessment of Workplaces Manufacturing Nanosized Tio2 and Silver." Inhalation Toxicology **23**(4): 226-236.

Linkov, I., F. K. Satterstrom, J. Steevens, E. Ferguson and R. C. Pleus (2007). "Multi-Criteria Decision Analysis and Environmental Risk Assessment for Nanomaterials." Journal of Nanoparticle Research **9**(4): 543-554.

Linkov, I., J. Steevens, M. Chappell, T. Tervonen, J. R. Figueira and M. Merad (2009). Classifying Nanomaterial Risks Using Multi-Criteria Decision Analysis. Nanomaterials: Risks and Benefits. I. Linkov and J. Steevens, Springer Netherlands**:** 179-191.

Liu, H. H., M. Bilal, A. Lazareva, A. Keller and Y. Cohen (2015). "Simulation Tool for Assessing the Release and Environmental Distribution of Nanomaterials." Beilstein Journal of Nanotechnology **6**: 938.

Liu, H. H. and Y. Cohen (2014). "Multimedia Environmental Distribution of Engineered Nanomaterials." Environmental Science & Technology **48**(6): 3281-3292.

Lorenz, C., N. Von Goetz, M. Scheringer, M. Wormuth and K. Hungerbühler (2011). "Potential Exposure of German Consumers to Engineered Nanoparticles in Cosmetics and Personal Care Products." Nanotoxicology **5**(1): 12-29.

Mackevica, A. and S. Foss Hansen (2016). "Release of Nanomaterials from Solid Nanocomposites and Consumer Exposure Assessment - a Forward-Looking Review." Nanotoxicology **10**(6): 641-653.

Marvin, H. P., H. Bouwmeester, M. Bakker, E. D. Kroese, D. van de Meent, F. Bourgeois, R. Lokers, H. van der Ham and L. Verhelst (2013). "Exploring the Development of a Decision Support System (Dss) to Prioritize Engineered Nanoparticles for Risk Assessment." Journal of Nanoparticle Research **15**(8): 1-13.

Maynard, A. D. and R. J. Aitken (2016). "'Safe Handling of Nanotechnology' Ten Years On." Nature Nanotechnology **11**(12): 998-1000.

Minetto, D., A. Volpi Ghirardini and G. Libralato (2016). "Saltwater Ecotoxicology of Ag, Au, CuO, TiO2, ZnO and C_{60} Engineered Nanoparticles: An Overview." Environment International **92-93**: 189-201.

Nazarenko, Y., T. W. Han, P. J. Lioy and G. Mainelis (2011). "Potential for Exposure to Engineered Nanoparticles from Nanotechnology-Based Consumer Spray Products." Journal of Exposure Science & Environmental Epidemiology **21**(5): 515-528.

Nazarenko, Y., H. Zhen, T. Han, P. J. Lioy and G. Mainelis (2012). "Potential for Inhalation Exposure to Engineered Nanoparticles from Nanotechnology-Based Cosmetic Powders." Environmental Health Perspectives **120**(6): 885-892.

NIOSH (2011). Occupational Exposure to Titanium Dioxide**:** 119. https://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf

Nowack, B. (2014). Emissions from Consumer Products Containing Engineered Nanomaterials over Their Lifecycle. Safety of Nanomaterials Along Their Lifecycle. W. Wohlleben, T. A. J. Kuhlbusch, J. Schnekenburger and C.-M. Lehr, CRC Press**:** 335-354.

Nowack, B. (2017). "Evaluation of Environmental Exposure Models for Engineered Nanomaterials in a Regulatory Context." NanoImpact **8**: 38-47.

Nowack, B., M. Baalousha, N. Bornhöft, Q. Chaudhry, G. Cornelis, J. Cotterill, A. Gondikas, M. Hassellöv, J. Lead and D. M. Mitrano (2015). "Progress Towards the Validation of Modeled Environmental Concentrations of Engineered Nanomaterials by Analytical Measurements." Environmental Science: Nano **2**(5): 421-428.

Nowack, B., C. Brouwer, R. E. Geertsma, E. H. W. Heugens, B. L. Ross, M. C. Toufektsian, S. W. P. Wijnhoven and R. J. Aitken (2013). "Analysis of the Occupational, Consumer and Environmental Exposure to Engineered Nanomaterials Used in 10 Technology Sectors." Nanotoxicology **7**(6): 1152-1156.

Nowack, B., J. F. Ranville, S. Diamond, J. A. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A. A. Koelmans and S. J. Klaine (2012). "Potential Scenarios for Nanomaterial Release and Subsequent Alteration in the Environment." Environmental Toxicology and Chemistry **31**(1): 50-59.

NRC (2003). The Measure of Star: Review of the U.S. Environmental Protection Agency's Science to Achieve Results (Star) Research Grants Program. Washington, DC, The National Academies Press. **2017:** 192.

NSET (2006). Environmental, Health and Safety Research Needs for Engineered Nanoscale Materials. R. Rusell and R. Cresanti. Washington, D.C., Nanoscale Science, Engineering and Technology Subcommittee Committee on Technology.

O'Brien, N. J. and E. J. Cummins (2011). "A Risk Assessment Framework for Assessing Metallic Nanomaterials of Environmental Concern: Aquatic Exposure and Behavior." Risk Analysis **31**(5): 706-726.

OECD (2016). Approaches on Nano Grouping/ Equivalence/ Read-across Concepts Based on Physical-Chemical Properties (GERA-PC) for Regulatory Regimes. Paris.

Oh, E., R. Liu, A. Nel, K. B. Gemill, M. Bilal, Y. Cohen and I. L. Medintz (2016). "Meta-Analysis of Cellular Toxicity for Cadmium-Containing Quantum Dots." Nature Nanotechnology **11**(5): 479-486.

Oomen, A. G., K. G. Steinhäuser, E. A. J. Bleeker, F. van Broekhuizen, A. Sips, S. Dekkers, S. W. P. Wijnhoven and P. G. Sayre (2017). "Risk Assessment Frameworks for Nanomaterials: Scope, Link to Regulations, Applicability, and Outline for Future Directions in View of Needed Increase in Efficiency." NanoImpact **9**: 1-13.

Pirela, S. V., G. A. Sotiriou, D. Bello, M. Shafer, K. L. Bunker, V. Castranova, T. Thomas and P. Demokritou (2015). "Consumer Exposures to Laser Printer-Emitted Engineered Nanoparticles: A Case Study of Life-Cycle Implications from Nano-Enabled Products." Nanotoxicology **9**(6): 760-768.

Powers, C. M., G. Dana, P. Gillespie, M. R. Gwinn, C. O. Hendren, T. C. Long, A. Wang and J. M. Davis (2012). "Comprehensive Environmental Assessment: A Meta-Assessment Approach." Environmental Science & Technology **46**(17): 9202-9208.

Raies, A. B. and V. B. Bajic (2016). "In Silico Toxicology: Computational Methods for the Prediction of Chemical Toxicity." Wiley Interdisciplinary Reviews: Computational Molecular Science **6**(2): 147-172.

Raunio, H. (2011). "In Silico Toxicology – Non-Testing Methods." Frontiers in Pharmacology **2**: 33.

Robichaud, C. O., D. Tanzil, U. Weilenmann and M. R. Wiesner (2005). "Relative Risk Analysis of Several Manufactured Nanomaterials: An Insurance Industry Context." Environmental Science & Technology **39**(22): 8985-8994.

Romero-Franco, M., H. A. Godwin, M. Bilal and Y. Cohen (2017). "Needs and Challenges for Assessing the Environmental Impacts of Engineered Nanomaterials (ENMs)." Beilstein Journal of Nanotechnology **8**: 989-1014.

SCENIHR (2005). Opinion On: The Appropriateness of the Risk Assessment Methodology in Accordance with the Technical Guidance Documents for New and Existing Substances for Assessing the Risks of Nanomaterials.

SCENIHR (2007). Opinion On: The Appropriateness of the Risk Assessment Methodology in Accordance with the Technical Guidance Documents for New and Existing Substances for Assessing the Risks of Nanomaterials.

SCENIHR (2009). Opinion On: The Appropriateness of Existing Methodologies to Assess the Potential Risks Associated with Engineered and Adventitious Products of Nanotechnologies.

Schiff, K., D. Diehl and A. Valkirs (2004). "Copper Emissions from Antifouling Paint on Recreational Vessels." Marine Pollution Bulletin **48**(3-4): 371-377.

Schulte, P. A., C. L. Geraci, L. L. Hodson, R. D. Zumwalde, E. D. Kuempel, V. Murashov, K. F. Martinez and D. S. Heidel (2013). "Overview of Risk Management for Engineered Nanomaterials." J Phys Conf Ser **429**(012062): 1-10.

Schulte, P. A., G. Roth, L. L. Hodson, V. Murashov, M. D. Hoover, R. Zumwalde, E. D. Kuempel, C. L. Geraci, A. B. Stefaniak, V. Castranova and J. Howard (2016). "Taking Stock of the Occupational Safety and Health Challenges of Nanotechnology: 2000–2015." Journal of Nanoparticle Research **18**(6): 159.

Shafer, G. (1992). Dempster-Shafer Theory. Encyclopedia of Artificial Intelligence. S. Shapiro, John Wiley & Sons. **1:** 330-331.

Shandilya, N., O. Le Bihan, C. Bressot and M. Morgeneyer (2015). "Emission of Titanium Dioxide Nanoparticles from Building Materials to the Environment by Wear and Weather." Environmental Science & Technology **49**(4): 2163-2170.

Shi, H., R. Magaye, V. Castranova and J. Zhao (2013). "Titanium Dioxide Nanoparticles: A Review of Current Toxicological Data." Particle and Fibre Toxicology **10**.

Spinazzè, A., A. Cattaneo, M. Limonta, V. Bollati, P. A. Bertazzi and D. M. Cavallo (2016). "Titanium Dioxide Nanoparticles: Occupational Exposure Assessment in the Photocatalytic Paving Production." Journal of Nanoparticle Research **18**(6): 1-12.

Sun, T. Y., F. Gottschalk, K. Hungerbühler and B. Nowack (2014). "Comprehensive Probabilistic Modelling of Environmental Emissions of Engineered Nanomaterials." Environmental Pollution **185**: 69-76.

Tervonen, T., I. Linkov, J. R. Figueira, J. Steevens, M. Chappell and M. Merad (2009). "Risk-Based Classification System of Nanomaterials." Journal of Nanoparticle Research **11**(4): 757-766.

Tsuji, J. S., F. S. Mowat, S. Donthu and M. Reitman (2009). Application of Toxicology Studies in Assessing the Health Risks of Nanomaterials in Consumer Products. Nanotoxicity from in Vivo and in Vitro Models to Health Risks. S. C. Sahu and D. A. Casciano. Chichester, West Sussex, UK, John Wiley**:** 630.

U.S.Congress (2003). 21st Century Nanotechnology Research and Development Act. (2003-2004). **Public Law No: 108-153**.

U.S.Congress (2011). Pesticide Registration Improvement Renewal Act. Title 7 - Agriculture.

UNECE (2015). Globally Harmonized System of Classification and Labelling of Chemicals (Ghs). New York and Geneva, United Nations Economic Commission for Europe**:** 527.

Vale, G., K. Mehennaoui, S. Cambier, G. Libralato, S. Jomini and R. F. Domingos (2016). "Manufactured Nanoparticles in the Aquatic Environment-Biochemical Responses on Freshwater Organisms: A Critical Overview." Aquatic Toxicology **170**: 162-174.

Vaquero, C., N. Gelarza, J. L. L. de Ipiña, C. Gutierrez-Cañas, I. Múgica, G. Aragón, M. Jaen, R. Pina, I. Larraza and A. Esteban-Cubillo (2015). "Occupational Exposure to Nano-TiO_2 in the Life Cycle Steps of New Depollutant Mortars Used in Construction." Journal of Physics: Conference Series **617**: 012006.

Wang, L.-F., N. Habibul, D.-Q. He, W.-W. Li, X. Zhang, H. Jiang and H.-Q. Yu (2015). "Copper Release from Copper Nanoparticles in the Presence of Natural Organic Matter." Water Research **68**: 12-23.

Wang, Z., L. Zhang, J. Zhao and B. Xing (2016). "Environmental Processes and Toxicity of Metallic Nanoparticles in Aquatic Systems as Affected by Natural Organic Matter." Environmental Science: Nano **3**(2): 240-255.

Weir, A., P. Westerhoff, L. Fabricius, K. Hristovski and N. von Goetz (2012). "Titanium Dioxide Nanoparticles in Food and Personal Care Products." Environmental Science & Technology **46**(4): 2242-2250.

Xia, T., D. Malasarn, S. J. Lin, Z. X. Ji, H. Y. Zhang, R. J. Miller, A. A. Keller, R. M. Nisbet, B. H. Harthorn, H. A. Godwin, H. S. Lenihan, R. Liu, J. Gardea-Torresdey, Y. Cohen, L. Madler, P. A. Holden, J. I. Zink and A. E. Nel (2013). "Implementation of a Multidisciplinary Approach to Solve Complex Nano EHS Problems by the UC Center for the Environmental Implications of Nanotechnology." Small **9**(9-10): 1428-1443.

Yang, J.-B. and D.-L. Xu (2002). "Nonlinear Information Aggregation Via Evidential Reasoning in Multiattribute Decision Analysis under Uncertainty." IEEE Transactions on Systems, Man, and Cybernetics. Part A, Systems and Humans **32**(3): 376-393.

Yang, J.-B. and D.-L. Xu (2002). "On the Evidential Reasoning Algorithm for Multiple Attribute Decision Analysis under Uncertainty." IEEE Transactions on Systems, Man, and Cybernetics. Part A, Systems and Humans **32**(3): 289-304.

Yung, M., C. Mouneyrac and K. Leung (2014). Ecotoxicity of Zinc Oxide Nanoparticles in the Marine Environment. Encyclopedia of Nanotechnology. B. Bhushan. Netherlands, Springer**:** 1-17.

Zuverza-Mena, N., D. Martínez-Fernández, W. Du, J. A. Hernandez-Viezcas, N. Bonilla-Bird, M. L. López-Moreno, M. Komárek, J. R. Peralta-Videa and J. L. Gardea-Torresdey (2017). "Exposure of Engineered Nanomaterials to Plants: Insights into the Physiological and Biochemical Responses-a Review." Plant Physiology and Biochemistry **110**: 236-264.

CHAPTER 4: PROGRESS TOWARDS UNDERSTANDING WHETHER ENMS POSE A SIGNIFICANT RISK TO PUBLIC HEALTH

(I am planning to submit a modified version of this chapter as a manuscript for publication in *American Journal of Public Health.* Authors: Michelle Romero-Franco, Yoram Cohen, Hilary Godwin*)*

ABSTRACT

Although many advances have been made in the field of the Environmental Health and Safety of Nanomaterials (Nano EH&S) over the last two decades, not all of these have been communicated effectively back to key stakeholders, particularly public health professionals. There is a particular need for work that synthesizes knowledge from the broad range of studies on Nano EH&S that help to answer address large, overarching concerns that are of interest to the public health community, such as whether engineered nanomaterials (ENMs) pose a significant risk to human health. Here, we present a critical analysis of how the scientific and policy milestones in Nano EH&S over the last 2 decades have advanced our understanding of the potential public health implications of ENMs.

INTRODUCTION

Since the early 2000's, the interest in the potential benefits of nanotechnology applications has been accompanied by a growing concern regarding the identification of risks these materials may pose to human health and the environment. Early identification of knowledge gaps about the potential harmful effects of ENMs have led to a growing number of public and private research

initiatives in this area. These initiatives have resulted in a significant growth of the number of publications in the field of Environmental Health and Safety implications of Engineered Nanomaterials (Nano EH&S).

As discussed in **Chapter 1**, major accomplishments have been achieved in terms of outreach and risk communication in the field of Nano EH&S. In this regard, a key group of stakeholders who is important to the sustainability of nanotechnology, is the public health professionals. Given the major implications of the potential risks and benefits of ENMs, a communication bridge must be built between Nano EH&S researchers and the public health community. Here, a review is presented through the lens of answering a series of key questions of concern that can be relevant to public health professionals:

1. Have policy changes been put into place at state, federal and international levels to protect human health from engineered nanomaterials (ENMs)?
2. Do ENMs pose novel threats to human health that differ fundamentally from those of conventional chemicals?
3. Can conventional approaches to risk assessment that were developed for conventional chemicals/materials be applied to ENMs?
4. Are people and/or the environment currently exposed to concentrations of ENMs that pose significant risk (or are such exposures expected in the near future)?
5. Are there sufficient data to be able to assess the risks associated with ENMs?
6. Are there sufficient knowledge and tools to enable minimization of exposures and mitigate risks associated with ENMs?

To answer these questions and provide a rationale for such answers, we reviewed existing literature for the field of Nano EH&S for the period 2003-2017 and analyzed research highlights,

policies and the level of scientific consensus among scientific publications and reports. In the following sections, a discussion is provided of the how key changes in policies and regulations in the field of Nano EH&S over the last two decades, and how scientific advances in this same time period can be used to provide science-based responses to core public health concerns. To measure the progress achieved within the individual research topics and policies related to the questions of concern **(Table 4.1)**, three qualitative scales are provided. In the first scale, the level of progress/advances in the field is measured by a scale ranging from *low* to *high*, where *low* reflects that minimum or no progress has been achieved; *medium* reflects progress above minimum level (e.g., considerable understanding in terms of toxicity mechanisms, exposure and/or environmental fate and transport has been achieved) with considerable information gaps; and *high* reflects outstanding progress with only minor knowledge gaps. The second scale, also ranging from *low* to *high*, reflects our perceived measure of scientific consensus among the reviewed literature, where *low* level of consensus indicates that within the analyzed literature publications were identified expressing opposite or conflicting conclusions with respect to a research topic, and *high* level of consensus indicates that the majority of the reviewed publications are consistent. The last scale indicates the perceived level of concern from the public health community derived from our analysis of the literature. In this scale a *low* level of concern is linked to a substantial amount of progress in the field(s) related to the question and existing scientific consensus (e.g., medium – high), while a *high* level of concern is linked to a combination of low progress in the field related to the question and lack of scientific consensus. In some cases, the level of concern is denoted as *highly variable*, which indicates that for there are specific ENMs or contexts, for which substantial evidence to guide decision-making or estimate potential risks is available, but in other cases/contexts, substantial information gaps still exist. In the last section, we summarize selected

examples of lessons learned from investments in Nano EH&S and their relevance to conventional chemicals and/or other chemicals of emerging concern.

IMPLICATIONS OF PROGRESS IN NANO EH&S FOR THE PUBLIC HEALTH COMMUNITY

1. **Have policy changes been put into place at state, federal and international levels to protect human health from engineered nanomaterials (ENMs)?** To protect public health and the environment from the potential impacts of ENMs, several policy changes have taken place over the last two decades. See **Figure 4.1**. These policy changes have been driven by research advances (e.g., the improvement of analytical methods for characterization of ENMs, the adaptation and implementation of in silico toxicology approaches for ENM testing, among others) and collaborative efforts (e.g., research consortia devoted to standardization of toxicity testing, such as Nano Go Consortium (Xia et al. 2013), Quality Nano (Hole et al. 2013), and the International Alliance for Nano EHS (IANH) (Roebben et al. 2011)). It has been reported that policy documents (e.g., reports and analyses conducted by government agencies, international organizations, industry and advocacy oriented NGO's) can be used to measure the level of action toward responsible development of nanotechnology (Laas and Weil 2014). In their review, Laas et al. identified 63 reports from different organizations, with over 65% addressing Nano EH&S concerns. Further analysis indicated that the difference between work conducted in the European Union and the U.S. was the European focus on precautionary approaches relative to the U.S. focus on research of effects elicited by ENMs (Laas and Weil 2014).

At the national level, in the U.S., the EPA began their efforts to protect public health with a public meeting in 2005 to discuss the role of Nanotechnology within the Toxic Substances Control

Act (TSCA) (70 FR 24574) (EPA 2005). As a starting point, EPA developed a voluntary pilot information program (EPA 2005), which evolved to a stewardship program to incorporate ENMs into the Toxic Substances Control Act (TSCA) (Savage et al. 2007). The EPA later expanded these efforts to an information gathering rule on new and existing nanomaterials and pre-manufacture notices (PMNs) for new ENMs. These action aimed at ensuring that ENMs are manufactured and used in a manner that protects against unreasonable risks to human health and the environment (Bergeson and Plamondon 2007). As part of this regulatory approach, EPA has issued "significant new use rules" (SNURs) under section 5(a)(2) of the Toxic Substances Control Act (TSCA) for multi-walled Carbon Nanotubes (MWCNT) and single-walled Carbon Nanotubes SWCNTs, which were the subject of pre-manufacturing notices (EPA 2010). In 2016, the authority of EPA was further expanded with the amendment of TSCA (Frank R. Lautenberg Chemical Safety for the 21st Century Act) (CONGRESS 2016). The amendment of this act gave EPA the authority to require reporting, record-keeping and testing requirements and restrictions relating to chemical substances and/or mixtures. EPA now has the ability to regulate all industrial chemicals regardless of any material or form, which includes nanoscale materials. An innovative element to this act is the inclusion of Alternative Testing Strategies (ATS), computational toxicology, bioinformatics, high-throughput screening (HTS) and in vitro studies to inform regulatory decisions and support safer by design approaches (Nel and Malloy 2017).

Policy changes have been seen throughout Europe, where various international and European organizations (e.g., the European Commission (EC), the European Parliament (EP) and Working Parties of the OECD) have acted to develop regulations to address potential risks associated with ENMs. Initiatives such as the existing regulatory framework of Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) allow the EC to provide rules in terms of

risk management of ENMs (McManus and Eijmberts 2017). Specific regulatory measures in this context include products safety testing, market authorization, data-disclosure and labelling for ENMs and nano-enabled products (Justo-Hanani and Dayan 2015). These regulatory actions have been evolving since 2005 when the EC adopted an action plan for the development and regulation of nanotechnology (McManus and Eijmberts 2017) to address nanotechnology workers' health to the list of concerns. Since 2005, a particular regulatory change was the integration of nano-specific provisions into regulation (EC) no. 1333/2008 on food additives and cosmetics regulation (Regulation (EC) no.1223/2009) (Schwirn et al. 2014). This regulation also called on the EU to compile a publicly available catalogue of all ENMs used in cosmetic products placed on the market, including those used as colorants, UV filters, and preservatives (Schwirn et al. 2014). By 2010, the EC amended the cosmetics directive to include safety assessment of ENMs in cosmetic products, pre-market notification, labeling and compulsory registration (Justo-Hanani and Dayan 2015). Finally, a major milestone was reached when in 2012, the EC announced a review of REACH with consideration of specific relevant nano-criteria for market entry (e.g., previously registered materials such as carbon and graphite would require new chemical registrations for their nano-forms). To help promote compliance with these regulations, the European Union has implemented technical assistance through the Seventh Framework Program (FP7) and Horizon 2020 (Justo-Hanani and Dayan 2015). The Horizon 2020 and the FP7 programs include all the European research initiatives aimed at strengthening efforts for employment, growth and competitiveness in Europe. Horizon 2020 is a European program designed to support the safe deployment of different technologies, including nanomaterials to the consumer market through different areas such as industrial development, science and policy, and societal challenges (e.g., health and wellbeing, and the environment) (EC 2018).

The implementation of the above-mentioned policy programs is a clear example of progress achieved in terms of protection of public health and the environment. However, it is acknowledged that the nature of the existing and emerging policies is subject to changes in the political arena. For instance, while a major milestone has been achieved in the U.S. with the passing of the TSCA amendment (Frank R. Lautenberg Act), other changes to environmental protection regulations in the U.S. (e.g., withdrawal from the Paris Climate Accord, revocation of the Clean Power Plan to reduce emissions, and modification to certain pesticide regulations) proposed by the current administration (Dillon et al. 2018) may present serious obstacles to public health protection, and there is still continued reason for concern.

In summary, based on the reviewed information, the following scores are proposed for Question 1 (Have policy changes been put into place at state, federal and international levels to protect human health from engineered nanomaterials (ENMs)?):

- Number of studies, amount of work in this area: *High*
- Level of scientific consensus in reviewed studies: *High*
- Current level of concern vis a vis public health: *Medium*

2. Do engineered nanomaterials pose novel threats to human health that differ fundamentally from those of conventional chemicals? To answer this question, it is important to emphasize that ENMs are very diverse and their potential threats to human health depend on multiple factors including their properties and their potential for exposure. In occupational settings, seminal studies (NIOSH 2011, NIOSH 2013, Shi et al. 2013) on several high profile ENMs have demonstrated that there are some nanomaterials that are more toxic than their micron-sized or conventional analogs. Examples of these materials include various types of carbon nanotubes,

nano TiO_2 and carbon nanofibers (CNF) (NIOSH 2011, NIOSH 2013, Shi et al. 2013). As a result, the recommended exposure limits (RELs) for these materials are significantly lower than those of their micron-sized or conventional analogs (Shi et al. 2013).

However, there are also cases, in which the size of nanomaterials was not found to contribute to the main mechanism of toxicity. A classic example of the above is nano Ag, whose primary toxicity has been closely related to the release of silver ions (Ag^+) (McShan et al. 2014).

Furthermore, the ability to measure ENMs toxicity under specific exposure conditions has been facilitated by developments in ENM characterization including the impact of their physicochemical properties on toxicity, and how the influence of properties on toxicity differ from those of conventional analogs (non-nano-sized materials or conventional chemicals). The development of standardized protocols/materials, sample preparation and testing for ENMs has been an important driver in establishing the relationship between physicochemical properties and toxicity. Furthermore, the progress in the field of ENM characterization, via the application of novel analytical techniques (e.g., SEM, TEM, and AFM) has made it possible to improve the quality and reliability of studies focused on potential environmental impacts. Likewise, major advances have been made in our ability to assess the hazards of ENMs. Advances in predictive (in silico) toxicology and alternative testing strategy (ATS) approaches and their application to ENMs has helped to address the need for assessing the hazard potential of large numbers of emerging nanomaterials. A cost analysis by Choi et al. estimated that the costs of thoroughly testing new ENMs that currently exist would be between $249 million to $1.18 billion (Choi et al. 2009) and would take 34-53 years (Choi et al. 2009). The application of in silico methods, such as QSARs and HCS/HTS, has made it possible for scientists to rapidly identify hazard traits of new ENMs and reduce the time and costs of testing. Moreover, by incorporating the use of predictive

toxicology approaches/ATS in regulations (e.g., the Frank R. Lautenberg Act in 2016), decision-makers are taking a step toward improving the efficiency of safety screening processes for emerging ENMs.

While the methodologies to assess the hazard potential of ENMs are under continuous improvement, and the advances in characterization of ENMs have been key in developing a more comprehensive understanding of the hazard potential of ENMs, experts recommend careful review of the scientific evidence for development of regulatory policies (Nowack et al. 2011) and examination of hazard potential of ENMs on a case by case basis.

In summary, based on the reviewed information, the following scores are proposed for Question 2 (Do engineered nanomaterials pose novel threats to human health that differ fundamentally from those of conventional chemicals?):

- Number of studies, amount of work in this area: *High*
- Level of scientific consensus in reviewed studies: *Low*
- Current level of concern vis a vis public health: *Highly variable*

3. Can conventional approaches to risk assessment that were developed for conventional chemicals/materials be applied to ENMs? Although risk assessment methods for chemicals are well established, their adoption and/or adaptation for ENMs would require consideration of various issues that include, but are not limited to: (a) the behavior of ENMs in various media (e.g., dissolution, agglomeration/aggregation, adsorption); (b) persistence (techniques to predict aspects of degradation of certain ENMs; (c) transportation and distribution of ENMs across media; (d) predicted environmental concentrations (PECs) and ENM transformation products and impurities;

(e) bioaccumulation of ENMs; and (f) effects/predicted no effect concentration (PNEC) (Cohen et al. 2012).

With regard of specific data needs for risk assessment of ENMs, these include: (i) ENMs hazard properties, (ii) ENMs dose-response and dosimetry metrics, (ii) production volume and emission rates (including modes of release) of ENMs, (iii) environmental transformations, and (iv) distribution of ENMs in the environment and associated multimedia exposure levels.

At present quantitative risk assessment, integration of frameworks into decision-support tools, and available information to conduct risk assessments are still subject to data limitations. One example of current data limitations is quantitative exposure assessment, which involves the measurement/estimation of environmental concentrations, and establishment of dose response relationships for ENMs. Another example of limitations for quantitative risk assessment involves the application of certain QSAR models, which must be adopted for relevant endpoints to be validated according to OECD guidelines (Burello 2017). One example of a challenge in quantitative risk assessment is the current need for the incorporation of the existing frameworks and methodologies developed for risk assessment into actual decision-support tools that would allow analysts to conduct environmental impact assessments. Lastly, an example of the information that still remains scarce is the content of ENMs in commercial products (e.g., patents that prevent the disclosure of formulations including ENMs), which would allow for improved calculations of release and exposure.

In short, there are suitable frameworks to assess potential risks of ENMs, either adapted from chemical risk assessment or developed as novel approaches to include the implications of the physicochemical properties of ENMs and subsequent transformations in the environment. However, existing approaches to conduct an environmental impact/risk assessment are feasible

within specific contexts/scenarios (e.g., specific conditions of exposure/receptors) and are subject to the availability of information for the ENM under analysis (Romero-Franco et al. 2017). Furthermore, in the analysis presented here, existing approaches to conduct environmental impact/risk assessment of ENMs were reviewed from peer-review publications. This in turn may result in some frameworks not being readily available for public use or adapted to specific governance contexts (Trump et al. 2018).

In summary, based on the reviewed information, the following scores are proposed for Question 3 (Can conventional approaches to risk assessment that were developed for conventional chemicals/materials be applied to ENMs?):

- Number of studies, amount of work in this area: *Medium-High*
- Level of scientific consensus in reviewed studies: *High*

Current level of concern vis a vis public health: *Low*

4. Are people and/or the environment currently exposed to concentrations of ENMs that pose significant risk (or are such exposures expected in the near future)? To answer this question, it is necessary to breakdown the potential for exposure to ENMs into three major categories: occupational settings, human contact with consumer products containing ENMs, and environmental exposure derived from intentional or unintentional release of ENMs.

In workplace settings, major advances have been in terms of exposure assessment, particularly for inhalation exposure. These advances have led to implementation of exposure control measures to mitigate risk (see **Question 6**). Several seminal studies related to hazard potential due to inhalation exposure have been carried out in occupational settings (Schulte et al. 2016). Furthermore, at the national level, NIOSH has taken steps to protect workers' health by

establishing RELs for certain carbon based materials (NIOSH 2013) and nano TiO_2 (NIOSH 2011).

In the context of human exposure related to direct contact with consumer products containing ENMs, it is important to consider that not all contact with products containing ENMs will result in exposure. For example, human exposure to ENMs could occur directly from contact with commercial products via inhalation (e.g., from cleaning aids, spray cosmetics and coatings and dermal penetration (e.g., from cosmetics) (Tiede et al. 2016). However, the potential for human exposure from direct contact with products that contain ENMs in a solid matrix (e.g., electronics) is very unlikely (Hansen et al. 2008). Several models have been developed to estimate exposure to ENMs in sprays and cosmetics (Nazarenko et al. 2011, Nazarenko et al. 2012). However, the extent to which these exposure could lead to harmful effects has not been completely established. Furthermore, in cases such as nano TiO_2 used in sunscreen lotion the evidence regarding dermal absorption of nano TiO_2 has not been conclusive (EPA 2010). In addition, Nano EH&S professionals have developed approaches to produce ENMs following "safer by design" principles that allow for materials to be screened for safety (at the same time that they are screened for efficacy) to address concerns regarding exposure via intravenous routes (e.g. some medicines and diagnostic aids such as coating of silica nanoparticles for pancreatic cancer treatment (Liu et al. 2016)).

Advances in our understanding of the environmental fate and transport of ENMs have provided critical insights into the potential for human exposure to ENMs. In the ENM exposure assessment field, major advances include development of modeling approaches (e.g., probabilistic and mechanistic models) to estimate environmental concentrations of ENMs in different environmental compartments. However, a critical parameter to improve ENM exposure

assessment is the ability to directly estimate/measure and quantify environmental releases and concentrations in environmental media (Nowack 2017). Despite advances made in assessing the exposure potential of ENMs, the models developed for estimating the environmental concentrations of ENMs still need to be further validated with field measurements. This remains challenging because instruments for measuring exposures to ENMs in situ (environmental and biological monitoring) are still under development.

Overall, our current knowledge of the potential exposures to ENMs in the environment and human population has increased significantly over the last two decades (e.g., development of modeling approaches to estimate concentrations in different media) (Gottschalk et al. 2010, Sun et al. 2014, Liu et al. 2015), and extensive progress has been achieved in occupational health of ENMs (e.g., proposed occupational exposure limits (OEL)) (Kuempel et al. 2012, Schulte et al. 2016). However, information is still needed in terms of improved data regarding quantitative ENM environmental releases (Cohen et al. 2012, Nowack 2017) and ENM concentrations in commercial product formulations (Cuddy et al. 2015).

In summary, based on the reviewed information, the following scores are proposed for Question 4 (Are people and/or the environment currently exposed to concentrations of ENMs that pose significant risk (or are such exposures expected in the near future)?):

- Number of studies, amount of work in this area: *Medium*
- Level of scientific consensus in reviewed studies: *Low*
- Current level of concern vis a vis public health: *Highly variable*

5. **Are there sufficient data to be able to assess the risks associated with ENMs?** One of the biggest challenges in assessing the potential risks of ENMs has been the difficulty associated with determining whether or not there is sufficient information to conduct a risk assessment for a specific ENM (e.g., type and composition) and the desired context of the assessment (e.g., decision context and exposure scenarios). To provide a systematic process by which an analyst can determine when sufficient data are available to perform such an analysis, the IANano approach is presented in **Chapter 3** of this thesis. Also in **Chapter 3** the application of IANano to assess information adequacy for three ENMs was demonstrated (i.e., nano Cu-CuO, nano TiO_2 and nano ZnO) in a series of scenarios. This assessment revealed that the current body of knowledge, based on analysis of 274 peer review publications through 2016, is reasonable for conducting occupational risk assessment for nano TiO_2. In all the studied cases, the analysis demonstrated that additional information will be required regarding the fate and transport, environmental release and exposure data as the progress of the field of exposure assessment is still not up to par with that of hazard assessment of ENMs.

In summary, it is not possible to make a general statement with regard to the level of information availability to assess the risks associated with *all* existing ENMs as the extent to which information is suitable to conduct an environmental impact/risk assessment has to be determined on a case by case basis.

Based on the reviewed information, the following scores are proposed for Question 5 (Are there sufficient data to be able to assess the risks associated with ENMs?):

- Number of studies, amount of work in this area: *Medium*
- Level of scientific consensus in reviewed studies: *Low*
- Current level of concern vis a vis public health: *Highly variable*

6. Are there sufficient knowledge and tools to enable minimization of exposures and mitigate risks associated with ENMs? The risk assessment studies on ENMs to date have also led to the design and implementation of risk management strategies for some of these materials. These efforts have been most prominent for occupational settings. Projects such as NEAT (Nanoparticle Emission Assessment Technique) (Methner et al. 2010) have been useful in evaluating emissions and identifying engineering controls to minimize ENM emissions in industrial facilities and reduce worker exposure, and occupational exposure levels (OEL) derived from benchmark approaches (Kuempel et al. 2012) have been instrumental for establishing recommendations for exposure limits.

Overall, the implementation of specific risk mitigation strategies (e.g., exposure control) can be considered a major advance towards the protection of workers' health. However, the existing information gaps, for environmental exposures and or exposure via consumer products, require further research to expand the development and dissemination of risk mitigation strategies outside occupational settings.

In summary, based on the reviewed information, the following scores are proposed for Question 6 (Are there sufficient knowledge and tools to enable minimization of exposures and mitigate risks associated with ENMs?):

- Number of studies, amount of work in this area: *Medium-Low*
- Level of scientific consensus in reviewed studies: *Medium*
- Current level of concern vis a vis public health: *Medium*

Lessons that we have learned from our investments in Nano EH&S and their relevance to conventional chemicals and/or other chemicals of emerging concern

Lessons from the field of Nano EH&S that could be used to improve oversight of other emerging technologies and or chemicals include:

- *It is important to conduct context-dependent safety assessments.* A major consideration to be made when assessing potential impacts of ENMs is the specific exposure scenario/conditions and the exposure targets (e.g., the conditions of the hazard and exposure assessment studies should reflect, to the extent possible, realistic conditions in the environment) (Holden et al. 2016). For instance, the conditions under which an ENM is assessed will determine the specific information needed and the methodology or framework to be applied (Romero-Franco et al. 2017, Schimpel et al. 2018). These same considerations could be reasonably expected to be true for many of the chemicals and materials of emerging concern and can help to overcome concerns that arise when confronted with insufficient data to perform an all-encompassing assessment of risk potential.
- *The level of validation and characterization for hazard and exposure studies of ENMs required depends on the purpose or decision-context in which the information is being used.* In this regard, the OECD technical guidelines (TGs) or ISO standards can be used to dictate the extent of testing required. However, not all assays/tests included in the above mentioned guidelines are practical or appropriate for ENMs, as this depends on the regulatory or decision-making context (Hjorth et al. 2017). Thus, standard reference materials and standard protocols have been developed for ENMs that may not necessarily meet OECD or ISO standards but can still be useful in a wide range of contexts. These

standard reference materials and standardized protocols can be applied as long as the conclusions made from their use are consistent with the purpose for which they have been validated ("fit for purpose") (Nel et al. 2015). For instance, the European Commission recommended fit-for-purpose analytical methods in cases where consumer products are tested to determine whether these products contain ENMs and the resulting information is used to enforce labeling requirements (Stamm et al. 2012). The "fit for purpose" selection of assays and validation approaches has been broadly embraced by the ATS community (ICCVAM 2018) and it is envisioned such approach should greatly improve the efficiency in the ability to assess the hazard potential of ENMs, as well as other emerging chemicals of concern.

- *Strategic allocation of funding for EH&S research can help drive scientific and policy milestones.* Increases in funding allocated for Nano EH&S over the last two decades has helped to drive critical scientific and policy milestones. Starting in 2005, calls made by various organizations to increase Nano EH&S funding to meet the emerging research needs (Denison 2005) resulted in allocation of significant additional funds to strengthen the research efforts in areas such as the ethical, legal and societal implications (ELSI) of nanotechnology. This work has promoted education, outreach and involvement of stakeholders, which has in turn influenced public perceptions of the risks of nanotechnology.
- *Better integration of science into decision-making* is another important element into the safe deployment of nanotechnology that can be extrapolated to other emerging chemicals and vice versa. Bosso et al. has argued that products containing ENMs have arrived to market with a mature regulatory framework of statutes and rules that have resulted from

regulatory actions toward toxic chemicals (Bosso 2016). However, the outputs of risk assessment and life cycle analysis of ENMs feed into decision-making, such approaches are meant as decision-support tools and the weight of making regulatory decisions falls into the decision-makers/regulators, who must take into account the current state of data limitations that restrict quantitative assessments (Hjorth 2017). Incorporating input from multi-stakeholder groups (e.g., representatives from industry, government agencies, NGOs, and academia) can help to strengthen the decision-making process. For this purpose, qualitative approaches have been proposed to provide guidance on communication among stakeholders of how alternative testing strategies (ATS) can be incorporated into decision-making (Godwin et al. 2015). Furthermore, the incorporation of ATS into the TSCA amendment to inform decisions, and the change in key regulatory definitions (to be more inclusive of all the nano-sized materials) (Nel and Malloy 2017), is expected to further strengthen the link between science and decision-making. In summary, regulatory decision-making requires the involvement of key stakeholders (and understanding how these stakeholders manage risks associated with ENMs) to achieve safer deployment of nanotechnology. Hence, a key lesson learned with regard to regulatory decisions affecting ENMs is to encourage and improve communication between the scientific and regulatory communities to design research strategies that could be more effectively applied into decision-making.

CONCLUSIONS

The unique properties of ENMs and their applications are tied to promising benefits tied but also to potential hazards, particularly given the novel ways that ENMs interacts with biological entities and the environment. This has resulted in increasing concerns from key stakeholders, and

addressing these concerns is necessary not only to improve public perception but also to ensure ethical and safe deployment of nanotechnology. Over the last decade and a half, the field of Nano EH&S has undergone significant growth, thus, communication strategies to connect key findings of this community with public health practitioners are needed. This work aims at addressing overarching questions of concern posed by the public health community by providing public health practitioners with a qualitative analysis of key progress in the field of Nano EH&S.

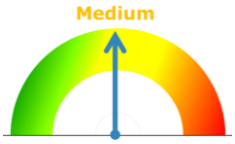
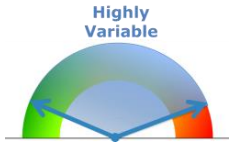
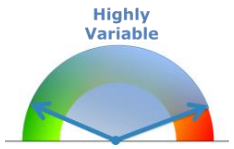
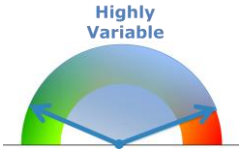
Significant progress in our understanding of the potential environmental and human health impacts of ENMs have been addressed by a large body of studies on toxicity of ENMs and ongoing development of sophisticated models to predict environmental fate and transport of ENMs in order to estimate exposure to these materials. Our understanding/assessment of the potential effects of specific ENMs is subject to the information available on exposure and hazard for a particular class of ENMs. Moreover, the application of the available information to conduct environmental impact/risk assessments for ENMs is dependent on what the likely exposure scenario/conditions are, as well as decision-making contexts being considered. While extensive information exists regarding occupational exposure to certain ENMs, and subsequent potential health effects, additional information is still required for many environmental exposure routes and for many classes of ENMs that have not yet been studied extensively.

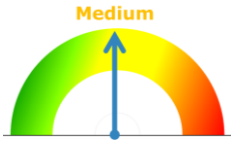
Early public health concerns/needs included the determination of appropriateness of existing risk assessment framework/approaches for ENMs. Progress has been achieved in terms of standardization of toxicity testing/characterization protocols of ENMs, development of models/tools and approaches to characterize the presence of ENMs in the environment and their potential impacts. However, additional work is still required to improve quantitative

measurement/tracking of ENMs in the environment (e.g., accounting for naturally-occurring nano-sized particles).

One of the goals of this chapter has been to communicate the advances in the field of Nano EH&S as it pertains to questions relevant to the public health community. In doing so, the research highlights/advances analyzed here serve as scientific basis for better informing decision-making and regulatory responses. It is envisioned that by incorporating high-quality scientific evidence into this process, the growth of commercial applications of nanomaterials can continue while also ensuring the safety of consumers.

Table 4.1 Summarized responses to questions relevant to the public health community regarding Nano EH&S

Question	Number of studies/ amount of work in this area	Level of scientific consensus in reviewed studies	Current level of concern vis a vis Public Health	Comments
1. Have policy changes been put into place to protect human health from engineered nanomaterials (ENMs)?				Major advances have been accomplished in terms of policies to protect public health. In Europe, these policies have expanded to labeling consumer products containing ENMs.
2. Do ENMs pose novel threats to human health that differ fundamentally from those of conventional chemicals?				Highly dependent on type of material and context/scenarios.
3. Can conventional approaches to risk assessment that were developed for conventional chemicals/materials be applied to ENMs?				Adequate frameworks exist; practical implementation tools for specific contexts/decision scenarios are still needed.
4. Are people and/or the environment currently exposed to concentrations of ENMs that pose significant risk (or are such exposures expected in the near future)?				Highly dependent on type of the material and context/scenarios.
5. Are there sufficient data to be able to assess the risks associated with ENMs?				Highly dependent on type of the material and context/scenarios.

Question	Number of studies/ amount of work in this area	Level of scientific consensus in reviewed studies	Current level of concern vis a vis Public Health	Comments
6. Are there sufficient knowledge and tools to enable minimization of exposures and mitigate risks associated with ENMs?				Advances made in occupational settings. Information is still needed for environmental risks/subject to the availability of improved quantitative assessments.

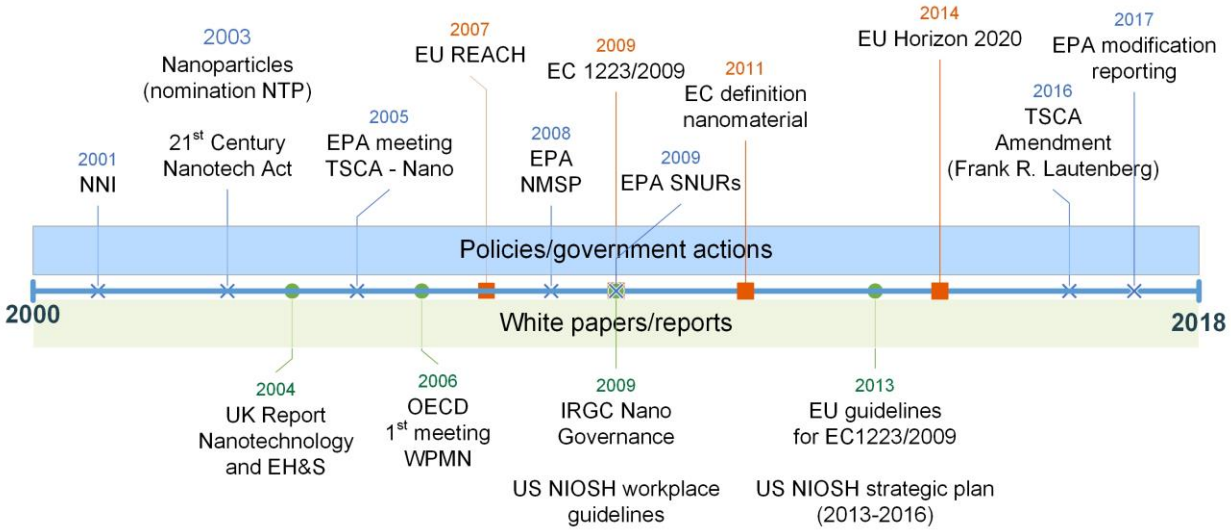


Figure 4.1 Timeline showing selected policies/government agencies' actions related to Nano EH&S in the U.S. and Europe

Note: The rectangles shown in orange represent policies developed in the European Union (EU) related to Nano EH&S. The marks in blue represent policies or policy-related events in the U.S. The green circles below the timeline represent important white papers or reports related to Nano EH&S policy.

Table 4.2 Summarized description of the policy events related to Nano EH&S over the last decade and a half shown in Figure 4.1

Year	Name of the policy/ action	Description/relevance to progress in Nano EH&S
2001	▪ Launch of the U.S. National Nanotechnology Initiative (NNI)	▪ The NNI was established as an organization for communication, cooperation, and collaboration for all federal agencies engaged in nanotechnology research (www.nano.gov)
2003	▪ 21st Century Nanotechnology Research and Development Act	▪ This Act included allocation of federal funding for Nano EH&S. As part of this act, the Nanoscale Science, Engineering, and Technology (NSET) subcommittee was required to develop categories of investment called Program Component Areas (PCA) to provide a means by which Congress and the executive branch could be informed of and direct the relative investments in these areas. The activities performed through the PCAs were required to ensure U.S. global leadership in the development and application of nanotechnology and advance the productivity and industrial competitiveness, while taking specified steps to ensure that ethical, legal, environmental, and other appropriate societal concerns are considered during the development of nanotechnology (NSET 2006)
	▪ NTP nomination of nanomaterials for testing	▪ In 2003, the Center for Biological and Environmental Nanotechnology (CBEN) nominated ENMs for testing by the National Toxicology Program (NTP) (Colvin 2003), which resulted in studies by the NTP evaluating nanoparticle translocation, characterizing the inhalation toxicology of high aspect ratio materials and determining the immune responses to ENMs (Colvin 2003)
2004	UK Royal Society and the Royal Academy of Engineering publication Nanoscience and Nanotechnologies	▪ This was one of the earliest reports that focused on the need to address potential health, environmental, social, ethical, and regulatory issues associated with nanotechnology. In this report, the UK Royal Academy of Engineering also provided definitions for nanoscience and ‘nanotechnologies (Maynard 2007)
2005	Initial public meeting on TSCA and Nanotechnology	▪ This was the first meeting held by U.S. EPA to discuss a potential voluntary pilot program for certain nanoscale materials and the information needed to adequately inform the pilot program (EPA 2005). The meeting was followed by the publication of the EPA Nanotechnology white paper, which aimed at informing EPA management of the science needs associated with nanotechnology, to support related EPA program office needs, and to communicate these nanotechnology science issues to stakeholders and the public (EPA 2005).

Year	Name of the policy/ action	Description/relevance to progress in Nano EH&S
2006	OECD 1st Meeting of the Working Party on Manufactured Nanomaterials (WPMN)	▪ The main objective of the 1st Meeting of the Working Party on Manufactured Nanomaterials (WPMN) was to agree a draft program of work, for 2006-2008. Another objective of the meeting was to collect information from member countries (delegates) on current or planned developments on the safety of manufactured nanomaterials in their countries or organizations (OECD 2006). One of the resulting projects of this initiative, was published in 2007: a collaborative framework "NanoRisk" was launched by DuPont aiming at providing guidance on the analysis of exposure and hazard potential of nanomaterials (DUPONT 2007)
2007	REACH passed in EU	▪ The passing of the REACH legislation (Registration, Evaluation, Authorization and Restriction of Chemicals) raised concerns about the fact that nanoparticles and nanomaterials were not specifically mentioned or defined in the legislation. Also, concerns were related to the fact that nanomaterials may not be considered as a new substance/substance of concern under such legislation given their production levels and their chemical formulation (Handy and Shaw 2007)
2008	EPA Nanomaterial Stewardship program (NMSP)	▪ The Nanomaterial Stewardship Program (NMSP) was announced, by the Assistant Administrator of the Office of Prevention, Pesticides and Toxic Substances (OPPTS), to ''complement and support [the] new and existing chemicals programs under the Toxic Substances Control Act (TSCA)''. The development of this program was based in part on input received from EPA's National Pollution Prevention and Toxics Advisory Committee, including a document outlining a Nanoscale Materials Voluntary Program delivered to the Agency in December 2005. The Stewardship Program was developed to enable participating companies that produce or use nanoscale materials, to submit, or in some cases, generate data to help EPA determine the hazards of nanoscale materials that are subject to regulation under TSCA (EPA 2007)

Year	Name of the policy/ action	Description/relevance to progress in Nano EH&S
2009	▪ Regulation (EC) N° 1223/2009 in EU	▪ The 2009 Regulation (EC) N° 1223/2009 on cosmetic products (the main regulatory framework for finished cosmetic products when placed on the EU market) was adopted to replace the Directive 76/768/EC, dated from 1976 to include ENMs and other emerging chemicals. EC 1223/2009 provided a regime to reinforce product safety taking into consideration the possible use of nanomaterials. The updated rules for the use of nanomaterials in cosmetic products included that products containing other nanomaterials not otherwise restricted by the Cosmetics Regulation were the object of a full safety assessment at EU level if the Commission has concerns. Also, ENMs in cosmetics must be labeled in the list of ingredients with the word "nano" in brackets following the name of the substance, e.g. "titanium dioxide (nano)" (EC 2009)
	▪ International Risk Governance Council (IRGC) Policy Brief on Appropriate Risk Governance Strategies for Nanotechnology Applications in Food and Cosmetics	▪ This policy brief was targeted at policy makers engaged in the planning, oversight, and funding of nanotechnology regulation, research and practical applications. It was aimed at assisting risk decision makers in developing the processes and regulations that are essential to assuring the development and public acceptance of the many benefits that nanotechnology promises to deliver (IRGC 2007)
	▪ U.S. National Institute for Occupational Safety and Health (NIOSH) publishes interim safety guidelines for working with nanomaterials in the workplace	▪ This report entitled "Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials" summarizes work conducted by NIOSH up to the publication date to assess potential hazards for human health resulting from exposure to nanomaterials in the workplace (Hodson et al. 2009).
	▪ EPA introduces Significant New Use Rules (SNURs) for multi-walled carbon nanotubes (MWCNTs)	▪ These rules (SNURs) provide additional information for industry on uses of multi-walled carbon nanotubes that are considered new activities and require the submission of pre-manufacturing notices to the EPA (EPA 2010)
2011	▪ European Commission recommendation on the definition of "Nanomaterial" for regulatory purposes	▪ The European Commission agreed on a single definition for ensuring conformity across legislative areas and sectors and made a first legal step toward harmonizing the nanomaterials trade in EU markets (Justo-Hanani and Dayan 2014)
2013	▪ EU Publication of guidelines for reporting safety information of ENMs in cosmetic products in the context of EC 1223/2009	▪ In these guidelines, it was established that the information reported under EC 1223/2009 should contain: "quantitative and qualitative composition of the cosmetic product (including the Physicochemical characteristics of substances or mixtures especially for nanomaterials), and exposure to the substances in the products, as well as the toxicological profile of the substances used" (EC 2013)
	▪ U.S. National Institute of Occupational Health and Safety (NIOSH) publishes	▪ This document was proposed by NIOSH as a roadmap to advance basic understanding of the toxicology and workplace exposures involved so that appropriate risk management practices can be

Year	Name of the policy/ action	Description/relevance to progress in Nano EH&S
	a nanotechnology strategic plan for 2013- 2016	implemented during discovery, development, and commercialization of engineered nanomaterials (NIOSH 2013)
2014	▪ Launch of Horizon 2020 (European Union Eighth Framework Program on Research and Technological Development)	▪ Horizon 2020 is the European Union's flagship program to support research and innovation. It brings previous EU research initiatives under a single umbrella and covers the period 2014-2020. Horizon 2020's leadership in enabling industrial technologies stream includes a focus on nanotechnologies and other emerging technologies (EC 2018)
2016	▪ U.S. TSCA amendment (Frank Lautenberg Act)	▪ With the amendment of TSCA (Frank R. Lautenberg Chemical Safety for the 21st Century Act), the authority of EPA was further expanded to require reporting, record-keeping and testing; as well as impose restrictions relating to chemical substances and/or mixtures, including nanomaterials. This amendment represents a new regulatory paradigm, which involves the consideration of Alternative Testing Strategies (ATS) for regulatory purposes (Nel and Malloy 2017)
2017	U.S. EPA action to modify information reporting for ENMs	▪ Under TSCA recordkeeping requirements (40 CFR Part 704), the EPA modified the recordkeeping requirements for certain chemical substances when they are manufactured or processed at the nanoscale. Specifically, the EPA requires persons that manufacture or process, or intend to manufacture or process these chemical substances to electronically report to EPA certain information, which includes insofar as known to or reasonably ascertainable by the person making the report, the specific chemical identity, production volume, methods of manufacture and processing, exposure and release information, and existing information concerning environmental and health effects. This rule involves one-time reporting for existing discrete forms of certain nanoscale materials, and a standing one-time reporting requirement for new discrete forms of certain nanoscale materials before those new forms are manufactured or processed (EPA 2017)

REFERENCES FOR CHAPTER 4

Bergeson, L. L. and J. E. Plamondon (2007). "TSCA and Engineered Nanoscale Substances." Nanotechnology Law & Business **4**: 51.

Bosso, C. (2016). "Settling into the Midstream? Lessons for Governance from the Decade of Nanotechnology." Journal of Nanoparticle Research **18**(6): 163.

Burello, E. (2017). "Review of (Q)SAR Models for Regulatory Assessment of Nanomaterials Risks." NanoImpact **8**: 48-58.

Choi, J.-Y., G. Ramachandran and M. Kandlikar (2009). The Impact of Toxicity Testing Costs on Nanomaterial Regulation, ACS Publications.

Cohen, Y., R. Rallo, R. Liu and H. H. Liu (2012). "In Silico Analysis of Nanomaterials Hazard and Risk." Accounts of Chemical Research **46**(3): 802–812.

Colvin, V. (2003). NTP Testing Nomination for Nanoscale Materials. N. T. Program/NIEHS. Houston, Texas.

US. Congess (2016). Frank, R. Lautenberg Chemical Safety for the 21st Century Act. Public Law 114–182—JUNE 22, 2016

Cuddy, M. F., A. R. Poda, R. D. Moser, C. A. Weiss, C. Cairns and J. A. Steevens (2015). "A Weight-of-Evidence Approach to Identify Nanomaterials in Consumer Products: A Case Study of Nanoparticles in Commercial Sunscreens." Journal Of Exposure Science And Environmental Epidemiology **26**: 26.

Denison, R. A. (2005). "A Proposal to Increase Federal Funding of Nanotechnology Risk Research to at Least $100 Million Annually." Washington, DC: Environmental Defense.

Dillon, L., Sellers, C., Underhill, V., Shapiro, N., Ohayon, J.L., Sullivan, M., Brown, P., Harrison, J., Wylie, S. and "EPA Under Siege" Writing Group, (2018). "The Environmental Protection Agency in the Early Trump Administration: Prelude to Regulatory Capture". American Journal of Public Health, *108*(S2), pp.S89-S94.

DUPONT. (2007). "Nanorisk Framework." from http://www.nanoriskframework.org/.

EC (2009). Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products 1223/2009. E. Parliament.

EC (2013). 2013/674/Eu: Commission Implementing Decision of 25 November 2013 on Guidelines on Annex I to Regulation (Ec) No 1223/2009 of the European Parliament and of the Council on Cosmetic Products E. Parliament.

EC (2018). "Horizon 2020." Retrieved January 23, 2018, from http://ec.europa.eu/programmes/horizon2020/en.

EPA (2005). Environmental Protection Agency Nanotechnology White Paper: External Review Draft, EPA.

EPA (2007). Concept Paper for the Nanoscale Materials Stewardship Program under TSCA (Supporting & Related Material Document) epa-Hq-Oppt-2004-0122-0058. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2004-0122-0058.

EPA (2010). Multi-Walled Carbon Nanotubes and Single-Walled Carbon Nanotubes; Significant New Use Rules. 40 CFR Parts 9 and 721. EPA. **[EPA–HQ–OPPT–2008–0252; FRL–8835–5]**.

EPA (2010). Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen.

EPA (2017). Chemical Substances When Manufactured or Processed as Nanoscale Materials: Tsca Reporting and Recordkeeping Requirements. 40 CFR Part 704.

Godwin, H., C. Nameth, D. Avery, L. L. Bergeson, D. Bernard, E. Beryt, W. Boyes, S. Brown, A. J. Clippinger and Y. Cohen (2015). "Nanomaterial Categorization for Assessing Risk Potential to Facilitate Regulatory Decision-Making." ACS Nano **9**(4): 3409-3417.

Gottschalk, F., R. W. Scholz and B. Nowack (2010). "Probabilistic Material Flow Modeling for Assessing the Environmental Exposure to Compounds: Methodology and an Application to Engineered Nano-TiO_2 Particles." Environmental Modelling & Software **25**(3): 320-332.

Handy, R. D. and B. J. Shaw (2007). "Toxic Effects of Nanoparticles and Nanomaterials: Implications for Public Health, Risk Assessment and the Public Perception of Nanotechnology." Health, Risk & Society **9**(2): 125-144.

Hansen, S., E. Michelson, A. Kamper, P. Borling, F. Stuer-Lauridsen and A. Baun (2008). "Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products." Ecotoxicology **17**(5): 438-447.

Hjorth, R. (2017). "The Shortfall of Risk Assessment for Decision-Making." Nature Nanotechnology **12**(12): 1109.

Hjorth, R., L. M. Skjolding, S. N. Sørensen and A. Baun (2017). "Regulatory Adequacy of Aquatic Ecotoxicity Testing of Nanomaterials." NanoImpact **8**: 28-37.

Hodson, L., M. Methner and R. D. Zumwalde (2009). Approaches to Safe Nanotechnology; Managing the Health and Safety Concerns Associated with Engineered Nanomaterials, NIOSH.

Holden, P. A., J. L. Gardea-Torresdey, F. Klaessig, R. F. Turco, M. Mortimer, K. Hund-Rinke, E. A. Cohen Hubal, D. Avery, D. Barcelo, R. Behra, Y. Cohen, L. Deydier-Stephan, P. L. Ferguson, T. F. Fernandes, B. Herr Harthorn, W. M. Henderson, R. A. Hoke, D. Hristozov, J. M. Johnston, A. B. Kane, L. Kapustka, A. A. Keller, H. S. Lenihan, W. Lovell, C. J. Murphy, R. M. Nisbet, E. J. Petersen, E. R. Salinas, M. Scheringer, M. Sharma, D. E. Speed, Y. Sultan, P. Westerhoff, J. C. White, M. R. Wiesner, E. M. Wong, B. Xing, M. Steele Horan, H. A. Godwin and A. E. Nel (2016).

"Considerations of Environmentally Relevant Test Conditions for Improved Evaluation of Ecological Hazards of Engineered Nanomaterials." Environmental Science & Technology **50**(12): 6124-6145.

Hole, P., K. Sillence, C. Hannell, C. M. Maguire, M. Roesslein, G. Suarez, S. Capracotta, Z. Magdolenova, L. Horev-Azaria, A. Dybowska, L. Cooke, A. Haase, S. Contal, S. Mano, A. Vennemann, J. J. Sauvain, K. C. Staunton, S. Anguissola, A. Luch, M. Dusinska, R. Korenstein, A. C. Gutleb, M. Wiemann, A. Prina-Mello, M. Riediker and P. Wick (2013). "Interlaboratory Comparison of Size Measurements on Nanoparticles Using Nanoparticle Tracking Analysis (NTA)." Journal of Nanoparticle Research **15**: 2101.

ICCVAM (2018). A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States, Interagency Coordinating Committee on the Validation of Alternative Methods.

IRGC (2007). Nanotechnology Risk Governance. Geneva, International Risk Governance Council. http://www.irgc.org/IMG/pdf/PB_nanoFINAL2_2_.pdf

Justo-Hanani, R. and T. Dayan (2014). "The Role of the State in Regulatory Policy for Nanomaterials Risk: Analyzing the Expansion of State-Centric Rulemaking in Eu and Us Chemicals Policies." Research Policy **43**(1): 169-178.

Justo-Hanani, R. and T. Dayan (2015). "European Risk Governance of Nanotechnology: Explaining the Emerging Regulatory Policy." Research Policy **44**(8): 1527-1536.

Kuempel, E. D., V. Castranova, C. L. Geraci and P. A. Schulte (2012). "Development of Risk-Based Nanomaterial Groups for Occupational Exposure Control." Journal of Nanoparticle Research **14**(1029): 1-15.

Kuempel, E. D., C. L. Geraci and P. A. Schulte (2012). "Risk Assessment and Risk Management of Nanomaterials in the Workplace: Translating Research to Practice." Annals of Occupational Hygiene **56**(5): 491-505.

Laas, K. and V. Weil (2014). "Taking a Proactive Approach Towards Responsibility: Indications of Nano Policy-Making around the World, 2001-2008." Annals of Economics and Statistics/Annales d'Économie et de Statistique (115-116): 255-275.

Liu, H. H., M. Bilal, A. Lazareva, A. Keller and Y. Cohen (2015). "Simulation Tool for Assessing the Release and Environmental Distribution of Nanomaterials." Beilstein Journal of Nanotechnology **6**: 938.

Liu, X., A. Situ, Y. Kang, K. R. Villabroza, Y. Liao, C. H. Chang, T. Donahue, A. E. Nel and H. Meng (2016). "Irinotecan Delivery by Lipid-Coated Mesoporous Silica Nanoparticles Shows Improved Efficacy and Safety over Liposomes for Pancreatic Cancer." ACS Nano **10**(2): 2702-2715.

Maynard, A. D. (2007). "Nanotechnology: The Next Big Thing, or Much Ado About Nothing?" Annals of Occupational Hygiene **51**(1): 1-12.

McManus, I. P. and J. Eijmberts (2017). "Multi-Level Governance of Nanotechnology in Europe: Policy Variation in Germany, the Uk, and the Netherlands." European Review **25**(2): 273-294.

McShan, D., P. C. Ray and H. Yu (2014). "Molecular Toxicity Mechanism of Nanosilver." Journal of Food and Drug Analysis **22**(1): 116-127.

Methner, M., L. Hodson, A. Dames and C. Geraci (2010). "Nanoparticle Emission Assessment Technique (Neat) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials—Part B: Results from 12 Field Studies." Journal of Occupational and Environmental Hygiene **7**(3): 163-176.

Nazarenko, Y., T. W. Han, P. J. Lioy and G. Mainelis (2011). "Potential for Exposure to Engineered Nanoparticles from Nanotechnology-Based Consumer Spray Products." Journal of Exposure Science & Environmental Epidemiology **21**(5): 515-528.

Nazarenko, Y., H. Zhen, T. Han, P. J. Lioy and G. Mainelis (2012). "Potential for Inhalation Exposure to Engineered Nanoparticles from Nanotechnology-Based Cosmetic Powders." Environmental Health Perspectives **120**(6): 885-892.

Nel, A. E. and T. F. Malloy (2017). "Policy Reforms to Update Chemical Safety Testing." Science **355**(6329): 1016-1018.

Nel, A. E., W. J. Parak, W. C. W. Chan, T. Xia, M. C. Hersam, C. J. Brinker, J. I. Zink, K. E. Pinkerton, D. R. Baer and P. S. Weiss (2015). "Where Are We Heading in Nanotechnology Environmental Health and Safety and Materials Characterization?" ACS Nano **9**(6): 5627-5630.

NIOSH (2011). Occupational Exposure to Titanium Dioxide**:** 119.

NIOSH (2013). Occupational Exposure to Carbon Nanotubes and Nanofibers.

NIOSH (2013). Protecting the Nanotechnology Workforce: Niosh Nanotechnology Research and Guidance Strategic Plan, 2013–2016. Cincinnati, OH, Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS

Nowack, B. (2017). "Evaluation of Environmental Exposure Models for Engineered Nanomaterials in a Regulatory Context." NanoImpact **8**: 38-47.

Nowack, B., H. F. Krug and M. Height (2011). "120 Years of Nanosilver History: Implications for Policy Makers." Environmental Science & Technology **45**(4): 1177-1183.

NSET (2006). Environmental, Health and Safety Research Needs for Engineered Nanoscale Materials. R. Rusell and R. Cresanti. Washington, D.C., Nanoscale Science, Engineering and Technology Subcommittee Committee on Technology. https://www.nano.gov/node/253

OECD (2006). Current Developments/ Activities on the Safety of Manufactured Nanomaterials. Tour De Table at the 1st Meeting of the Working Party on Manufactured Nanomaterials. London, UK., OECD. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2006)35&doclanguage=en

Roebben, G., S. Ramirez-Garcia, V. A. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, C. M. Garner, A. Rawle, A. Elder, V. L. Colvin, W. Kreyling, H. F. Krug, Z. A. Lewicka, S. McNeil, A. Nel, A. Patri, P. Wick, M. Wiesner, T. Xia, G. Oberdorster and K. A. Dawson (2011). "Interlaboratory Comparison of Size and Surface Charge Measurements on Nanoparticles Prior to Biological Impact Assessment." Journal of Nanoparticle Research **13**(7): 2675-2687.

Romero-Franco, M., H. A. Godwin, M. Bilal and Y. Cohen (2017). "Needs and Challenges for Assessing the Environmental Impacts of Engineered Nanomaterials (Enms)." Beilstein Journal of Nanotechnology **8**: 989-1014.

Savage, N., T. A. Thomas and J. S. Duncan (2007). "Nanotechnology Applications and Implications Research Supported by the Us Environmental Protection Agency Star Grants Program." Journal of Environmental Monitoring **9**(10): 1046-1054.

Schimpel, C., S. Resch, G. Flament, D. Carlander, C. Vaquero, I. Bustero and A. Falk (2018). "A Methodology on How to Create a Real-Life Relevant Risk Profile for a Given Nanomaterial." Journal of Chemical Health and Safety **25**(1): 12-23.

Schulte, P. A., I. Iavicoli, J. H. Rantanen, D. Dahmann, S. Iavicoli, R. Pipke, I. Guseva Canu, F. Boccuni, M. Ricci, M. L. Polci, E. Sabbioni, A. Pietroiusti and E. Mantovani (2016). "Assessing the Protection of the Nanomaterial Workforce." Nanotoxicology **10**(7): 1013-1019.

Schulte, P. A., G. Roth, L. L. Hodson, V. Murashov, M. D. Hoover, R. Zumwalde, E. D. Kuempel, C. L. Geraci, A. B. Stefaniak, V. Castranova and J. Howard (2016). "Taking Stock of the Occupational Safety and Health Challenges of Nanotechnology: 2000–2015." Journal of Nanoparticle Research **18**(6): 159.

Schwirn, K., L. Tietjen and I. Beer (2014). "Why Are Nanomaterials Different and How Can They Be Appropriately Regulated under REACH?" Environmental Sciences Europe **26**(1): 1-9.

Shi, H., R. Magaye, V. Castranova and J. Zhao (2013). "Titanium Dioxide Nanoparticles: A Review of Current Toxicological Data." Particle and Fibre Toxicology **10**.

Shi, H. B., R. Magaye, V. Castranova and J. S. Zhao (2013). "Titanium Dioxide Nanoparticles: A Review of Current Toxicological Data." Particle and Fibre Toxicology **10**: 33.

Stamm, H., N. Gibson and E. Anklam (2012). "Detection of Nanomaterials in Food and Consumer Products: Bridging the Gap from Legislation to Enforcement." Food Additives & Contaminants: Part A **29**(8): 1175-1182.

Sun, T. Y., F. Gottschalk, K. Hungerbühler and B. Nowack (2014). "Comprehensive Probabilistic Modelling of Environmental Emissions of Engineered Nanomaterials." Environmental Pollution **185**: 69-76.

Tiede, K., S. F. Hanssen, P. Westerhoff, G. J. Fern, S. M. Hankin, R. J. Aitken, Q. Chaudhry and A. B. Boxall (2016). "How Important Is Drinking Water Exposure for the Risks of Engineered Nanoparticles to Consumers?" Nanotoxicology **10**(1): 102-110.

Xia, T., R. F. Hamilton, J. C. Bonner, E. D. Crandall, A. Elder, F. Fazlollahi, T. A. Girtsman, K. Kim, S. Mitra, S. A. Ntim, G. Orr, M. Tagmount, A. J. Taylor, D. Telesca, A. Tolic, C. D. Vulpe, A. J. Walker, X. Wang, F. A. Witzmann, N. Q. Wu, Y. M. Xie, J. I. Zink, A. Nel and A. Holian (2013). "Interlaboratory Evaluation of in Vitro Cytotoxicity and Inflammatory Responses to Engineered Nanomaterials: The NIEHS Nano Go Consortium." Environmental Health Perspectives **121**(6): 683-690.

CHAPTER 5: OVERARCHING CONCLUSIONS AND POSSIBLE TOPICS FOR FUTURE WORK

OVERARCHING CONCLUSIONS

Over the last two decades, our understanding of the environmental health and safety implications of ENMs has grown significantly. One of the major contributions to this achievement has been the development of different frameworks to provide evidence-based approaches to help in the decision-making process regarding ENMs. In **Chapter 2**, the utility of these frameworks was assessed via decision-making contexts (scenarios). Through this analysis, we provided an overview of the most common and pressing needs faced by critical stakeholders to arrive at decisions with respect to the environmental health and safety of ENMs and how specific frameworks can be used to address such needs. In other words, the contribution of this analysis to the scientific community relies in the identification of frameworks that can meet, at least partly, the needs of potential decision makers in real life scenarios. Furthermore, the identification of intrinsic limitations to such frameworks allows for the specific components/elements that require improvement can be outlined and prioritized. In this regard, further research should focus on the development of integrative frameworks for assessing the risk potential of ENMs that: a) address the complexities of ENMs and their transformations, b) integrate quantitative and qualitative data, c) allow use of modeling tools to fill data gaps, d) minimize reliance on expert judgement, and e) enable quantification of uncertainties associated with the use of both quantitative and qualitative data/information. The development of new frameworks or improvement of existing ones should facilitate their adaptation to a variety of contexts required by decision makers and aid in the

prioritization of future research and manufacturing of ENMs and related products in support of environmentally sustainable nanotechnology.

A primary contribution of the work presented in this thesis to the field of Nano EH&S was the development and implementation of decision support tool (IANano) to systematically assess/evaluate the available information for a particular ENM and determine if this evidence is sufficient to conduct an environmental impact assessment. This work is presented in **Chapter 3**. The process of assessing the adequacy of available information to conduct an EIA of ENMs is particularly important in a problem formulation stage given that it allows the analyst to identify for which ENM the information is readily available and or to identify whether further testing required to conduct an EIA, and the specific type of information needed (e.g., environmental fate and transport, life cycle stages where release and exposure may occur, and exposure conditions/scenarios). In **Chapter 3**, IANano was applied to the analysis of the available evidence for conducting an EIA for a group of ENMs (i.e., nano Cu-CuO, nano ZnO, and nano TiO_2). This analysis revealed that the body of information available for exposure potential of these ENMs has not kept pace with that of hazard potential. The results show that the lowest aggregated scores are related to the exposure potential information for all ENMs, with the exception of nano TiO_2, for which exposure potential in occupational settings has been extensively studied. This study suggests that additional research is needed on the fate and transport, environmental release, and exposure assessment of ENMs, if quantitative environmental impact/risk assessments are to be feasible. These case studies suggest that IANano should be useful for a broad range of decision makers (regulators, manufacturers, and academics) who wish to use a systematic approach to evaluate whether sufficient data are available to perform an environmental impact/risk assessment for a particular ENM. However, future research is needed to explore the completeness of information

for additional exposure scenarios and for other classes of ENMs. Future studies could also focus on changing scoring and weighting approaches that reflect the relative relevance of different attributes to the overall process of EIA, to explore whether additional attributes and categories might be appropriate. Future studies include the use of data mining and artificial intelligence techniques to collect and curate the information available for ENMs. The conclusions from **Chapter 3,** with regard to the body of information available for exposure and hazard potential of ENMs, are also consistent with the analysis conducted of the literature for the period of 2005-2016 in **Chapter 1**, where a substantial amount of research efforts were identified for hazard assessment of ENMs (e.g., toxicity assay standardization and improvement in predictive toxicology assays). By contrast, exposure assessment of ENMs has been limited by both validation methods (e.g., modeling approaches are subject to the reliability of input data) and human exposure assessment/epidemiological studies. Another important field to Nano EH&S is informatics and computer science techniques (i.e., nanoinformatics), which has contributed to the overall progress in Nano EH&S over this period of time. Among its many contributions to the field of Nano EH&S, nanoinformatics has made it possible for researchers to analyze large datasets and overcome some critical challenges related to data gaps and mixed data sources.

Chapter 4 provides a synthesis and analysis of the body of information collected through **Chapters 1-3** from the perspective of how progress over the last decade and a half in Nano EH&S research answers questions that are relevant to the public health community. In this context, it is noted that while early public health concerns/needs included the determination of ENM testing regimes, substantial progress has been achieved in many areas of the field. Particularly, major progress has been achieved in terms of standardization of toxicity testing/characterization protocols of ENMs, development of models/tools and approaches to characterize the presence of

ENMs in the environment and their potential impacts. However, additional work is still required to improve quantitative measurement/tracking of ENMs in the environment (e.g., accounting for naturally-occurring nano sized particles) as well as to develop quantitative safety thresholds/exposure limits in environmental media. Further discussion is also needed in terms of what are the next steps/needs to protect public health. To do this, the communication with the public health community must be expanded and regulatory responses (driven by research) must be balanced against broader societal and economic implications of regulating some aspects of nanotechnology while considering scientific uncertainty.

Overall, the framework presented in this thesis is an example of how the scientific evidence can be analyzed and used in support of environmental health decision-making. As shown in this work, for the case of ENMs, the task of navigating through the scientific evidence can be done in a transparent way that can allow the analyst or decision-maker to identify scenarios of concern, available methods to assess risks and assess the available information depending on the decision-context.

POSSIBLE TOPICS FOR FUTURE WORK

It is envisioned that the work presented here can be further expanded in several ways. For example, the approach developed to assess the availability of information to conduct environmental impact assessments of ENMs (**Chapter 2**) could be modified to include additional parameters related to the quality of information. One possible extension would be to adapt the Dempster-Shafer algorithm (employed to integrate the information and calculate scores) to include an expanded set of variables to incorporate a semi-quantitative measure of the quality of the information assessed. Within the scope of evaluating existing information to conduct environmental impact assessment of ENMs, another important line of future work would be to

integrate IANano into statistical or mathematical approaches to assess datasets available from curated publications. In this regard, methods such as meta-analyses can be implemented to identify ENM hazard traits and or further prioritize ENMs with respect to their potential hazard potential.

Another area that warrants further research is the development of additional decision support tools to conduct quantitative or semi-quantitative EIA of ENMs. As discussed in **Chapter 1**, a decision support tool to conduct EIA of ENMs is needed to complete the integrative framework shown in **Figure 1.4**. The tool needed, as proposed within the scope of this work, would greatly benefit from: (a) allowing rapid evaluations of various environmental scenarios (Bilal et al. 2017), (b) providing an intelligent query system to explore the impact of parameter variations and sensitivity on predicted outcomes (Bilal et al. 2017), and (c) assessing the conditional dependence (causal relationships) of ENMs adverse outcomes with respect to various input (Money et al. 2012, Bilal et al. 2017). The above can, for example, be accomplished with the implementation of Bayesian Networks (BN) into a decision support tool as a path to constructing expert systems that would allow intelligent query for conducting case studies while providing useful insight even when confronted with the availability of partial information (as illustrated in the case study of **Chapter 3**). One of distinctive characteristics of BN models is that they are constructed directly from the available information and can be readily updated (i.e., via incremental learning) by incorporating rigorous learning algorithms as new data become available as opposed to most models which require rebuilding when presented with new information (Money et al. 2012, Bilal et al. 2017). The built-in capabilities of BNs (including sensitivity analysis) can provide the following three major outcomes that are relevant to EIA: (i) visual representation of the causal relationships that affect environmental impact assessment, (ii) rapid interrogation and handling of uncertainties associated with multiple input parameters and their impact on various factors, and (iii) robust

parameter sensitivity analysis identify the most significant attributes that correlate with target outcomes. BN models can also be easily constructed to provide a visual representation of bidirectional conditional dependency in the form of network links (Money et al. 2012, Bilal et al. 2017). In other words, BN based models include features within their network structure that can be utilized for both causal (e.g., predicting ENMs toxicity or exposure outcomes when ENMs physicochemical properties and other parameters are unknown) and diagnostic reasoning (e.g. the reverse approach of predicting ENMs properties that govern known toxicity or exposure profiles) (Money et al. 2012, Bilal et al. 2017). Such characteristics make the Bayesian Network models appropriate for the framework proposed here.

REFERENCES FOR CHAPTER 5

Bilal, M., H. Liu, R. Liu and Y. Cohen (2017). "Bayesian Network as a Support Tool for Rapid Query of the Environmental Multimedia Distribution of Nanomaterials." Nanoscale **9**(12): 4162-4174.

Money, E. S., K. H. Reckhow and M. R. Wiesner (2012). "The Use of Bayesian Networks for Nanoparticle Risk Forecasting: Model Formulation and Baseline Evaluation." Science of the Total Environment **426**: 436-445.

APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 1

Table A1. Detailed description of challenges in Nano EH&S, responses, remaining gaps and potential role of public health

General (intrinsic) challenges related to unique ENM properties, analytical methods for characterization and rapid development			
Challenge	**Response/ Advances**	**Remaining Gaps**	**Potential role of Public Health**
1. Initially, high batch to batch variability in materials and hazard properties. Need for standard materials and protocols for preparation and characterization.	• U.S. NIST and EC JRC focus on the development of standard reference materials, and protocols for sample preparation, physicochemical and biological measurements. • Application of analytical techniques such as SEM and TEM to track cellular and tissue uptake of ENMs and proposals for minimum critical characterization of ENM properties for Nano EH&S studies. • Development of protocols for sample preparation of Nano TiO_2 dispersion in bovine serum albumin (BSA).	• Extend reporting use of reference materials and standard protocols in peer review publications.	• Support the reporting of standard protocols and use of references materials in publications.
2. Large number of new materials being produced in a small amount of time and consequent safety data gaps.	• Alternative Testing Strategies (ATS) haven been used to address hazard data gaps, testing a large number of materials per assay (see challenge #6) • Read-across from chemicals, and adaptation of in silico toxicology to ENMs. • For exposure data gaps, the development of environmental fate and transport modelling.	• Limited percentage of the ATS assays that have been developed have been validated against animal studies.	• Promote discussion of the potential applications of ATS in research related to Nano EH&S.
3. Need for computational and statistical approaches for handling ENMs data sets.	• Data curation and management approaches. • Funding agencies establishing requirements for data repositories and data sharing. • Decision support tools (Risk Assessment, Risk banding/grouping, Ranking).	• Limited current use for regulatory purposes, which might expand with the TSCA/Frank R. Lautenberg update. • Limited use by scientific community related to concerns regarding data sharing (authorship), complex ontologies (taxonomy). • Quality/utility of data in publically-accessible repositories.	• Participate in discussions regarding the regulatory use of data repositories/curation and tools.

ENMs and Hazard potential challenges			
Challenge	**Response/Advances**	**Remaining Gaps**	**Potential role of Public Health**
4. Hazard potential/toxicity changes depending on environmental/biological conditions/interactions, these changes also influence fate & transport, therefore the hazard assessment of transformed ENMs may differ from pristine materials.	• Improved tracking of cellular and tissue uptake of ENM using novel analytical techniques (e.g., SERS and TEM). TEM cryomicroscopy is now used routinely to image intercellular structures and unstained biomolecules at the sub-nanometer level.	• Improve design of study conditions to reflect environmental "realism" (e.g., identification of relevant receptors and exposure conditions).	• Provide insights on environmental conditions of relevance to public health to help identify priorities for further research.
5. Hazard potential highly dependent on ENM properties (e.g., size, shape, composition, surface coatings, etc.). Need for approaches to predict hazard based on properties.	• Knowledge derived from UFP studies led to identification of properties that influence toxic response (e.g., surface area and size). • Finding of physicochemical properties to predict hazard of ENMs (e.g., metal oxides and CNTs).	• Substantial advance made with regard to the identification of physicochemical properties influencing hazard of first generation ENMs. However, new generation ENMs require further investigation (see challenges #15 and #16).	• Identify potential opportunities for the application of the predictive information/approaches to protect human health.
6. Need for validated hazard assessment strategies and protocols that consider the correct balance of in vitro and in vivo testing across organisms.	• International efforts working towards the development of reference materials and standard protocols (e.g., IANH, Quality Nano, Nano Go Consortium). • OECD work on validation and standardization of toxicity tests. • Improvement in reporting characterization and protocol of testing.	• While several efforts have been made with regard to standardization of protocols and materials, there is still a need to improve reporting practices.	• Support good practices of reporting the use of standard protocols and reference materials in peer review publications.

ENMs and Exposure potential challenges			
Challenge	**Response/ Advances**	**Remaining Gaps**	**Potential role of Public Health**
7. Need for data and models to quantify environmental releases and fate/transport of ENMs in the environment (considering that physicochemical properties ENMs change as a result of biological and environmental conditions therefore affect transport across media).	• Several probabilistic and mechanistic models have been developed to estimate environmental concentrations of ENMs in different environmental compartments. Relevant probabilistic models include material flow analysis, a mechanistic multimedia fate and transport modeling framework and applied Bayesian Network tools to model environmental	• Improve parameterization of release models (input for F&T models) and switch to measured data (less on qualitative assessments). • Improve quality of input data to reduce the uncertainties that result from missing or conflicting	• Provide insights on environmental conditions of relevance to public health to help identify priorities for further research.

	multimedia distribution of ENMs using probabilistic and mechanistic approaches. • Strategic research on understanding ENM behavior in the environment is leading to mechanistic models of nanomaterial transportation and transformation, and dynamic models of nanomaterial mass-flow.	knowledge. For the probabilistic environmental fate models, the inclusion of particle-specific processes needs to be further developed. • Validation of models with field exposure data in Challenge #8.	
8. Need for field monitoring/measurement data to track ENMs exposure in the environment, workplace and consumer products (issues with nanoparticle interference and proper dosimetry tools for ENMs in complex media).	• New characterization tools/techniques such as the following are emerging to evaluate the structure and dynamics of the environmental interface (e.g., LC-APPI-MS). • Development of rigorous methodologies for assessing the physical state of ENMs in complex media (dosimetry).	• Still limited instruments for measuring exposures to ENMs in situ (environmental and biological monitoring). • Biomarkers of exposure still to be developed.	• Provide insights on environmental conditions of relevance to public health to help identify priorities for further research.
9. Difficulty in estimating occupational exposure (e.g., interference with ENMs with other particulate matter in workplace settings), and need for methods to translate exposure levels in animals to exposure levels in humans.	• Development of monitoring approaches such as NEAT (Nanoparticle Emission Assessment Technique). • Studies designed to identify measures to mitigate workers' exposure.	• Need for better quality of data for existing exposure assessments reports. • Improve reporting of workers' exposure in low income countries.	• Promote the study of ENM worker exposure as a relevant component of the public health agenda.

ENMs and risk potential challenges			
Challenges	**Response/ Advances**	**Remaining Gaps**	**Potential role of Public Health**
10. Need to know whether/when/which traditional Risk Assessment approaches are suitable for ENMs (and develop alternative approaches when they are not).	• Several frameworks have been developed to address the specific conditions of risk assessment for ENMs (e.g., Nano Risk, MCDA, probabilistic/Monte Carlo, Bayesian Networks). • QSARs applied to improve quantitative risk assessments.	• Limitations have been reported with regard to quantitative risk assessments. Exposure assessment, environmental concentrations, and dose response. • QSAR models still lack consideration for relevant endpoints (according to OECD validation principles) for considering their use in regulatory risk assessment.	• Participate in the application of the existing frameworks into relevant exposure scenarios of concern.
11. Need for approaches/tools to estimate data sufficiency to conduct risk assessment of ENMs and to tailor the process depending on the analyst's goals.	• Several methodologies have been developed to address this challenge such as weight of evidence (WOE) assessment/ranking and ranking research needs through MCDA to fill in data gaps.	• Improve these current methodologies and integrate them into decision-support tools.	
12. Need to know whether/when/which traditional Life Cycle Analysis (LCA) tools can be	• Several frameworks have adopted or incorporated LCA into the analysis of potential impacts of ENMs.	• Information for commercial product use still scarce (patented) and	

used to understand the overall environmental impact of ENMs (and, if not, what modifications need to be made/gaps need to be filled, e.g., emissions data/estimates).		need for improvement of results communication.	
13. Need for epidemiological studies that link effects seen in animal models to human health effects.	• A series of epidemiological studies have been carried out to analyze workers' exposure to ENMs.	• Need for improved exposure assessment information.	• Explore potential collaborations between public health agencies and research groups to improve existing epidemiological studies.
14. Need for risk reduction strategies that can be implemented through data collection and Nano EH&S research linked to regulatory decision-making.	• Risk management strategies have been developed mainly in occupational settings mainly through industrial hygiene control measures.	• Need for a link between the results from these studies and regulatory/policy actions.	• Promote and support science-policy discussions and identify areas of opportunity to integrate science into regulatory decision-making.

Next Generation ENMs			
Challenges	**Response/ Advances**	**Remaining Gaps**	**Potential role of Public Health**
15. Improve safety screening and safe design of ENMs used in commercial applications (e.g., therapeutics and diagnostics).	• Implementation of testing nanomedicines at the U.S. Nanotechnology Characterization Laboratory (NCL) and European Medicines Agency (EMA).	• Address regulatory gaps related to nanomedicine incorporating testing information from NCL and EMA, guided by a balance of innovation and R&D with public health protection.	• Develop risk communication strategies for existing approved nanomedicines to benefit public's perception.
16. Need safety assessment procedures developed for relatively simple ENMs to be validated as suitable for 3rd and 4th generation ENMs.	• Minor advance in terms of complete safety assessment strategies. • The potential of self-organization towards self-replication in new generation ENMs is considered critical with respect to precautionary risk management.	• Further research is required to identify how to assess safety of remotely activate nanostructures, environmentally responsive nanostructures and transforming active nanostructures.	• Start a conversation on potential relevance to public health, including potential benefits.

ENMs and outreach strategies			
Challenges	**Response/Advances**	**Remaining Gaps**	**Potential role of Public Health**
17. Involvement of relevant stakeholders (e.g., NGOs, industry and policy makers) in Nano EH&S (including risk assessment).	• Dialogue among stakeholders has been encouraged by U.S. and European agencies as part of the Nanotechnology development strategies. • Several workshops and meetings have brought together a variety of stakeholders (national and international leaders from government, industry, and academia) to discuss utility of ATS for decision-making analyses of ENMs. • Meetings held by OECD working parties and the development of the DuPont-Nano Risk Framework as a result of such meetings. • Policy workshops in Europe to involve stakeholders in the implementation of nano-regulations.	• Address the overlook of the Public Health community.	• Provide expertise in current discussions by identifying opportunities for benefits of Nanotechnology in public health.
18. Communication of results back to key stakeholder groups, including the public health community.	• Development of risk communication strategies by federal agencies. • Increase in the media coverage of Nanotechnology and potential risks and benefits. • Education and outreach programs such as NanoDays and college training/fellowship programs.	• Improve existing risk communication strategies by addressing uncertainties. • Expand and update current risk communication strategies.	• Collaborate in the development of improved risk communication strategies and education programs.

APPENDIX B: SUPPORTING INFORMATION FOR CHAPTER 2

Table B1. Analysis and classification of reviewed hazard identification frameworks.

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps[(a)]	Software tools[(b)]
Swiss precautionary matrix (Höck J. et al. 2013) *Application:* ENM containing products	Decision tree/questionnaire, based on published data, suitable for pre-screening of available ENMs data (e.g., physicochemical properties, hazard traits) and determination of further actions that may be needed (e.g., additional data, actions to control exposure)	Nano-relevance (*e.g.*, whether or not the analyzed ENM meets the definition under EU regulations), effects (*e.g.*, potential of the ENM for ROS formation, redox potential), potential for exposure (considering the maximum amounts released to the environment, frequency of exposure) and available information about the ENM life cycle (*e.g.*, source of the ENM).	Categories/classification of the hazard: group A - no need for review of (unspecified) risk management measures; group B - need for review of (unspecified) risk management measures or need for additional information.	No	Web application
Risk Classification System based on Multi Criteria Decision Analysis (MCDA risk classification) (Linkov et al. 2007, Linkov et al. 2009, Tervonen et al. 2009) *Application:* ENMs	Outranking: given a set of alternatives that are compared in terms of performance for selected criteria designed via expert judgment. The user must assign scores (e.g., 1-4, 60-100, 5-200) that correspond to categories (e.g., low-very low, medium-low, medium, high, extremely high) for each pre-determined criterion (e.g., Agglomeration, Reactivity/charge, Critical function groups, Contaminant dissociation, Bioavailability potential, Bioaccumulation potential, Toxic potential, Size) based on data or expert elicitation.	Data regarding specific properties of the ENM (*e.g.*, size, reactivity/charge, critical function groups) and transformation in the environment (*e.g.*, agglomeration, contaminant dissociation, bioavailability potential, bioaccumulation potential, toxicity). Data input can be from experimental studies or via expert judgment.	Categories/classification of the hazard (*e.g.*, toxicity): very low, low, medium, high, and extremely high.	Yes	MCDA general software (not specific for risk classification of ENMs)
Hazard and exposure potential identification for ENMs in consumer products (NanoRiskCat) (Hansen et al. 2014) *Application*: ENM containing products	Decision tree/flowchart, where each of the steps is depicted in a flowchart, which requires a specific choice to be made by the analyst on the basis of available evidence.	Data regarding the potential hazard posed by the ENM based on: human health (*e.g.*, acute toxicity, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity due to single or multiple exposures, skin corrosion and eye irritation) and environmental hazards (e.g., ecotoxicity, persistence and bioaccumulation).	Categories/classification of the hazard: a color-coded system/visual representation of the potential hazards that includes a short description of the evidence (grey color assigned to insufficient data, green-low hazard potential, yellow-medium potential hazard and red-high hazard potential).	Yes	No

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps[(a)]	Software tools[(b)]
DF4Nano grouping (Arts et al. 2015) *Application*: ENMs	Guidance for classification is given to the analyst in tables developed based on published data and reports. The analyst must choose the categories for evaluating the available information comparing with values and thresholds provided in the framework.	Data regarding intrinsic ENM properties (water solubility, shape and aspect ratio, composition), system-related properties (use, release and exposure, uptake, biodistribution and biopersistence and bio-physical interactions) and toxic effects (endpoints from short term in vivo studies or toxicity evidence from in vitro studies) induced by the ENM.	Categories/classification of the ENMs: four main categories: 1) soluble ENMs, 2) biopersistent high-aspect ratio, 3) passive ENMs and 4) active ENMs. ENMs classified in 3 and 4 are considered to require a further analysis/risk assessment.	Yes	No

(a) Capability of a framework to address data gaps: whether the framework includes a detailed methodology to obtain missing data (*e.g.*, consideration of expert judgment to fill in data gaps or modeling tools incorporated in the framework); (b) Availability of software tools specifically designed to conduct the analysis: whether software tools were developed or adapted to conduct the analysis. Specific tools included in this category include, spreadsheets (e.g., EXCEL), web applications (e.g., online software), desktop software.

Table B2. Analysis and classification of reviewed environmental risk assessment frameworks. ** The guidelines described by the American Institute of Chemical Engineers AICHE and or the Health and Safety Executive of the UK. ***To analyze the effect of nano Ag in the microbial community, the parameters included the effect on the microbial decomposition of organic matter, reduction in decomposer and denitrifier community redundancy, as well as the overall microbial community in the sediment (Goksøyr 1975)

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps	Software tools
Life Cycle Analysis (Som et al. 2010, Theis et al. 2011, Eckelman et al. 2012, Gavankar et al. 2012, Hischier and Walser 2012) *Application:* ENMs, ENM containing products	LCA refers to a class of approaches of assessing environmental impact of ENMs, whereby hazard identification, exposure assessment and risk characterization may be analyzed throughout the life cycle of an ENM. Depending on the scope of the analysis, as shown in case studies, the approach can be modified to estimate environmental impacts (e.g., with production of ENMs or nano enabled products or release of the ENMs to the environment related to consumer products).	Depending on the scope/outcome of the analysis: a) global warming/CO_2 emissions (detailed steps of manufacturing processes and their CO_2 emissions)(Walser et al. 2011, Hischier and Walser 2012); b) potentially affected fraction of aquatic organisms per unit mass of CNTs (EC_{50} values, degradation rates, partition coefficients, releases to environmental compartments and bioaccumulation factors)(Eckelman et al. 2012).	Depending on the design/desired outcome (e.g., environmental impacts from ENM production or release): Quantitative global warming potential (CO_2 emissions related to the synthesis of the assessed ENM) or potential detrimental impacts on aquatic organisms as a consequence of the ENM release.	No	LCA general software (not specific for ENMs)
DUPONT's nanorisk (DUPONT 2007) *Application:* Processes	Combines a systematic collection and organization of information with a chemical process risk assessment (CPQRA)(AIChE 2000), when information is available. CPQRA** focuses on acute rather than chronic hazards. Risk = F (s,c,f), s = hypothetical scenario, c = estimated consequence(s), f = estimated frequency	Inputs include ENM properties of the ENM (*e.g.*, name(s), form, chemical composition, surface coatings, molecular and crystal structure, physical form/shape, particle size, size distribution and surface-area, particle density, solubility (in water and biologically relevant fluids), dispersibility) and information about the industrial processes relevant to the ENM.	Depending on the available information, the results can be presented as lifecycle profiles that include information on physicochemical properties, ecotoxicity, and environmental fate to be used for risk management strategies; or include a quantitative risk analysis of the industrial processes related to the ENM.	No	No
EPA's Comprehensive Environmental Assessment CEA (Powers et al. 2012, Powers et al. 2014) *Application*: ENMs	Collective "judgment process" designed to compile extensive information and provide guidance to decision makers such as research planners and risk managers. The framework is presented as a roadmap to guide the user through the process of systematic data collection and identification of critical data gaps.	Product lifecycle, Environmental fate and transport, exposure routes, in addition to dose related information (e.g., toxicokinetics: absorption, distribution, metabolism, and excretion), if available (EPA 2013)	Summary of available information regarding a specific ENM evaluated by a group of experts indicating recommendations for research priorities and risk management.	No	Web application

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps	Software tools
Ranking initial environmental and human health risk: Nano HAZ framework (O'Brien and Cummins 2010) *Application:* ENMs	Ecological and human health risk assessment adapted to ENMs and applying benchmark doses (BMD) calculations based on published data.	Predicted nanomaterial environmental concentrations from published exposure studies (published modelled data(Gottschalk et al. 2010)). Published ecotoxicological studies were used to develop provisional benchmark dose lower confidence limits (BMDLs) through the application of the U.S. EPA Bench Mark Dose Software. A similar approach was conducted with animal studies to obtain BMDLs for human health risk calculations.	Classification/categories of the ENMs: Relative Risk Ranking groups. 0–2 (low environmental or health risk on a relative basis), 3–4 (concentrations that require monitoring and potential action), 5 + (environmental concentration above those provisional regulatory and toxicological limits as set in a published case study (O'Brien and Cummins 2010)).	Yes	No
Nanomaterial risk screening (Beaudrie et al. 2015) *Application*: ENMs	The framework provides a template for the analyst in order to compare data for the target ENM with reference information for each category/level. Framework was developed via expert judgment.	ENM properties categorized as intrinsic (chemical composition, crystal structure, size (average), shape (aspect ratio), charge (zeta potential), specific surface area) and extrinsic properties (reactivity, solubility, hydrophobicity, agglomeration, sorption tendency). Hazard indicators: ROS potential, movement through cells; Exposure indicators: persistence, and mobility.	Classification/categories of the ENMs: risk-rating groups (from lowest concern 1 to highest concern 5).	No	Spreadsheet
Human health and Ecological Risk Assessment as adapted from REACH (Aschberger et al. 2011) *Application:* ENMs	Follows the steps of a risk assessment for 90-day exposure studies and modeled environmental concentrations (from probabilistic material flow analysis (PMFA)).	Dose descriptors, overall assessment factors and estimated human indicative no-effect level (INELs) for workers of different ENM for chronic inhalation exposure based on experimental data and modeled environmental releases of ENMs.	Quantitative: Risk Quotient obtained by comparing predicted environmental concentrations with human no effect levels (PEC/INEC).	No	No
Risk quantification based on probabilistic flow modeling analysis (Gottschalk et al. 2013) *Application:* ENMs	Risk assessment combines predicted environmental concentrations (via PMFA) with a species sensitivity distribution (*e.g.*, probability distribution of harmful effects as a function of concentration for a given ENM).	Probability distributions of ENMs environmental concentrations (obtained via PMFA) with the probability distribution of adverse effects developed from literature review (species sensitivity distributions SSD).	Quantitative: Risk Index calculated as the product of probability of critical environmental concentrations and the probability that organisms would potentially be negatively impacted at such concentrations.	Yes	No

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps	Software tools
FINE (Forecasting the Impacts of Nanomaterials in the Environment applied to nano Ag) (Money et al. 2012) using Bayesian Networks *Application:* ENMs	Probability of risk calculation based on Bayes' principle; the network was designed via expert judgment.	Particle behavior under a set of aquatic and sediment environmental conditions (e.g., temperature, pH, fluid flow, organic matter, conductivity, time) in addition to NP properties (e.g., NP coating, zeta potential, fractal dimension, NP diameter, collision rate efficiency, homogeneous and heterogeneous NP attachment efficiencies, NP aggregation potential, biodegradation factors, deposition and dissolution) and the interaction surfaces; 2) Exposure related parameters (e.g., NP concentrations (including dissolved form) in water and sediment; 3) Hazard (Bioavailability potential, amount of bio-uptake, stage of development, mortality, growth/fitness, effects on microbial biomass in sediment and water, trace metal presence, reduction in decomposer community redundancy, reduction in denitrifier community redundancy, overall sediment community, overall water community, effect on decomposition***, methanogenesis, denitrification, primary production, carbon sequestration, trace gas emissions, eutrophication).	Quantitative: a modified version of the deterministic Risk Quotient shown as a probabilistic expression (probability measure in a scale of 0-1).	Yes	Software generic (not specific for ENMs)

Table B3. Analysis and classification of reviewed occupational risk assessment frameworks.

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps	Software tools
Risk based classification for occupational exposure control (Kuempel et al. 2012) *Application:* ENMs	The process follows a quantitative risk assessment (QRA) approach based on benchmark doses (BMD).	Particle size, shape, and density utilized in estimation of inhalation and lung region-specific deposition fraction; toxicity assays (multiple exposure or dose groups to describe dose–response relationship; estimated benchmark dose); biological significance of response (to evaluate severity and relevance to humans); body and lung weight; target lung region surface area and volume (to normalize dose from animals to humans).	Quantitative: Excess Risk - defined as the percent of excess risk for a specific health outcome.	Yes	No
Risk classification based on an Industry Insurance Protocol (Robichaud et al. 2005) *Application*: Processes	Comparison of scores assigned to characterize the industrial process of manufacturing ENMs with pre-established scores based on an insurance protocol.	Qualitative relative risk ranking required data on physicochemical properties and quantities of inventoried materials; relative risk assessment is also based on factors such as toxicity, flammability, persistence in the environment. Additional required data include toxicity metrics (e.g., LC_{50} and LD_{50}), and environmental fate and transport parameters (e.g., water solubility, log K_{ow}, flammability, and expected emissions).	Classification/categories: relative risk ranking - a comparison of an industrial chemical process vs. an ENM process.	Yes	No
Control Banding: CB Nanotool (Paik et al. 2008) *Application:* Processes	Classification based on the characteristics of the process and the hazard evidence for the target ENM.	ENM properties (surface chemistry, particle shape, particle diameter, solubility), toxicity evidence (carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, toxicity of parent material (bulk material, non nano sized material that is considered similar to the ENM); estimated amount of ENM used for a given task, dustiness/mistiness, number of employees with similar exposure, frequency of operation, duration of operation.	Classification/categories: Risk Level for occupational risk expressed as risk bands that indicate recommendations regarding the pursuit of risk management strategies needed for exposure control.	Yes	Spreadsheet (not publicly available)

Name of the framework and scope	Intrinsic characteristics	Input	Output	Address data gaps	Software tools
Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012) *Application:* Processes	Classification based on the characteristics of the process and the hazard evidence for the target ENM.	ENM properties (e.g., particle shape, diameter, length, solubility, composition, bioavailability, reactivity); toxicity evidence (e.g., carcinogenicity, reproductive toxicity, mutagenic); industrial process parameters (e.g., duration of the handling, frequency of the handling, background concentration), characteristics of the matrix of ENM (e.g., dustiness of powders and fraction of the ENM in powders) and engineering controls (e.g., use of personal protective equipment).	Classification/categories: Priority bands that indicate the priority for risk management.	No	Web application

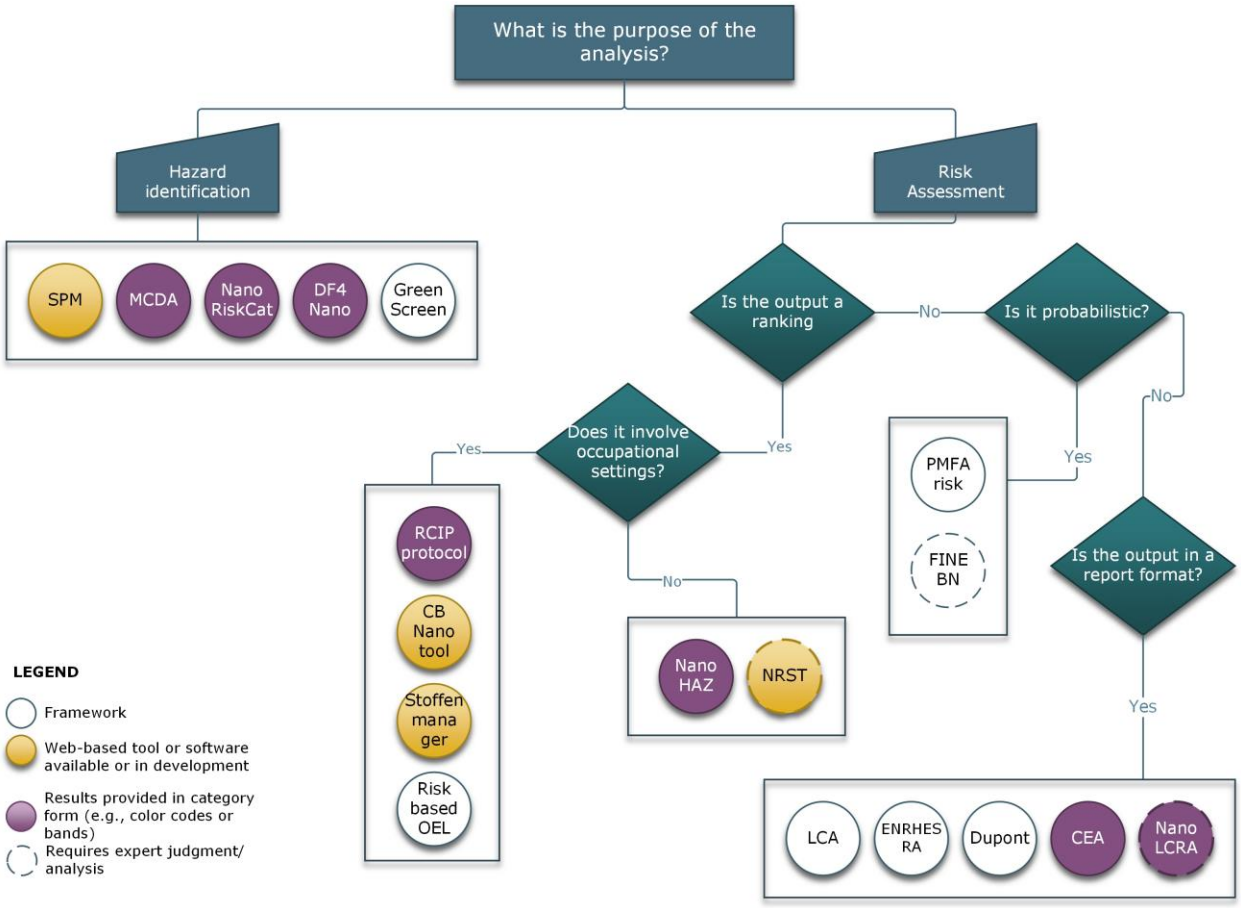


Figure B1. Flowchart depicting the process used to select which frameworks are suitable for specific decision-making scenarios.

Table B4. Information elements and criteria used to identify suitable frameworks for each decision-making scenario (decision context)

Scenario 1: Company is producing a new ENM and wants to know what controls to put into place to protect their workers during manufacturing or processing				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Hazard identification or Risk assessment in occupational settings to develop an internal risk management strategy including recommended engineering controls, administrative controls	• Details about process to be used/how material will be handled during manufacturing; • Potential for exposure during this process/handling	Swiss Precautionary Matrix (Höck J. et al. 2013)	The Swiss Precautionary Matrix (SPM) framework and its web tool require detailed data input regarding the physicochemical properties of the ENM, duration of the processes involving the ENM, and the potential effects/toxicity of the ENM. The SPM can be used as a screening framework to identify the need for actions in terms of ENM risk management in occupational settings (e.g., gathering additional information related to potential hazards of ENMs).	Tools (e.g., in silico methods or alternative testing strategies) are needed to predict or obtain information for those ENMs for which complete characterization and toxicity information is not available. Modifications to the SPM are needed that would allow users to consider existing protective measures when developing risk management approaches
		DuPont's Nanorisk (DUPONT 2007)	The DuPont Nanorisk framework allows the analyst to develop lifecycle profiles that include information on physicochemical properties, ecotoxicity and environmental fate to develop risk management strategies. The design of this framework considers a chemical process risk analysis, which makes it suitable for this scenario.	Tools (e.g., in silico methods or alternative testing strategies) are needed to predict or obtain information for those ENMs for which complete characterization and toxicity information is not available.
		Control Banding: CB Nanotool (Paik et al. 2008)	The CB Nanotool provides guidelines to classify ENMs in different groups according to the potential for worker exposure, depending on the number of exposed workers, the conditions of the exposure and the frequency and duration of these processes.	Tools (e.g., in silico methods or alternative testing strategies) are needed to predict or obtain information for those ENMs for which complete characterization and toxicity information is not available.
		Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012)	This web-based tool focuses on a classification similar to the banding approach. This framework is suitable in this scenario because the analysis considers the characteristics of industrial processes (*e.g.*, duration and frequency of the ENM handling, background concentration engineering controls such as use of personal	Tools (e.g., in silico methods or alternative testing strategies) are needed to predict or obtain information for those ENMs for which complete characterization and toxicity information is not available.

Scenario 1: Company is producing a new ENM and wants to know what controls to put into place to protect their workers during manufacturing or processing				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
			protective equipment) and the hazard evidence of the ENM (*e.g.*, properties including particle shape, diameter, length, solubility, composition, bioavailability, reactivity; and human toxicity).	The Stoffenmanager Nano only considers the potential risks derived from inhalation exposure.

Scenario 2: OSHA deciding whether to establish a OEL/PEL for a specific class of ENMs				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Hazard identification/ risk assessment in occupational settings to support a legally defensible recommendation or requirement for allowed exposure	• Human toxicity (quantitative dose-response) information for that specific class of ENMs; • Limit of detection for monitoring exposure for specific class of ENMs	Swiss Precautionary Matrix (Höck J. et al. 2013)	While the Swiss Precautionary Matrix (SPM) does not provide a quantitative approach to estimate occupational exposure limits, in this context, the SPM and its web tool can be used as a first tier screening framework to identify the need for actions in terms of risk management (e.g., gathering additional information).	Tools (e.g., in silico methods or alternative testing strategies) are needed to predict or obtain information for those ENMs for which complete characterization and toxicity information is not available.
		Risk based classification for occupational exposure control (Kuempel et al. 2012)	The framework follows a quantitative risk assessment (QRA) similar to that of conventional chemicals and requires ENM data (*e.g.*, properties including particle size, shape, density; toxicity assays; dose–response relationships to extrapolate animal studies data to human health outcomes) to calculate an excess risk of developing an adverse outcome for human health. While this framework requires extensive toxicity data (e.g., dose-response data, which can be extrapolated from ultrafine particles), it allows the analyst to determine occupational exposure limits (OEL), which is the desired output in this scenario.	Incorporation of methods to extrapolate or calculate dose-response data from in silico studies would expand the utility of this tool.

Scenario 3: Company needs to assess the potential impacts of the production of a nano-enabled product and how to manage risks if any				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Risk assessment of a particular ENM to develop risk management strategies.	• Potential for exposure across life cycle • Hazards for humans and the environment	Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects (Stoffenmanager Nano) (Van Duuren-Stuurman et al. 2012)	While Stoffenmanager can be used to classify the ENMs according to their potential hazards, it does not provide a strategy to manage such risks. Furthermore, the potential impacts are assessed only in terms of production and not through the lifecycle of the ENM. Thus, the application of this framework in this context could be complemented by other approaches.	The availability of a web-based tool for this framework makes it more widely accessible and allows for rapid screening.
		Risk classification based on an Industry Insurance Protocol (RCIP) (Robichaud et al. 2005)	The RCIP framework is suitable in this context because it was tailored to industrial processes and allows the user to identify a risk category (e.g., low, medium, high) for the production of a specific ENM compared to that of an analogous traditional chemical. Hence, this framework could be applied in this context to classify ENMs into risk categories and compare to the production of chemicals.	The RCIP framework is based on a protocol that is not publicly accessible, hence its application is limited.
		Nano LCRA	The Nano LCRA framework could be used in this scenario as a "roadmap" to identify the data gaps that need to be filled. However, it does not provide a methodology for risk characterization or a strategy to develop risk management actions. Additionally, to perform the assessment, the ability to use expert judgment to assess of the available information is required.	The suitability of Nano LCRA in this context would improve if a strategy to characterize risk with limited data was provided. In addition, the framework would need to be adapted for use by non-experts.

Scenario 4: Company deciding which nanoparticle or nano-enabled product poses less risk than alternatives for a particular application (company taking a precautionary approach to make safe-by-design applications)				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Hazard Risk Assessment/Hazard identification that allows for an assessment or comparison of alternatives in terms of environmental impacts and technical performance	• Relative hazards for humans and the environment for different alternatives; • Differences in exposure potential for different alternatives	Risk Classification System based on Multi Criteria Decision Analysis (MCDA risk classification) (Linkov et al. 2007, Linkov et al. 2009, Tervonen et al. 2009)	The MCDA framework allows the analyst to perform assessment of a group of alternatives and directly make comparisons/draw conclusions on the best alternative. To be able to perform an assessment, the analyst must have sufficient data available for each parameter involved (e.g., occupational and environmental impacts, technical performance and stakeholders' preferences).	MCDA relies on expert judgment to compare the risk potential of a series of ENMs, based on their different parameters/traits. However, the assessment requires expert judgment to perform the assessment.
		Life Cycle Analysis (Som et al. 2010, Theis et al. 2011, Eckelman et al. 2012, Gavankar et al. 2012, Hischier and Walser 2012)	LCA allows for analysis of hazard identification, exposure assessment and risk characterization through the life cycle stages of an ENM.	LCA is a flexible approach that allows the analyst to assess different environmental impacts of ENMs. However, the analysis does not include a method to compare ENMs with each other.
		FINE (Forecasting the Impacts of Nanomaterials in the Environment applied to nano Ag) (Money et al. 2012) using Bayesian Networks	FINE provides a measure of potential risk (e.g., a modified version of the deterministic Risk Quotient shown as a probabilistic expression, in a measure or probability of 0-1).	The probabilistic measure of risk allows the analyst to compare potential concerns for a particular ENM on a scale 0-1 and Bayesian Networks can also be designed and implemented to calculate other parameters such as technical performance for specific ENMs.
		Modified GreenScreen (Sass et al. 2016)	The output of the modified GreenScreen is the identification of hazards/hazard traits associated with ENMs based on available toxicity information and physicochemical properties. This information is then used to optimize product development and to identify suitable replacements, if necessary.	While the modified Green Screen allows the analyst to identify hazards related to a specific ENM. The data availability may limit its application.
		Hazard and exposure potential identification for ENMs in	The output of this framework is partially useful in this context as the categories or classification of the hazard and exposure are presented in a color-coded	The visual representation of the categories of potential hazard and exposure are useful in this scenario as it can be used by the analyst to

Scenario 4: Company deciding which nanoparticle or nano-enabled product poses less risk than alternatives for a particular application (company taking a precautionary approach to make safe-by-design applications)				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
		consumer products (NanoRiskCat) (Hansen et al. 2014)	system/visual representation of the potential hazards.	identify traits of concern. However, the NanoRiskCat framework does not include a methodology to assess alternatives.

Scenario 5: EPA deciding whether to issue a SNUR (significant new use rule) under TSCA (toxic substances control act) for a particular type of ENM				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Risk assessment to provide substantial evidence to indicate that a specific ENM will present an unreasonable risk to people or the environment.	• Potential for exposure across life cycle (including potential for releases into the environment); • Hazards for humans and the environment	EPA's Comprehensive Environmental Assessment CEA (Powers et al. 2012, Powers et al. 2014)	CEA is a theoretical approach that has been proposed to gather and analyze information regarding the potential impacts of ENMs.	Given that the significant new use rule (SNUR) must consider volume(s) of manufacturing and processing of the ENM and human exposure, the CEA is a suitable framework in this scenario at the analysis includes this information. However, the analysis of the information relies solely on the expertise of the analyst.
		Ranking initial environmental and human health risk: Nano HAZ framework (O'Brien and Cummins 2010)	This framework focuses on an adaptation of risk assessment (ecological and human health risk). The output of this framework is presented as classes or categories of the ENMs into Relative Risk Ranking groups. For example, 0–2 (low environmental or health risk on a relative basis), 3–4 (concentrations that require monitoring and potential action), 5 + (environmental concentration above limits of concern).	In this framework, the analyst may use provisional regulatory and toxicological limits from ultrafine particles if necessary. However, the applicability of this framework would improve if in silico toxicity data was considered for those ENMs, whose complete characterization information is not available.

Scenario 5: EPA deciding whether to issue a SNUR (significant new use rule) under TSCA (toxic substances control act) for a particular type of ENM				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
		Nanomaterial risk screening (Beaudrie et al. 2015)	The framework consists of individual rating/scoring of hazard and exposure potential of an ENM which relies solely on expert judgment.	To improve the applicability in this context, this framework would need to be adapted for use by non-experts.
		Risk quantification based on probabilistic flow modeling analysis (Gottschalk et al. 2013)	This framework focuses on risk assessment and combines predicted environmental concentrations (via environmental modeling) with a species sensitivity distribution (e.g., probability distribution of harmful effects shown at different concentrations for a given ENM).	This framework is limited by the information available as input for the exposure modeling (e.g., production volumes and/or estimations of release) as well as toxicity data available.
		FINE (Forecasting the Impacts of Nanomaterials in the Environment applied to nano Ag) (Money et al. 2012) using Bayesian Networks	This framework provides a Risk Quotient shown as a probabilistic expression, in a measure or probability of 0-1.	The development of a Bayesian Network requires expertise to establish the causal diagram that is the conceptual foundation of this approach. As presented in the case study by Money et al, the framework lacks exposure potential information in the Bayesian Network.

Scenario 6: FDA deciding whether to allow registration of a new nano-enabled product in food (whole food, dietary supplement, food ingredient or additive), medical devices, drugs or cosmetics				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
Hazard identification/ Risk Assessment, for example, a Risk Evaluation and Mitigation Strategy (REMS) is required for	• Potential routes of exposure and properties that impact exposure potential (e.g., size distribution potential for aggregation and agglomeration	Hazard and exposure potential identification for ENMs in consumer products (NanoRiskCat) (Hansen et al. 2014)	This framework was designed to identify scenarios of potential exposure to ENMs via consumer products (qualitative assessment), which makes it particularly suitable for this scenario, where products are evaluated. However, NanoRiskCat does not provide a combined result of the exposure and hazard potential of a determined ENM.	To be more widely useful in this context, this framework would need to be adapted to incorporate quantitative information in the analysis.

Scenario 6: FDA deciding whether to allow registration of a new nano-enabled product in food (whole food, dietary supplement, food ingredient or additive), medical devices, drugs or cosmetics				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
new drugs that contain ENMs	of ENMs in the final product; • Human hazard data (including dosimetry for in vitro and in vivo toxicological studies and in vitro and in vivo toxicological data on ENM ingredients and their impurities, dermal penetration, potential inhalation, irritation and sensitization studies and mutagenicity/genotoxicity studies); physicochemical characteristics of ENMs under the conditions of toxicity testing and as expected in the final product, impurities			
		Engineered Nanoparticles - Review of Health and Environmental Safety: Human health and Ecological Risk Assessment (ENRHES – RA adapted from REACH) (Aschberger et al. 2011)	This framework focuses on a risk assessment using 90-day exposure studies and modeled environmental concentrations. This framework is limited by available toxicity studies and the properties of the ENMs as assessed in these assays are not the same as those of the ENMs used in foods or personal care products	The applicability of this framework in this context would improve if it was adapted to consider consumer products such as those regulated by FDA

Scenario 6: FDA deciding whether to allow registration of a new nano-enabled product in food (whole food, dietary supplement, food ingredient or additive), medical devices, drugs or cosmetics				
Desired Output	**Data Information Needed in this scenario**	**Potentially applicable framework**	**How does the desired output from framework meet needs from decision-context?**	**Recommendations Observations**
		DF4Nano grouping (Arts et al. 2015)	The output of this framework is a classification of the ENM based on its hazard traits, derived from tables obtained from published data and international organizations. The analyst must choose the categories evaluating the available information for the analysis while comparing with the values and thresholds provided by the authors.	This framework is partially suitable in this scenario as it can be used as a first tier screening for the analyst to identify ENMs of concern.

REFERENCES FOR APPENDIX B

AIChE (2000). Guidelines for Chemical Process Quantitative Risk Analysis (2nd Edition), Center for Chemical Process Safety/AIChE.

Arts, J. H. E., M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, W. Wohlleben and R. Landsiedel (2015). "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (Df4nanogrouping)." Regulatory Toxicology and Pharmacology **71**(2, Supplement): S1-S27.

Aschberger, K., C. Micheletti, B. Sokull-Kluttgen and F. M. Christensen (2011). "Analysis of Currently Available Data for Characterising the Risk of Engineered Nanomaterials to the Environment and Human Health - Lessons Learned from Four Case Studies." Environment International **37**(6): 1143-1156.

Beaudrie, C. E. H., M. Kandlikar, R. Gregory, G. Long and T. Wilson (2015). "Nanomaterial Risk Screening: A Structured Approach to Aid Decision Making under Uncertainty." Environment Systems & Decisions **35**(1, Sp. Iss. SI): 88-109.

DUPONT. (2007). "Nanorisk Framework." from http://www.nanoriskframework.org/.

Eckelman, M. J., M. S. Mauter, J. A. Isaacs and M. Elimelech (2012). "New Perspectives on Nanomaterial Aquatic Ecotoxicity: Production Impacts Exceed Direct Exposure Impacts for Carbon Nanotoubes." Environmental Science & Technology **46**(5): 2902-2910.

U.S. EPA (2013). Comprehensive Environmental Assessment Applied to Multiwalled Carbon Nanotube Flame-Retardant Coatings in Upholstery Textiles: A Case Study Presenting Priority Research Gaps for Future Risk Assessments (Final Report).

Gavankar, S., S. Suh and A. F. Keller (2012). "Life Cycle Assessment at Nanoscale: Review and Recommendations." International Journal of Life Cycle Assessment **17**(3): 295-303.

Goksøyr, J. (1975). Decomposition, Microbiology, and Ecosystem Analysis. Fennoscandian Tundra Ecosystems: Part 1 Plants and Microorganisms. F. E. Wielgolaski. Berlin, Heidelberg, Springer Berlin Heidelberg**:** 230-238.

Gottschalk, F., E. Kost and B. Nowack (2013). "Engineered Nanomaterials in Water and Soils: A Risk Quantification Based on Probabilistic Exposure and Effect Modeling." Environmental Toxicology and Chemistry **32**(6): 1278-1287.

Gottschalk, F., R. W. Scholz and B. Nowack (2010). "Probabilistic Material Flow Modeling for Assessing the Environmental Exposure to Compounds: Methodology and an Application to Engineered Nano-TiO_2 Particles." Environmental Modelling & Software **25**(3).

Hansen, S. F., K. A. Jensen and A. Baun (2014). "Nanoriskcat: A Conceptual Tool for Categorization and Communication of Exposure Potentials and Hazards of Nanomaterials in Consumer Products." Journal of Nanoparticle Research **16**(1): 1-25.

Hischier, R. and T. Walser (2012). "Life Cycle Assessment of Engineered Nanomaterials: State of the Art and Strategies to Overcome Existing Gaps." Science of the Total Environment **425**.

Höck J., E. T., Furrer E., Gautschi. M, Hofmann H., Höhener K., Knauer K., Krug H., Limbach, G. P. L., Nowack B., Riediker M., Schirmer K., Schmid K., Som C., Stark W., Studer C., Ulrich A., and W. A. von Götz N., Wengert S., Wick P. (2013). Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Berne, Federal Office of Public Health and Federal Office for the Environment. **Version 3.0:** 26.

Kuempel, E. D., V. Castranova, C. L. Geraci and P. A. Schulte (2012). "Development of Risk-Based Nanomaterial Groups for Occupational Exposure Control." Journal of Nanoparticle Research **14**(9).

Linkov, I., F. K. Satterstrom, J. Steevens, E. Ferguson and R. C. Pleus (2007). "Multi-Criteria Decision Analysis and Environmental Risk Assessment for Nanomaterials." Journal of Nanoparticle Research **9**(4): 543-554.

Linkov, I., J. Steevens, M. Chappell, T. Tervonen, J. R. Figueira and M. Merad (2009). Classifying Nanomaterial Risks Using Multi-Criteria Decision Analysis. Nanomaterials: Risks and Benefits. I. Linkov and J. Steevens, Springer Netherlands**:** 179-191.

Money, E. S., K. H. Reckhow and M. R. Wiesner (2012). "The Use of Bayesian Networks for Nanoparticle Risk Forecasting: Model Formulation and Baseline Evaluation." Science of the Total Environment **426**: 436-445.

O'Brien, N. and E. Cummins (2010). "Ranking Initial Environmental and Human Health Risk Resulting from Environmentally Relevant Nanomaterials." Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering **45**(8): 992-1007.

Paik, S. Y., D. M. Zalk and P. Swuste (2008). "Application of a Pilot Control Banding Tool for Risk Level Assessment and Control of Nanoparticle Exposures." Annals of Occupational Hygiene **52**(6): 419-428.

Powers, C. M., G. Dana, P. Gillespie, M. R. Gwinn, C. O. Hendren, T. C. Long, A. Wang and J. M. Davis (2012). "Comprehensive Environmental Assessment: A Meta-Assessment Approach." Environmental Science & Technology **46**(17): 9202-9208.

Powers, C. M., K. D. Grieger, C. O. Hendren, C. A. Meacham, G. Gurevich, M. G. Lassiter, E. S. Money, J. M. Lloyd and S. M. Beaulieu (2014). "A Web-Based Tool to Engage Stakeholders in Informing Research Planning for Future Decisions on Emerging Materials." Science of the Total Environment **470**: 660-668.

Robichaud, C. O., D. Tanzil, U. Weilenmann and M. R. Wiesner (2005). "Relative Risk Analysis of Several Manufactured Nanomaterials: An Insurance Industry Context." Environmental Science & Technology **39**(22).

Sass, J., L. Heine and N. Hwang (2016). "Use of a Modified Greenscreen Tool to Conduct a Screening-Level Comparative Hazard Assessment of Conventional Silver and Two Forms of Nanosilver." Environmental Health: A Global Access Science Source **15**(1): 105.

Som, C., M. Berges, Q. Chaudhry, M. Dusinska, T. F. Fernandes, S. I. Olsen and B. Nowack (2010). "The Importance of Life Cycle Concepts for the Development of Safe Nanoproducts." Toxicology **269**(2-3): 160-169.

Tervonen, T., I. Linkov, J. R. Figueira, J. Steevens, M. Chappell and M. Merad (2009). "Risk-Based Classification System of Nanomaterials." Journal of Nanoparticle Research **11**(4): 757-766.

Theis, T. L., B. R. Bakshi, D. Durham, V. M. Fthenakis, T. G. Gutowski, J. A. Isaacs, T. Seager and M. R. Wiesner (2011). "A Life Cycle Framework for the Investigation of Environmentally Benign Nanoparticles and Products." Physica Status Solidi-Rapid Research Letters **5**(9): 312-317.

Van Duuren-Stuurman, B., S. R. Vink, K. J. M. Verbist, H. G. A. Heussen, D. H. Brouwer, D. E. D. Kroese, M. F. J. Van Niftrik, E. Tielemans and W. Fransman (2012). "Stoffenmanager Nano Version 1.0: A Web-Based Tool for Risk Prioritization of Airborne Manufactured Nano Objects." Annals of Occupational Hygiene **56**(5).

Walser, T., E. Demou, D. J. Lang and S. Hellweg (2011). "Prospective Environmental Life Cycle Assessment of Nanosilver T-Shirts." Environmental Science & Technology **45**(10): 4570-4578.

APPENDIX C: SUPPORTING INFORMATION FOR CHAPTER 3

C1. DEMPSTER SHAFFER ALGORITHM

An important factor in the aggregation and overall score calculation is the handling uncertainties when attributes are assessed based on their levels of information availability. Moreover, it is important to ensure that an attribute is not erroneously characterized (i.e., scored inaccurately) relative to other attributes of significantly differing uncertainty. To address this, the Dempster-Shafer algorithm (Yang and Xu 2002) involves the following steps: (1) individual scores (i.e., values ranging from 0 to 1 representing the availability of information) and weights (*w*, relevance of the attribute to the parent sub-category) are assigned to each attribute, sub-category and category. Next, a probability mass is calculated for the scores and weights of available and unavailable information (2), followed by calculation of the joint probability (K) (for available and unavailable information) (3), which is then used to calculate the aggregated score (4) $\beta_n = \frac{m_{n,I(L)}}{1-\bar{m}_{H,I(L)}}$, this aggregate score represents a measure of the adequacy of information for an EIA. The equations are listed below,

Step 1:

$\beta_{n,i} =$ score for information availability/unavailability of attribute *i*

$m_{n,i} = \omega_i \times \beta_{n,i}$

Step 2:

$$m_{H,i} = 1 - \sum_{n=1}^{N} m_{n,i} = 1 - \omega_i \sum_{n=1}^{N} \beta_{n,i} \qquad , N = 1,2$$

$$\bar{m}_{H,i} = 1 - \omega_i$$

$$\widetilde{m}_{H,i} = \omega_i \left(1 - \sum_{n=1}^{N} \beta_{n,i}\right) \qquad , N = 1,2$$

$$m_{H,i} = \widetilde{m}_{H,i} + \overline{m}_{H,i}$$

Step 3:

$$m_{n,I(i+1)} = K_{I(i+1)}\left[m_{n,I(i)} \cdot m_{n,i+1} + m_{H,I(i)} \cdot m_{n,i+1} + m_{n,I(i)} \cdot m_{H,i+1}\right]$$

$$\widetilde{m}_{H,I(i+1)} = K_{I(i+1)}\left[\widetilde{m}_{H,I(i)} \cdot \widetilde{m}_{H,i+1} + \overline{m}_{H,I(i)} \cdot \widetilde{m}_{H,i+1} + \widetilde{m}_{H,I(i)} \cdot \overline{m}_{H,i+1}\right]$$

$$\overline{m}_{H,I(i+1)} = K_{I(i+1)}\left[\overline{m}_{H,I(i)} \cdot \overline{m}_{H,i+1}\right]$$

$$K_{I(i+1)} = \left[1 - \sum_{t=1}^{N} \sum_{\substack{j=1 \\ j \neq t}}^{N} m_{t,I(i)} \cdot m_{j,i+1}\right]^{-1} \qquad , N = 1,2$$

$$i = \{1,2 \ldots, L-1\}$$

Step 4:

$$\beta_n = \frac{m_{n,I(L)}}{1 - \overline{m}_{H,I(L)}}$$

Nomenclature:

n = index representing the status of information availability as well as the information value.

The present study considers only information availability (n=1) and unavailability (n=2)

i = attributes identifier (variable for each branch)

I = represents a sub-category or a category in the hierarchical information tree

$\beta_{n,i}$ = score for the case of available/unavailable information for attribute *i*

ω_i = weight assigned to attribute *i*

$m_{n,i}$ = metric representing the availability/unavailability of information for attribute *i*

$m_{H,i}$= metric representing the aggregate uncertainty regarding the availability of information

$m_{n,I(L)}$= aggregate score of sub-categories under the indicated category

$\overline{m}_{H,I(L)}$ = aggregate metric of uncertainty for all subcategories under the category

$K_{I(i+1)}$ = normalizing factor

L = number of attributes under a sub-category or number of sub-categories under a category

C2. SCORING APPROACH RULES

In the present work, we provide, as an illustration of the approach, two different scoring scales. The first is based on a logarithmic scale. The logarithmic scoring rule was set by using the expression, $s = 1 - \frac{1}{e^{aN}}$ where s represents a score assigned for a specific information attribute based on the number of publications available (N), and $a = \frac{1}{N}\ln\left(\frac{1}{1-s}\right)$ is a constant determined by N (the maximum number of publications available for one information attribute (i.e., 25 for the example used in the present work). The attribute score was then assigned based on the number of publications providing information for the attribute. Accordingly, $\beta_{n,i}$ scores of 0.2, 0.3, 0.6, 0.7, 0.85, 0.85 and 1 were assigned for a given information attribute that was supported by one publication, two to three publications, four to five publications, between six and nine publications, more than ten and less than fifteen publications, sixteen to twenty publications, and more than twenty one publications, respectively. The second scoring scale was set as a simple linear scoring rule whereby $\beta_{n,i}$ scores of 0.25, 05, 0.75 and 1 were assigned for a given information attribute when the documented literature consisted of only one publication, at least two but less than five publications, five or less than ten publications, and more than ten publications, respectively.

Table C1. Scoring scales used in IANano
Comparison of scoring scales used in IANano based on the categories/bins derived from the number of publications.

Log scoring scale			**Simple scoring scale**	
Number of publications (categories/bins)	Average score categories	Score assigned	Number of publications (categories/bins)	Score
>21 (21-25)	0.99	1	>10	1
16-20	0.96	0.95		
10-15	0.89	0.85		
6-9	0.74	0.7	6-9	0.75
4-5	0.56	0.6	2-5	0.5
2-3	0.37	0.3		
1	0.17	0.2	1	0.25

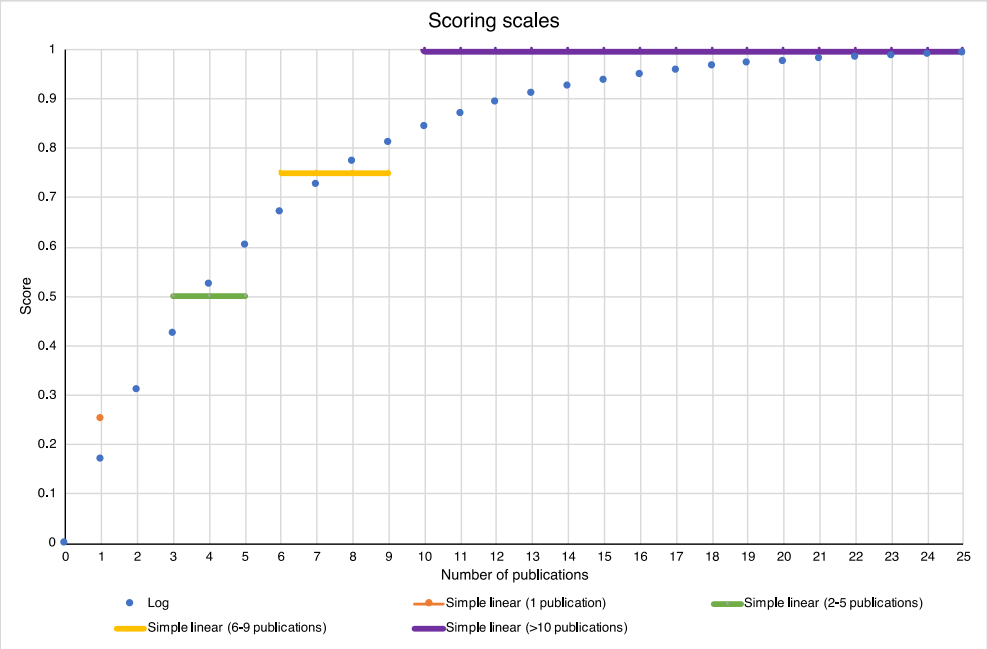


Figure C1. Scoring scales used in the presented IANano case studies for determining the adequacy of information based on the number of publications available per attribute.
The log scale establishes attributes $\beta_{n,i}$ scores of 0.2, 0.3, 0.6, 0.7, 0.85, 0.95 and 1 when supported by one publication, two to three publications, four to five publications, between six and nine publications, more than ten and less than fifteen publications, sixteen to twenty publications, and more than twenty one publications, respectively. For the simple linear scale, the $\beta_{n,i}$ attribute scores are set as 0.25, 05, 0.75 and 1 when the documented literature for the attribute is based on only one publication, at least two but less than five publications, five or less than ten publications, and more than ten publications, respectively.

Table C2. Detailed data collection process to assess the information available to conduct EIA of ENMs for the case studies described in Chapter 3

1. Literature search
The literature search was conducted for exposure and hazard information for each ENM included in the case studies (i.e., Nano TiO_2, Cu-CuO, and ZnO). Search terms included, for example, "exposure", "fate and transport", "applications", "physical form" "environmental release", "life cycle", "applications/commercial applications/consumer products" and ENM identifiers (e.g., "CuO nanoparticles", "CuO nanomaterial", "Copper nanoparticles/nanomaterials", and Copper nanoparticles/nanomaterial(s)).
2. Examination
Each identified publication was examined and included for subsequent IANano analysis if it provided the following information: - Nanomaterial identification/characterization: The nanomaterial under consideration was indeed a nanomaterial, nanoparticle or a nano-sized material. If the study referred of an ultrafine particle, additional evaluation of the publication was done to verify that the material was of a size ≤100 nm. - Physicochemical parameters: Clear documentation of the methodology that led to the reported outcomes. - Toxicity parameters: Clear documentation of the assay/experimental conditions and/or statistical analysis/model used, and toxicity/bioactivity outcomes. - Fate and Transport: Information regarding the approach used to quantify the reported source releases estimates (e.g., measurements, models, marketing/manufacturer reports, etc.), exposure concentrations (e.g., field monitoring, models, surveys).
3. Initial information extraction
The compiled published information sources were cataloged (author names and publication dates) with specific summary information categories included regarding main findings (e.g., experimental conditions, outcome, assay, species where tested if applicable) and category/sub-category/ attribute and where it could be used (e.g., toxicity evidence of in vitro toxicity human health related outcomes).
4. Information curation and analysis
Following information extraction (item 3), the information attributes were organized in a table for noting the availability or unavailability of information (i.e., Yes/No characterization) in the particular source regarding the attributes. Each publication/study was counted for the scoring process as per the selected scoring scale.

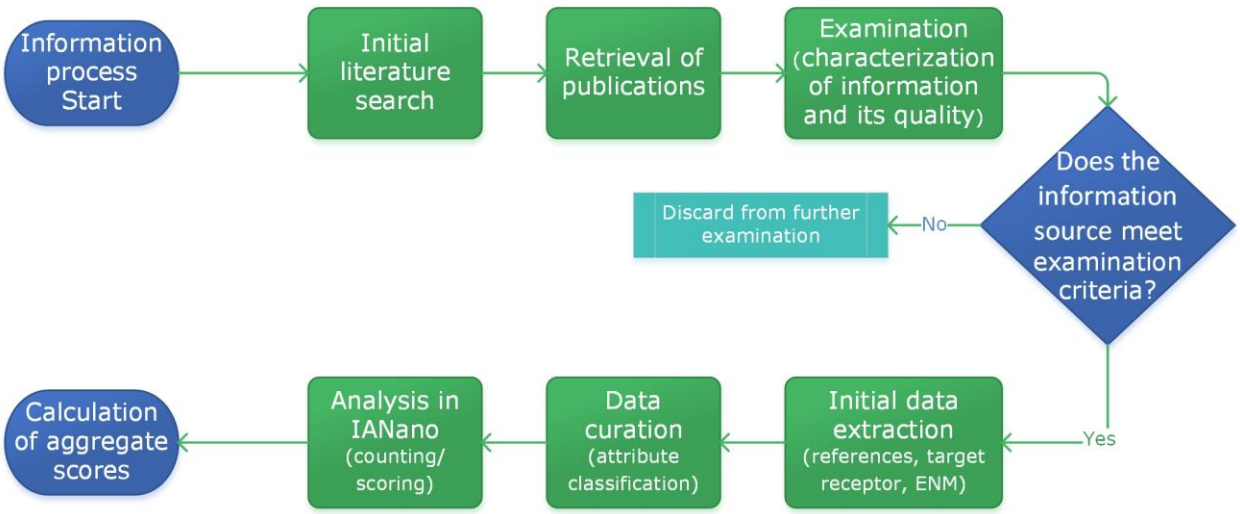


Figure C2. Compilation and curation of information

Steps taken to collect and curate the publications used in IANano (**Chapter 3**). This process describes the analysis of the publications in terms of data quality (e.g., criteria used to include publications in the study, such as characterization of ENM properties, description of statistical analysis for the results, and target organism).

Table C3. Weights assigned to categories, sub-categories and attributes for exposure potential in the general EIA scenario (Scenario I)

Categories	Weight	Sub-categories	Weight	Attributes	Weight
ENM characteristics	0.333	ENM state as applied in matrix/product	0.5	1. Airborne	0.333
				2. Suspended in liquids (dispersive/formulation)	0.333
				3. Suspended in solids (solid article)	0.333
		ENM state as released from matrix/product	0.5	4. Free nanoparticle/Free NM	0.333
				5. Homoaggregates	0.333
				6. Heteroaggregates (NM attached to larger particles)	0.333
Fate and transport parameters	0.333	Potential release to the environment (at different life cycle stages)	0.333	7. Manufacture	0.333
				8. Use	0.333
				9. Disposal	0.333
		Environmental compartment where release occurs	0.333	10. Water	0.333
				11. Soil	0.333
				12. Air	0.333
		Geographical scale	0.333	13. Geographical information	0.5
				14. Meteorological information	0.5
Exposure scenarios	0.333	Exposure conditions	0.5	15. Occupational	0.333
				16. Environmental	0.333
				17. Consumer product use	0.333
		Exposure receptors	0.5	18. Human	0.5
				19. Ecological	0.5

Table C4. Weights assigned to categories, sub-categories and attributes for hazard potential in the general EIA scenario (Scenario I)

Categories	Weight	Sub-categories	Weight	Attributes	Weight
In vivo	0.333	In vivo studies focusing on human health outcomes	0.5	1. Carcinogenicity, Mutagenicity, Development, and or Reproductive	0.333
				2. Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0.333
				3. Chronic toxicity (caused by multiple exposures)	0.333
		In vivo studies focusing on ecological impacts	0.5	4. Complex systems (mesocosm, microcosm, field and benthic), Bioaccumulation, and or Persistence	0.333
				5. Species Sensitivity Distribution (SSD)	0.333
				6. Toxicity in environmentally relevant species	0.333
In vitro	0.333	In vitro studies aimed at human health	0.5	7. Mutagenicity/ Genotoxicity	0.333
				8. Oxidative stress and or Inflammation	0.333
				9. Cytotoxicity (e.g., cell viability)	0.333
		In vitro studies aimed at non-human health outcomes	0.5	10. Cell viability or Cell lethality	0.5
				11. ENM transport across membranes	0.5
In silico and expert judgment	0.333	QSARs	0.333	12. QSARs (related to cytotoxicity or other outcomes)	0.333
		Other modeling	0.333	13. Meta-analysis, toxicokinetics, read-across	0.333
		Expert judgment	0.333	14. Expert elicitation/judgment	0.333

Table C5. Weights assigned to categories, sub-categories and attributes for exposure potential in Scenarios II and III ENM (direct) release into aquatic environments and associated potential impact on ecological receptors

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
ENM characteristics	0.333	ENM state as applied in matrix/product	0.5	1. Airborne	0	Airborne ENMs are not considered in this scenario given that intermedia transport is excluded from the assessment
				2. Suspended in liquids (dispersive/formulation)	0.5	
				3. Suspended in solids (solid article)	0.5	
		ENM state as released from matrix/product	0.5	4. Free nanoparticle/Free ENM	0.333	All these forms are included as relevant in the scenario, given the potential for transformation of the ENM in the aquatic environment
				5. Homoaggregates	0.333	
				6. Heteroaggregates (ENM attached to larger particles)	0.333	
Fate and transport parameters	0.333	Potential release to the environment (at different life cycle stages)	0.333	7. Manufacture	0	N/A for this scenario
				8. Use	1	Only direct release from ENM use to aquatic environments is considered as relevant in the scenario
				9. Disposal	0	N/A for this scenario
		Environmental compartment where release occurs	0.333	10. Water	1	The target environmental compartment for the analysis is water and intermedia air/water, water/sediment is not considered
				11. Soil	0	N/A for this scenario
				12. Air	0	N/A for this scenario
		Geography	0.333	13. Geographical information	1	These fate and transport parameters are excluded given that air/water, water/sediment are not considered in the analysis
				14. Meteorological information	0	N/A for this scenario
Exposure scenarios	0.333	Exposure conditions	0.5	15. Occupational	0	N/A for this scenario
				16. Environmental	1	Environmental (outdoor) exposure of ecological receptors.
				17. Consumer product use	0	N/A for this scenario
		Exposure receptors	0.5	18. Human	0	N/A for this scenario
				19. Ecological	1	

Table C6. Weights assigned to categories, sub-categories and attributes for hazard potential in Scenarios II and III ENM (direct) release into aquatic environments and associated potential impact on ecological receptors

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
In vivo	0.333	In vivo studies focused on human health outcomes	0	1. Carcinogenicity, Mutagenicity, Development, and or Reproductive	0	Given that the main target of this scenario were ecological receptors, the studies grouped in this category were excluded from the analysis
				2. Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0	
				3. Chronic toxicity (caused by multiple exposures)	0	
		In vivo studies focused on ecological impacts	1	4. Complex systems (mesocosm, microcosm, field and benthic), Bioaccumulation, and or Persistence	0.333	
				5. Species Sensitivity Distribution (SSD)	0.333	
				6. Toxicity in environmentally relevant species	0.333	
In vitro	0.333	In vitro studies aimed at human health	0	7. Mutagenicity/ Genotoxicity	0	Given that the main target of this scenario were ecological receptors, the studies grouped in this category were excluded from the analysis
				8. Oxidative stress and or Inflammation	0	
				9. Cytotoxicity (e.g., cell viability)	0	
		In vitro studies aimed at non-human health outcomes	1	10. Cell viability or Cell lethality	0.5	
				11. NM transport across membranes	0.5	
In silico and expert judgment	0.333	QSARs	0.333	12. QSARs (related to cytotoxicity or other outcomes)	0.333	
		Other modeling	0.333	13. Meta-analysis, toxicokinetics, read-across	0.333	
		Expert judgment	0.333	14. Expert elicitation/judgment	0.333	

Table C7. Weights assigned to categories, sub-categories and attributes for exposure potential in Scenario IV ENM (direct) release to soil and water from waste water treatment plants and associated potential impact on ecological receptors

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
ENM characteristics	0.333	ENM state as applied in matrix/product	0.5	1. Airborne	0	N/A for this scenario
				2. Suspended in liquids (dispersive/formulation)	0.5	
				3. Suspended in solids (solid article)	0.5	
		ENM state as released from matrix/product	0.5	4. Free nanoparticle/Free ENM	0.333	All these forms are included as relevant in the scenario, given the potential for transformation of the ENM in the aquatic environment
				5. Homoaggregates	0.333	
				6. Heteroaggregates (ENM attached to larger particles)	0.333	
Fate and transport parameters	0.333	Life cycle stage where release occurs	0.333	7. Manufacture	0	N/A for this scenario
				8. Use	1	Only direct release from ENM use to aquatic environments is considered as relevant in the scenario
				9. Disposal	0	N/A for this scenario
		Environmental compartment where release occurs	0.333	10. Water	0.5	
				11. Soil	0.5	
				12. Air	0	N/A for this scenario
		Geography Meteorology	0.333	13. Geographical information	0.5	
				14. Meteorological information	0.5	
Exposure scenarios	0.333	Exposure conditions	0.5	15. Occupational	0	N/A for this scenario
				16. Environmental	1	The target exposure scenario is environmental given the focus on ecological receptors
				17. Consumer product use	0	N/A for this scenario
		Exposure receptors	0.5	18. Human	0	N/A for this scenario
				19. Ecological	1	

Table C8. Weights assigned to categories, sub-categories and attributes for hazard potential in Scenario IV ENM (direct) release to soil and water from waste water treatment plants and associated potential impacts on ecological receptors

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
In vivo	0.333	In vivo studies focused on human health outcomes	0	1. Carcinogenicity, Mutagenicity, Development, and or Reproductive	0	Ecological receptors are the target in this scenario. Therefore, the studies grouped in this category were excluded from the analysis.
				2. Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0	
				3. Chronic toxicity (caused by multiple exposures)	0	
		In vivo studies focused on ecological impacts	1	4. Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence	0.333	
				5. Species Sensitivity Distribution (SSD)	0.333	
				6. Toxicity in environmentally relevant species	0.333	
In vitro	0.333	In vitro studies aimed at human health	0	7. Mutagenicity/ Genotoxicity	0	Ecological receptors are the target in this scenario. Therefore, the studies grouped in this category were excluded from the analysis.
				8. Oxidative stress and or Inflammation	0	
				9. Cytotoxicity (e.g., cell viability)	0	
		In vitro studies aimed at non-human health outcomes	1	10. Cell viability or Cell lethality	0.5	
				11. ENM transport across membranes	0.5	
In silico and expert judgment	0.333	QSARs	0.333	12. QSARs (related to cytotoxicity or other outcomes)	0.333	
		Other modeling	0.333	13. Meta-analysis, toxicokinetics, read-across	0.333	
		Expert judgment	0.333	14. Expert elicitation/judgment	0.333	

Table C9. Weights assigned to categories, sub-categories and attributes for exposure potential in Scenario V.A and V.B (nano TiO_2 release into air and associated impact on the human receptor)

Categories	Weight	Sub-categories	Weight V.A	Weight V.B	Attributes	Weight (V.A)	Weight (V.B)	Assumptions/ conditions considered in the scenario
ENM characteristics	0.333	ENM state as applied in matrix/ product	0.5	0.5	1. Airborne	1	1	Airborne ENMs are considered in this scenario as the only potential form relevant to inhalation exposure
					2. Suspended in liquids (dispersive/formulation)	0	0	N/A for this scenario
					3. Suspended in solids (solid article)	0	0	N/A for this scenario
		ENM state as released from matrix/ product	0.5	0.5	4. Free nanoparticle/ Free ENM	0.333	0.333	
					5. Homoaggregates	0.333	0.333	
					6. Heteroaggregates (ENM attached to larger particles)	0.333	0.333	
Fate and transport parameters	0.333	Life cycle stage where release occurs	0.333	0.5	7. Manufacture	0	1	In the workplace risk assessment scenario, the lifecycle stage of manufacture is considered as the only one relevant to the analysis
					8. Use	1	0	In the environmental risk assessment scenario, the lifecycle stage of use is considered as the only one relevant to the analysis, given that only direct release is considered
					9. Disposal	0	0	N/A for this scenario
		Environmental compartment where release occurs	0.333	0.5	10. Water	0	0	Only air is considered as the relevant environmental compartment given that the focus of this scenario is inhalation exposure
					11. Soil	0	0	
					12. Air	1	1	
		Geography Meteorology	0.333	0	13. Geographical information	1	0	N/A for Scenario V.B
					14. Meteorological information	0	0	N/A for this scenario
Exposure scenarios	0.333	Exposure conditions	0.5	0.5	15. Occupational	0	1	
					16. Environmental	1	0	N/A for this scenario

Categories	Weight	Sub-categories	Weight V.A	Weight V.B	Attributes	Weight (V.A)	Weight (V.B)	Assumptions/ conditions considered in the scenario
					17. Consumer product use	0	0	N/A for this scenario
		Exposure receptors	0.5	0.5	18. Human	1	1	
					19. Ecological	0	0	N/A for this scenario

Table C10. Weights assigned to categories, sub-categories and attributes for hazard potential in Scenario V.A and V.B (nano TiO_2 release into air and associated potential impact on the human receptor)

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
In vivo	0.333	In vivo studies focused on human health outcomes	1	1. Carcinogenicity, Mutagenicity, Development, and or Reproductive	0.333	
				2. Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0.333	
				3. Chronic toxicity (caused by multiple exposures)	0.333	
		In vivo studies focused on ecological impacts	0	4. Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence	0	The scenario focus is on human exposure. Therefore, studies grouped in this category were excluded from the analysis
				5. Species Sensitivity Distribution (SSD)	0	
				6. Toxicity in environmentally relevant species	0	
In vitro	0.333	In vitro studies aimed at human health	1	7. Mutagenicity/ Genotoxicity	0.333	
				8. Oxidative stress and or Inflammation	0.333	
				9. Cytotoxicity (e.g., cell viability)	0.333	
		In vitro studies aimed at non-human health outcomes	0	10. Cell viability or Cell lethality	0	The scenario focus is on human exposure. Therefore, studies grouped in this category were excluded from the analysis
				11. ENM transport across membranes	0	
In silico and expert judgment	0.333	QSARs	0.333	12. QSARs (related to cytotoxicity or other outcomes)	0.333	
		Other modeling	0.333	13. Meta-analysis, toxicokinetics, read-across	0.333	
		Expert judgment	0.333	14. Expert elicitation/judgment	0.333	

Table C11. Weights assigned to categories, sub-categories and attributes for exposure potential in Scenario VI (Exposure to nano TiO_2 via consumer product use and associated potential impacts on the human receptor)

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
ENM characteristics	0.333	ENM state as applied in matrix/product	0.5	1. Airborne	0.5	
				2. Suspended in liquids (dispersive/formulation)	0.5	
				3. Suspended in solids (solid article)	0	This scenario excludes exposure from direct contact with solid applications that may not result in exposure (e.g., electronic materials). However, studies reporting food containing nano TiO_2 are included in the analysis.
		ENM state as released from matrix/product	0.5	4. Free nanoparticle/Free ENM	0.333	
				5. Homoaggregates	0.333	
				6. Heteroaggregates (NM attached to larger particles)	0.333	
Fate and transport parameters	0.333	Potential release to the environment (at different life cycle stages)	1	7. Manufacture	0	
				8. Use	1	Only use is considered relevant given that this scenario includes exposure directly related to contact with consumer products/consumer product use
				9. Disposal	0	
		Environmental compartment where release occurs	0	10. Water	0	
				11. Soil	0	
				12. Air	0	
		Geographical scale	0	13. Geographical information	0	
				14. Meteorological information	0	
Exposure conditions	0.333	Potential exposure scenarios	0.5	15. Occupational	0	
				16. Environmental	0	
				17. Consumer product use	1	
		Potential exposure receptors	0.5	18. Human	1	
				19. Ecological	0	

Table C12. Weights assigned to categories, sub-categories and attributes for hazard potential in Scenario VI) exposure to nano TiO_2 via consumer product use and its potential impacts in human receptors

Categories	Weight	Sub-categories	Weight	Attributes	Weight	Assumptions/conditions considered in the scenario
In vivo	0.333	In vivo studies focused on human health outcomes	1	1. Carcinogenicity, Mutagenicity, Development, and or Reproductive	0.333	
				2. Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0.333	
				3. Chronic toxicity (caused by multiple exposures)	0.333	
		In vivo studies focused on ecological impacts	0	4. Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence	0	The scenario focus is on human exposure. Therefore, studies grouped in this category were excluded from the analysis
				5. Species Sensitivity Distribution (SSD)	0	
				6. Toxicity in environmentally relevant species	0	
In vitro	0.333	In vitro studies aimed at human health	1	7. Mutagenicity/ Genotoxicity	0.333	
				8. Oxidative stress and or Inflammation	0.333	
				9. Cytotoxicity (e.g., cell viability)	0.333	
		In vitro studies aimed at non-human health outcomes	0	10. Cell viability or Cell lethality	0	The scenario focus is on human exposure. Therefore, studies grouped in this category were excluded from the analysis
				11. ENM transport across membranes	0	
In silico and expert judgment	0.333	QSARs	0.333	12. QSARs (related to cytotoxicity or other outcomes)	0.333	
		Other modeling	0.333	13. Meta-analysis, toxicokinetics, read-across	0.333	
		Expert judgment	0.333	14. Expert elicitation/judgment	0.333	

Table C13. Number of publications available (as of 2016) per attribute of exposure in different EIA scenarios

Categories	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
ENM characteristics	ENM state as applied in matrix/ product	Airborne	0	2	1	0	0	0	0	2	2	2
		Suspended in liquids (dispersive)	3	7	1	3	2	1	3	0	0	7
		Suspended in solids (solid article)	1	4	1	1	1	1	1	0	0	1
	ENM state as released from matrix/ product	Free nanoparticle/ Free ENM	1	5	0	1	0	0	1	3	5	2
		Homoaggregates	4	4	2	4	2	2	4	0	0	4
		Heteroaggregates (ENM attached to larger particles)	1	1	2	1	0	2	1	0	0	1
Fate and transport parameters	Life cycle stage where release occurs	Manufacture	1	2	2	0	0	0	0	0	2	0
		Use	2	6	3	2	1	3	2	6	0	6
		Disposal	1	2	1	0	0	0	0	0	0	0
	Environmental compartment where release occurs	Water	5	10	7	5	2	6	4	0	0	0
		Soil	3	6	5	0	0	0	3	0	0	0
		Air	2	8	3	0	0	0	0	8	4*	0
	Geography Meteorology	Geographical information	1	1	1	1	1	1	1	1	0	0
		Meteorological information	1	1	1	0	0	0	1	0	0	0
Exposure scenarios	Exposure conditions	Occupational	1	12	2	0	0	0	0	0	12	0
		Environmental	1	4	2	1	1	2	1	2	0	0
		Consumer product use	2	6	4	0	0	0	0	0	0	6
	Exposure receptors	Human	2	7	2	0	0	0	0	4 **	7	7
		Ecological	2	2	2	2	2	2	2	0	0	0
TOTAL			**34**	**88**	**42**	**21**	**12**	**20**	**24**	**25**	**45**	**35**

* Occupational settings ** inhalation only

Table C14. Number of publications available (as of 2016) per attribute of hazard for the different EIA scenarios

Category	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
In vivo	In vivo studies focused on human health outcomes	Carcinogenicity, Mutagenicity, Development, and or Reproductive	1	11	2	0	0	0	0	5	11	11
		Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	5	4	11	0	0	0	0	1	4	4
		Chronic toxicity (caused by multiple exposures)	1	6	2	0	0	0	0	0 *	6	6
	In vivo studies focused on ecological impacts	Complex systems (e.g., mesocosm), Bioaccumulation, Persistence	7	2	1	7	1	1	7	0	0	0
		Species Sensitivity Distribution (SSD)	2	0	1	2	0	1	2	0	0	0
		Toxicity in environmentally relevant species	21	12	17	15	8	17	21	0	0	0
In vitro	In vitro studies aimed at human health	Mutagenicity/ Genotoxicity	10	25	12	0	0	0	0	25	25	25
		Oxidative stress and or Inflammation	4	6	15	0	0	0	0	6	6	6
		Cytotoxicity (e.g., cell viability)	6	6	17	0	0	0	0	6	6	6
	In vitro studies aimed at non-human health outcomes	Cell viability or Cell lethality	9	2	2	9	9	2	9	0	0	0
		ENM transport across membranes	3	1	1	3	3	1	3	0	0	0
In silico and expert judgment	QSARs	QSARs (related to cytotoxicity or other outcomes)	2	4	3	2	2	3	2	4	4	4
	Other modeling	Meta-analysis, toxicokinetics, read-across	3	4	6	3	3	6	3	4	4	4
	Expert elicitation	Expert judgment, elicitation	0	2	1	0	0	1	0	2	2	2
Total			**74**	**85**	**91**	**41**	**26**	**32**	**47**	**53**	**68**	**68**

*only intratracheal installation studies were included to explore the impact of the body of information available for this route of exposure

Table C15. Scores assigned per attribute of exposure information based on the simple linear scoring scale for various EIA scenarios

Categories	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
ENM characteristics	ENM state as applied in matrix/product	Airborne	0	0.5	0.25	0	0	0	0	0.5	0.5	0.5
		Suspended in liquids (dispersive/formulation)	0.5	0.75	0.25	0.5	0.5	0.25	0.5	0	0	0.75
		Suspended in solids (solid article)	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0	0	0.25
	ENM state as released from matrix/product	Free nanoparticle/Free ENM	0.25	0.5	0	0.25	0	0	0.25	0.5	0.5	0.5
		Homoaggregates	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5
		Heteroaggregates (ENM attached to larger particles)	0.25	0.25	0.5	0.25	0	0.5	0.25	0	0	0.25
Fate and transport parameters	Life cycle stage where release occurs	Manufacture	0.25	0.5	0.5	0	0	0	0	0	0.5	0
		Use	0.5	0.75	0.5	0.5	0.25	0.5	0.5	0.75	0	0.75
		Disposal	0.25	0.5	0.25	0	0	0	0	0	0	0
	Environmental compartment where release occurs	Water	0.5	1	0.75	0.5	0.5	0.75	0.5	0	0	0
		Soil	0.5	0.75	0.5	0	0	0	0.5	0	0	0
		Air	0.5	0.75	0.5	0	0	0	0	0.75	0.5	0
	Geography Meteorology	Geographical information	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0	0
		Meteorological information	0.25	0.25	0.25	0	0	0	0.25	0	0	0
Exposure scenarios	Exposure conditions	Occupational	0.25	1	0.5	0	0	0	0	0	1	0
		Environmental	0.25	0.5	0.5	0.25	0.25	0.5	0.25	0.5	0	0
		Consumer product use	0.5	0.75	0.5	0	0	0	0	0	0	0.75
	Exposure receptors	Human	0.5	0.75	0.5	0	0	0	0	0.5	0.75	0.75
		Ecological	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0
Aggregate score			**0.26**	**0.64**	**0.34**	**0.36**	**0.24**	**0.39**	**0.31**	**0.45**	**0.66**	**0.72**

Table C16. Scores assigned per attribute of hazard information based on the simple linear scoring scale for the various EIA scenarios

Categories	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
In vivo	In vivo studies focused on human health outcomes	Carcinogenicity, Mutagenicity, Development, and or Reproductive	0.25	1.0	0.50	0	0	0	0	0.5	1	1
		Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0.50	0.5	1.00	0	0	0	0	0.25	0.5	0.5
		Chronic toxicity (caused by multiple exposures)	0.25	0.75	0.50	0	0	0	0	0	0.75	0.75
	In vivo studies focused on ecological impacts	Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence	0.75	0.5	0.25	0.75	0.25	0.25	0.75	0	0	0
		Species Sensitivity Distribution (SSD)	0.50	0.0	0.25	0.5	0	0.25	0.5	0	0	0
		Toxicity in environmentally relevant species	1.00	1.0	1.00	1	0.75	1	1	0	0	0
In vitro	In vitro studies aimed at human health	Mutagenicity/ Genotoxicity	1.00	1.0	1.00	0	0	0	0	1	1	1
		Oxidative stress and or Inflammation	0.50	0.5	1.00	0	0	0	0	0.75	0.75	0.75
		Cytotoxicity (e.g., cell viability)	0.75	0.5	1.00	0	0	0	0	0.75	0.75	0.75
	In vitro studies aimed at non-human health outcomes	Cell viability or Cell lethality	0.75	0.5	0.50	0.75	0.75	0.5	0.75	0	0	0
		ENM transport across membranes	0.50	0.25	0.25	0.5	0.5	0.25	0.5	0	0	0
In silico and expert judgment	QSARs	QSARs (related to cytotoxicity or other outcomes)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Other modeling	Meta-analysis, toxicokinetics, read-across	0.5	0.5	0.75	0.5	0.5	0.75	0.5	0.5	0.5	0.5
	Expert elicitation	Expert judgment, elicitation	0	0.5	0.25	0	0	0.25	0	0.5	0.5	0.5
Aggregate score			**0.55**	**0.58**	**0.64**	**0.60**	**0.39**	**0.44**	**0.60**	**0.53**	**0.77**	**0.77**

Table C17. Scores assigned per attribute of exposure information based on the log scoring scale for the various EIA scenarios

Categories	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
ENM characteristics	ENM state as applied in matrix/product	Airborne	0	0.3	0.2	0	0	0	0	0.3	0.3	0.2
		Suspended in liquids (dispersive/formulation)	0.3	0.7	0.2	0.3	0.3	0.2	0.3	0	0	0.7
		Suspended in solids (solid article)	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0	0	0.2
	ENM state as released from matrix/product	Free nanoparticle/Free ENM	0.2	0.6	0	0.2	0	0	0.2	0.3	0.6	0.3
		Homoaggregates	0.6	0.6	0.3	0.6	0.3	0.3	0.6	0	0	0.6
		Heteroaggregates (ENM attached to larger particles)	0.2	0.2	0.3	0.2	0	0.3	0.2	0	0	0.2
Fate and transport parameters	Life cycle stage where release occurs	Manufacture	0.2	0.3	0.3	0	0	0	0	0	0.3	
		Use	0.3	0.7	0.3	0.3	0.2	0.3	0.3	0.6	0	0.6
		Disposal	0.2	0.3	0.2	0	0	0	0	0	0	0
	Environmental compartment where release occurs	Water	0.6	0.85	0.7	0.6	0.3	0.7	0.6	0	0	0
		Soil	0.3	0.7	0.6	0	0	0	0.3	0	0	0
		Air	0.3	0.7	0.3	0	0	0		0.7	0.6	0
	Geography Meteorology	Geographical information	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0
		Meteorological information	0.2	0.2	0.2	0	0	0	0.2	0	0	0
Exposure scenarios	Exposure conditions	Occupational	0.2	0.85	0.3	0	0	0	0	0	0.85	0
		Environmental	0.2	0.6	0.3	0.2	0.2	0.3	0.2	0.3	0	0
		Consumer product use	0.3	0.7	0.6	0	0	0	0	0	0	0.7
	Exposure receptors	Human	0.3	0.7	0.3	0	0	0	0	0.6	0.7	0.7
		Ecological	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	0
Aggregate score			**0.15**	**0.54**	**0.19**	**0.22**	**0.13**	**0.22**	**0.19**	**0.33**	**0.50**	**0.58**

Table C18. Scores assigned per attribute of hazard information based on the log scoring scale for the various EIA scenarios

Categories	Sub-Categories	Attributes	Scenario									
			I Cu-CuO	**I TiO_2**	**I ZnO**	**II.A**	**II.B**	**III**	**IV**	**V.A**	**V.B**	**VI**
In vivo	In vivo studies focused on human health outcomes	Carcinogenicity, Mutagenicity, Development, and or Reproductive	0.2	0.85	0.3	0	0	0	0	0.6	0.85	0.85
		Acute toxicity, Systemic toxicity, Neuro-toxicity, and or Skin/eye irritation	0.6	0.6	0.85	0	0	0	0	0.2	0.6	0.6
		Chronic toxicity (caused by multiple exposures)	0.2	0.7	0.3	0	0	0	0	0	0.7	0.7
	In vivo studies focused on ecological impacts	Complex systems (mesocosm, microcosm, and field studies), Bioaccumulation, Persistence	0.7	0.3	0.2	0.7	0.2	0.2	0.7	0	0	0
		Species Sensitivity Distribution (SSD)	0.3	0	0.2	0.3	0	0.2	0.3	0	0	0
		Toxicity in environmentally relevant species	0.96	0.85	0.95	0.85*	0.7	0.95	1	0	0	0
In vitro	In vitro studies aimed at human health	Mutagenicity/ Genotoxicity	0.85	1	0.85	0	0	0	0	1	1	1
		Oxidative stress and or Inflammation	0.6	0.7	0.85	0	0	0	0	0.7	0.7	0.7
		Cytotoxicity (e.g., cell viability)	0.7	0.7	0.95	0	0	0	0	0.7	0.7	0.7
	In vitro studies aimed at non-human health outcomes	Cell viability or Cell lethality	0.7	0.3	0.3	0.7	0.7	0.3	0.7	0	0	0
		ENM transport across membranes	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0	0	0
In silico and expert judgment	QSARs	QSARs (related to cytotoxicity or other outcomes)	0.3	0.6	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.6
	Other modeling	Meta-analysis, toxicokinetics, read-across	0.3	0.6	0.7	0.3	0.3	0.7	0.3	0.6	0.6	0.6
	Expert elicitation	Expert judgment, elicitation	0	0.3	0.2	0	0	0.2	0	0.3	0.3	0.3
Aggregate score			**0.42**	**0.56**	**0.46**	**0.42**	**0.27**	**0.31**	**0.44**	**0.53**	**0.74**	**0.74**

Table C19. Summary of information available for nano Cu-CuO related to exposure potential

Attribute name and number	References
1. Airborne	
2. Suspended in liquids (dispersive/formulation)	(Wilson 2013), (Ding, Meneses et al. 2013), (Adeleye, Oranu et al. 2016)
3. Embedded in a solid matrix (solid article)	(Chapman, Le Nor et al. 2013)
4. Free Nanoparticles	(Ding, Meneses et al. 2013)
5. Homoaggregates	(Conway, Adeleye et al. 2015), (Adeleye, Oranu et al. 2016), (Miao, Wang et al. 2016), (Wang, von dem Bussche et al. 2013)
6. Heteroaggregates (NPs attached to other/larger particles)	(Wang, Habibul et al. 2015)
7. Manufacture	(Keller, McFerran et al. 2013)
8. Use	(Ding, Meneses et al. 2013), (Adeleye, Oranu et al. 2016)
9. Disposal	(Keller, McFerran et al. 2013)
10. Water	(Keller, McFerran et al. 2013), (Pu, Tang et al. 2016), (Lazareva and Keller 2014), (Taylor and Walker 2016), (Liu and Cohen 2014)
11. Soil	(Navratilova, Praetorius et al. 2015), (Liu and Cohen 2014), (Lazareva and Keller 2014)
12. Air	(Keller, McFerran et al. 2013), (Liu and Cohen 2014)
13. Geographical	(Liu and Cohen 2014)
14. Meteorological	(Liu and Cohen 2014)
15. Occupational	(Zúñiga Rojas, Blamey Benavides et al. 2013)
16. Environmental	(Chio, Chen et al. 2012)
17. From commercial product use	(Cushen, Kerry et al. 2014), (Nazarenko, Han et al. 2011)
18. Human	(Cushen, Kerry et al. 2014), (Hannon, Kerry et al. 2016)
19. Ecological	(Hanna, Miller et al. 2014), (Navarro, Baun et al. 2008)

Table C20. Summary of information available for nano Cu-CuO related to hazard potential as of 2016

Attribute name and number	References
1.Carcinogenicity, mutagenicity, development, reproductive	(Adamcakova-Dodd, Monick et al. 2015)
2.Acute toxicity, Systemic toxicity, Skin/eye irritation	(Cho, Duffin et al. 2010, Cho, Duffin et al. 2012),(Chen, Meng et al. 2006),(Pettibone, Adamcakova-Dodd et al. 2008),(Gosens, Cassee et al. 2016)
3.Chronic toxicity (caused by multiple exposures)	(Cho, Duffin et al. 2010)
4.Complex systems (mesocosm, microcosm, field and benthic), bioaccumulation, persistence	(Zhang, Hua et al. 2014),(Buffet, Richard et al. 2013),(Hu, Culloty et al. 2014),(Dai, Syberg et al. 2013),(Isani, Falcioni et al. 2013),(Gomes, Novais et al. 2012),(Hanna, Miller et al. 2014)
5.Species Sensitivity Distribution (SSD)	(Garner, Suh et al. 2015),(Adam, Schmitt et al. 2015)
6.Toxicity in environmentally relevant species	(Griffitt, Weil et al. 2007, Griffitt, Luo et al. 2008),(Wu, Torres-Duarte et al. 2015),(Vicario-Pares, Castanaga et al. 2014),(Blinova, Ivask et al. 2010),(Manusadžianas, Caillet et al. 2012), (Bielmyer-Fraser, Jarvis et al. 2014), (Aruoja, Dubourguier et al. 2009), (Jo, Choi et al. 2012), (Gomes, Pinheiro et al. 2011, Gomes, Novais et al. 2012, Gomes, Araújo et al. 2013), (Hanna, Miller et al. 2013), (Pradhan, Seena et al. 2012), (Mortimer, Kasemets et al. 2011), (Heinlaan, Ivask et al. 2008), (Zuverza-Mena, Medina-Velo et al. 2015), (Collins, Luxton et al. 2012), (Unrine, Tsyusko et al. 2010), (Anjum, Adam et al. 2015), (Lin, Taylor et al. 2015)
7.Mutagenicity/ Genotoxicity	(Semisch, Ohle et al. 2014), (Perreault, Pedroso Melegari et al. 2012), (Karlsson, Gustafsson et al. 2009, Midander, Cronholm et al. 2009), (Alarifi, Ali et al. 2013), (Di Bucchianico, Fabbrizi et al. 2013), (Akhtar, Kumar et al. 2013), (Carmona, Inostroza-Blancheteau et al. 2015), (Abudayyak, Guzel et al. 2016), (Jose, Santra et al. 2011)
8.Oxidative stress, inflammation	(Misra, Nuseibeh et al. 2014), (Wang, Li et al. 2012, Wang, von dem Bussche et al. 2013), (Cho, Duffin et al. 2012)
9.Cytotoxicity (e.g., cell viability)	(Studer, Limbach et al. 2010), (Chusuei, Wu et al. 2013), (Alarifi, Ali et al.

	2013), (Karlsson, Cronholm et al. 2008), (Wongrakpanich, Mudunkotuwa et al. 2016), (Wang, Li et al. 2012)
10. Cell viability, Lethality (bactericidal or fungicidal effect)	(Ivask, Bondarenko et al. 2010), (Ren, Hu et al. 2009), (Kaweeteerawat, Ivask et al. 2015), (Kasemets, Ivask et al. 2009), (Hu, Cook et al. 2009), (Gunawan, Teoh et al. 2011), (Bayat, Rajapakse et al. 2014), (Ananth, Dharaneedharan et al. 2015), (Baek and An 2011)
11. ENM transport across membranes	(Karlsson, Cronholm et al. 2013), (Hedberg, Karlsson et al. 2016), (Tamayo, Zapata et al. 2014)
12. QSARs (related to cytotoxicity or other outcomes)	(Liu, Zhang et al. 2013), (Pathakoti, Huang et al. 2014)
13. Meta-analysis, toxicokinetics, read-across	(Notter, Mitrano et al. 2014), (Gajewicz, Cronin et al. 2015), (Sayes, Smith et al. 2013)
14. Expert judgment/elicitation	

Table C21. Summary of information available for nano TiO_2 related to exposure potential as of 2016

Attribute name and number	References
1. Airborne	(Boxall, Chaudhry et al. 2007), (Chen, Afshari et al. 2010)
2. Suspended in liquids (dispersive/formulation)	(EPA 2010, EPA 2010), (Shandilya, Le Bihan et al. 2015), (Al-Kattan, Wichser et al. 2013), (Zhang, Leu et al. 2015), (Lim, Sisco et al. 2015), (Hsu and Chein 2007)
3. Embedded in a solid matrix (solid article)	(EPA 2010), (Windler, Lorenz et al. 2012), (Von Goetz, Lorenz et al. 2013), (Lin, Li et al. 2014)
4. Free Nanoparticles	(Kaegi, Ulrich et al. 2008), (Al-Kattan, Wichser et al. 2014), (Koivisto, Lyyränen et al. 2012), (Pelclova, Barosova et al. 2015), (Lee, Kwon et al. 2011)
5. Homoaggregates	(Aruoja, Dubourguier et al. 2009), (Botta, Labille et al. 2011), (Kaegi, Ulrich et al. 2008), (Sharma 2009)
6. Heteroaggregates (NPs attached to other/larger particles)	(Chowdhury, Cwiertny et al. 2012)
7. Manufacture	(Gottschalk, Sonderer et al. 2009), (Sun, Gottschalk et al. 2014)
8. Use	(Shandilya, Le Bihan et al. 2015), (Al-Kattan, Wichser et al. 2013), (Pirela, Sotiriou et al. 2015), (Sun, Gottschalk et al. 2014), (Nowack, Ranville et al. 2012), (Chen, Afshari et al. 2010)
9. Disposal	(EPA 2010), (Nowack, Ranville et al. 2012)
10. Water	(EPA 2010), (O'Brien and Cummins 2011), (Pu, Tang et al. 2016), (Sun, Gottschalk et al. 2014), (Gottschalk, Lassen et al. 2015), (Boxall, Tiede et al. 2007); (Mueller and Nowack 2008), (Markus, Parsons et al. 2016), (Gottschalk, Scholz et al. 2010), (Praetorius, Scheringer et al. 2012)
11. Soil	(Keller, McFerran et al. 2013), (Sun, Gottschalk et al. 2014), (Gottschalk, Lassen et al. 2015), (Boxall, Tiede et al. 2007), (Mueller and Nowack 2008), (Gottschalk, Scholz et al. 2010)
12. Air	(EPA 2010), (Gottschalk, Lassen et al. 2015), (Mueller and Nowack 2008), (Boxall, Chaudhry et al. 2007), (Sun, Gottschalk et al. 2014), (Liu and Cohen 2014) (Liu, Bilal et al. 2015) ,(Gottschalk, Scholz et al. 2010)
13. Geographical	(Liu and Cohen 2014)

14. Meteorological	(Liu and Cohen 2014)
15. Occupational	(EPA 2010), (Christensen, Johnston et al. 2011), (Lee, Kwon et al. 2011), (Gangwal, Brown et al. 2011), (Yang, Mao et al. 2011), (Pini, Salieri et al. 2016), (Liao, Chiang et al. 2008), (Koivisto, Lyyränen et al. 2012), (Pelclova, Barosova et al. 2015), (Liou, Tsai et al. 2015), (Spinazzè, Cattaneo et al. 2016), (Vaquero, Gelarza et al. 2015), (Ham, Yoon et al. 2012)
16. Environmental	(O'Brien and Cummins 2010), (Vílchez, Fernández-Rosas et al. 2015), (Pini, Salieri et al. 2016), (Holbrook, Motabar et al. 2013)
17. From commercial product use	(Schilling, Bradford et al. 2010), (Weir, Westerhoff et al. 2012), (Lorenz, Von Goetz et al. 2011), (Chen, Afshari et al. 2010), (Von Goetz, Lorenz et al. 2013), (Lin, Li et al. 2014)
8. Human	(Weir, Westerhoff et al. 2012), (Lorenz, Von Goetz et al. 2011), (Boxall, Chaudhry et al. 2007), (Chen, Afshari et al. 2010), (Pini, Salieri et al. 2016), (Holbrook, Motabar et al. 2013), (Von Goetz, Lorenz et al. 2013)
9. Ecological	(Liu and Cohen 2014), (Botta, Labille et al. 2011)

Table C22. Summary of information available for nano TiO_2 related to hazard potential as of 2016

Attribute name and number	References
1. Carcinogenicity, mutagenicity, development, reproductive	(Shimizu, Tainaka et al. 2009), (Takahashi, Mizuo et al. 2010), (Hong, Si et al. 2015), (Chen, Yan et al. 2014), (Sycheva, Zhurkov et al. 2011), (Trouiller, Reliene et al. 2009)
Carcinogenicity, mutagenicity, development, reproductive (null findings)	(Dobrzyńska, Gajowik et al. 2014), (Lindberg, Falck et al. 2012, Naya, Kobayashi et al. 2012), (Saber, Jacobsen et al. 2012)
2. Acute toxicity, Systemic toxicity, Skin/eye irritation	(Bonner, Silva et al. 2013),(Setyawati, Tay et al. 2013),(Dobrzyńska, Gajowik et al. 2014),(Shrivastava, Raza et al. 2014)
3. Chronic toxicity (caused by multiple exposures)	(Park, Yoon et al. 2009),(Kobayashi, Naya et al. 2009),(Fu, Zhang et al. 2014),(Sang, Zheng et al. 2012),(Sun, Tan et al. 2012),(Hong, Hong et al. 2015)
4. Complex systems (mesocosm, microcosm, field and benthic), bioaccumulation, persistence	(Ge, Schimel et al. 2011),(D'Agata, Fasulo et al. 2014)
5. Species Sensitivity Distribution (SSD)	
6. Toxicity in environmentally relevant species	(Li, Wallis et al. 2014), (Zhu, Chang et al. 2010), (Aruoja, Dubourguier et al. 2009), (Minetto, Libralato et al. 2014), (Ghosh, Bandyopadhyay et al. 2010), (Hu, Li et al. 2010), (Kumari, Khan et al. 2011), (Pakrashi, Jain et al. 2014)
Toxicity in environmentally relevant species (null findings)	(Sekar, Falcioni et al. 2014), (Vevers and Jha 2008), (Lee, Kim et al. 2009), (Clemente, Castro et al. 2013)
7. Mutagenicity/ Genotoxicity	(Tavares, Louro et al. 2014), (Asare, Instanes et al. 2012), (Bhattacharya, Davoren et al. 2009), (Botelho, Costa et al. 2014), (Chen, Wang et al. 2014), (Di Virgilio, Reigosa et al. 2010), (Petković, Küzma et al. 2011, Petković, Zegura et al. 2011), (Falck, Lindberg et al. 2009), (Ghosh, Bandyopadhyay et al. 2010), (Jugan, Barillet et al. 2012), (Karlsson, Cronholm et al. 2008), (Osman, Baumgartner et al. 2010), (Prasad, Wallace et al. 2013), (Roszak, Stępnik et al. 2013), (Saquib, Al-Khedhairy et al. 2012), (Shukla, Sharma et al. 2011, Shukla, Kumar et al. 2013)
Mutagenicity/ Genotoxicity (null findings)	(Guichard, Schmit et al. 2012), (Hamzeh and Sunahara 2013), (Karlsson, Gustafsson et al. 2009), (Hackenberg, Friehs et al. 2010, Hackenberg, Friehs et

	al. 2011), (Woodruff, Li et al. 2012), (Wan, Mo et al. 2012)
8. Oxidative stress, inflammation	(Monteiller, Tran et al. 2007), (Xia, Hamilton et al. 2013), (Kermanizadeh, Gaiser et al. 2012), (Saquib, Al-Khedhairy et al. 2012), (Srivastava, Rahman et al. 2011), (Shen, Turney et al. 2014)
9. Cytotoxicity (e.g., cell viability)	(Komatsu, Tabata et al. 2008), (Guichard, Schmit et al. 2012), (Hamzeh and Sunahara 2013), (Saquib, Al-Khedhairy et al. 2012), (Farcal, Andon et al. 2015)
Cytotoxicity (e.g., cell viability) (null findings)	(Shi, Karlsson et al. 2012)
10. Cell viability, Lethality (bactericidal or fungicidal effect)	(Bayat, Rajapakse et al. 2014), (Barillet, Simon-Deckers et al. 2010)
11. ENM transport across membranes	(Brun, Barreau et al. 2014)
12. QSARs (related to cytotoxicity or other outcomes)	(Puzyn, Rasulev et al. 2011), (Pathakoti, Huang et al. 2014), (Sizochenko, Rasulev et al. 2014), (Toropova and Toropov 2013)
13. Meta-analysis, toxicokinetics, read-across	(Wang, Yang et al. 2014), (Gajewicz, Cronin et al. 2015), (Papa, Doucet et al. 2015), (Bachler, von Goetz et al. 2015)
14. Expert judgment/elicitation	(Hansen, Jensen et al. 2014), (Zimmer, Hertel et al. 2010)

Table C23. Summary of information available for nano ZnO related to exposure potential as of 2016

Attribute name and number	References
1. Airborne	(Lorenz, Hagendorfer et al. 2011)
2. Suspended in liquids (dispersive/formulation)	(Zhang, Leu et al. 2015),(Wilson 2013)
3. Embedded in a solid matrix (solid article)	(Shi, Li et al. 2014)
4. Free Nanoparticles	
5. Homoaggregates	(Bian, Mudunkotuwa et al. 2011), (Yung, Mouneyrac et al. 2014)
6. Heteroaggregates (NPs attached to other/larger particles)	(Yung, Mouneyrac et al. 2014), (Keller, Wang et al. 2010)
7. Manufacture	(Sun, Gottschalk et al. 2014), (Gottschalk, Sonderer et al. 2009)
8. Use	(Osmond and McCall 2010), (Pirela, Sotiriou et al. 2015), (Sun, Gottschalk et al. 2014)
9. Disposal	(Dale, Lowry et al. 2015)
10. Water	(Yung, Mouneyrac et al. 2014), (Pu, Tang et al. 2016), (Sun, Gottschalk et al. 2014), (Gottschalk, Lassen et al. 2015), (Boxall, Tiede et al. 2007), (Markus, Parsons et al. 2016), (Liu and Cohen 2014)
11. Soil	(Gottschalk, Sonderer et al. 2009), (Keller, McFerran et al. 2013), (Sun, Gottschalk et al. 2014), (Gottschalk, Lassen et al. 2015), (Liu and Cohen 2014)
12. Air	(Kim, Fazlollahi et al. 2010), (Gottschalk, Lassen et al. 2015), (Liu and Cohen 2014)
13. Geographical	(Liu and Cohen 2014)
14. Meteorological	(Liu and Cohen 2014)
15. Occupational	(Osmond and McCall 2010), (Ogura, Sakurai et al. 2011)
16. Environmental	(Osmond and McCall 2010), (Ge, Schimel et al. 2011)
17. From commercial product use	(Nohynek and Dufour 2012), (Schilling, Bradford et al. 2010), (Lorenz, Von Goetz et al. 2011), (Keller, Vosti et al. 2014)
18. Human	(Nohynek and Dufour 2012), (Lorenz, Von Goetz et al. 2011)
19. Ecological	(Lv, Zhang et al. 2015), (Navarro, Baun et al. 2008)

Table C24. Summary of information available for nano ZnO related to hazard potential as of 2016

Attribute name	References
1. Carcinogenicity, mutagenicity, development, reproductive	(Sharma, Singh et al. 2012), (Kwon, Lee et al. 2014)
2. Acute toxicity, Systemic toxicity, Skin/eye irritation	(Chuang, Juan et al. 2014), (Ho, Wu et al. 2011), (Li, Shen et al. 2012), (Chang, Ho et al. 2013), (Chen, Ho et al. 2015), (Cho, Duffin et al. 2012), (Fukui, Horie et al. 2012), (Hong, Tripathy et al. 2013), (Sharma, Singh et al. 2012), (Shrivastava, Raza et al. 2014), (Pasupuleti, Alapati et al. 2012)
3. Chronic toxicity (caused by multiple exposures)	(Chuang, Juan et al. 2014), (Cho, Duffin et al. 2010)
4. Complex systems (mesocosm, microcosm, field and benthic), bioaccumulation, persistence	(Ge, Schimel et al. 2011)
5. Species Sensitivity Distribution (SSD)	(Adam, Schmitt et al. 2015)
6. Toxicity in environmentally relevant species	(Hanna, Miller et al. 2013), (Heinlaan, Ivask et al. 2008), (Aruoja, Dubourguier et al. 2009), (Xiong, Fang et al. 2011), (Brayner, Dahoumane et al. 2010), (Franklin, Rogers et al. 2007), (Wu, Torres-Duarte et al. 2015), (Ali, Alarifi et al. 2012), (Hu, Li et al. 2010), (Isani, Falcioni et al. 2013), (Fabrega, Tantra et al. 2011), (Ates, Daniels et al. 2013), (Manzo, Miglietta et al. 2013), (Miao, Zhang et al. 2010), (Peng, Palma et al. 2011), (Trevisan, Delapedra et al. 2014), (Wong, Leung et al. 2010)
Soil	(Lv, Zhang et al. 2015), (Collins, Luxton et al. 2012)
7. Mutagenicity/ Genotoxicity	(Karlsson, Cronholm et al. 2008), (Sharma, Shukla et al. 2009, Sharma, Singh et al. 2011), (Alarifi, Ali et al. 2013), (Demir, Akça et al. 2014), (Hackenberg, Zimmermann et al. 2011), (Karlsson, Cronholm et al. 2008), (Osman, Baumgartner et al. 2010), (Valdiglesias, Costa et al. 2013), (Sharma, Anderson et al. 2012), (Wilhelmi, Fischer et al. 2013), (Hackenberg, Scherzed et al. 2011)
8. Oxidative stress, inflammation	(Lin, Xu et al. 2009), (Chang, Ho et al. 2013), (Chen, Ho et al. 2015), (Cho, Duffin et al. 2012), (Fukui, Horie et al. 2012), (Guo, Bi et al. 2013), (Hong, Tripathy et al. 2013), (Jeong, Kim et al. 2013), (Juang, Lai et al. 2014), (Prach, Stone et al. 2013), (Roy, Parashar et al. 2014), (Roy, Singh et al. 2014), (Sahu,

	Kannan et al. 2013), (Shen, Turney et al. 2014), (Wang, Deng et al. 2014)
9. Cytotoxicity (e.g., cell viability)	(Zhang, Nguyen et al. 2014), (Hackenberg, Scherzed et al. 2011), (Karlsson, Cronholm et al. 2008), (Kermanizadeh, Gaiser et al. 2012), (Buerki-Thurnherr, Xiao et al. 2013), (Goncalves and Girard 2014), (Hsiao and Huang 2013), (Luo, Shen et al. 2014), (Mihai, Chrisler et al. 2015), (Osmond-McLeod, Osmond et al. 2013), (Sahu, Kannan et al. 2014), (Shen, James et al. 2013), (Shi, Karlsson et al. 2012), (Yu, Yoon et al. 2013), (Sarkar, Ghosh et al. 2014), (Farcal, Andon et al. 2015), (Raemy, Grass et al. 2012)
10. Cell viability, Lethality (bactericidal or fungicidal effect)	(Kwon, Lee et al. 2014), (Baek and An 2011)
11. ENM transport across membranes	(Cohen, Derk et al. 2014)
12. QSARs (related to cytotoxicity or other outcomes)	(Puzyn, Rasulev et al. 2011), (Pathakoti, Huang et al. 2014), (Sizochenko, Rasulev et al. 2014)
13. Meta-analysis, toxicokinetics, read-across	(Chung, Yu et al. 2013), (Chen, Cheng et al. 2015), (Gajewicz, Cronin et al. 2015, Papa, Doucet et al. 2015), (Sayes, Smith et al. 2013)
14. Expert judgment/elicitation	(Zimmer, Hertel et al. 2010)

REFERENCES FOR APPENDIX C

Abudayyak, M., E. E. Guzel and G. Özhan (2016). "Copper (II) oxide nanoparticles induced nephrotoxicity in vitro conditions." Applied in Vitro Toxicology **2**(3): 157-164.

Adam, N., C. Schmitt, L. De Bruyn, D. Knapen and R. Blust (2015). "Aquatic acute species sensitivity distributions of ZnO and CuO nanoparticles." Science of the Total Environment **526**: 233-242.

Adamcakova-Dodd, A., M. M. Monick, L. S. Powers, K. N. Gibson-Corley and P. S. Thorne (2015). "Effects of prenatal inhalation exposure to copper nanoparticles on murine dams and offspring." Particle and Fibre Toxicology **12**: 30.

Adeleye, A. S., E. A. Oranu, M. Tao and A. A. Keller (2016). "Release and detection of nanosized copper from a commercial antifouling paint." Water Research **102**: 374-382.

Akhtar, M. J., S. Kumar, H. A. Alhadlaq, S. A. Alrokayan, K. M. Abu-Salah and M. Ahamed (2013). "Dose-dependent genotoxicity of copper oxide nanoparticles stimulated by reactive oxygen species in human lung epithelial cells." Toxicology and Industrial Health **32**(5): 809-821.

Al-Kattan, A., A. Wichser, R. Vonbank, S. Brunner, A. Ulrich, S. Zuin and B. Nowack (2013). "Release of TiO2 from paints containing pigment-TiO2 or nano-TiO2 by weathering." Environmental Science Process Impacts **15**(12): 2186-2193.

Al-Kattan, A., A. Wichser, S. Zuin, Y. Arroyo, L. Golanski, A. Ulrich and B. Nowack (2014). "Behavior of TiO2 released from nano-TiO2-containing paint and comparison to pristine nano-TiO2." Environmental Science & Technology **48**(12): 6710-6718.

Alarifi, S., D. Ali, S. Alkahtani, A. Verma, M. Ahamed, M. Ahmed and H. A. Alhadlaq (2013). "Induction of oxidative stress, DNA damage, and apoptosis in a malignant human skin melanoma cell line after exposure to zinc oxide nanoparticles." International Journal of Nanomedicine **8**: 983-993.

Alarifi, S., D. Ali, A. Verma, S. Alakhtani and B. A. Ali (2013). "Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes cells." International Journal of Toxicology **32**(4): 296-307.

Ali, D., S. Alarifi, S. Kumar, M. Ahamed and M. A. Siddiqui (2012). "Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail Lymnaea luteola L." Aquatic Toxicology **124-125**: 83-90.

Ananth, A., S. Dharaneedharan, M.-S. Heo and Y. S. Mok (2015). "Copper oxide nanomaterials: synthesis, characterization and structure-specific antibacterial performance." Chemical Engineering Journal **262**: 179-188.

Anjum, N. A., V. Adam, R. Kizek, A. C. Duarte, E. Pereira, M. Iqbal, A. S. Lukatkin and I. Ahmad (2015). "Nanoscale copper in the soil–plant system–toxicity and underlying potential mechanisms." Environmental research **138**: 306-325.

Aruoja, V., H. C. Dubourguier, K. Kasemets and A. Kahru (2009). "Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata." Science of the Total Environment **407**(4): 1461-1468.

Asare, N., C. Instanes, W. J. Sandberg, M. Refsnes, P. Schwarze, M. Kruszewski and G. Brunborg (2012). "Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells." Toxicology **291**(1-3): 65-72.

Ates, M., J. Daniels, Z. Arslan, I. O. Farah and H. F. Rivera (2013). "Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (Artemia salina) larvae: effects of particle size and solubility on toxicity." Environmental Science Process Impacts **15**(1): 225-233.

Bachler, G., N. von Goetz and K. Hungerbuhler (2015). "Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO2) nanoparticles." Nanotoxicology **9**(3): 373-380.

Baek, Y.-W. and Y.-J. An (2011). "Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus." Science of the Total Environment **409**(8): 1603-1608.

Barillet, S., A. Simon-Deckers, N. Herlin-Boime, M. Mayne-L'Hermite, C. Reynaud, D. Cassio, B. Gouget and M. Carriere (2010). "Toxicological consequences of TiO2, SiC nanoparticles and multi-walled carbon nanotubes exposure in several mammalian cell types: an in vitro study." Journal of Nanoparticle Research **12**(1): 61-73.

Bayat, N., K. Rajapakse, R. Marinsek-Logar, D. Drobne and S. Cristobal (2014). "The effects of engineered nanoparticles on the cellular structure and growth of Saccharomyces cerevisiae." Nanotoxicology **8**(4): 363-373.

Bhattacharya, K., M. Davoren, J. Boertz, R. P. Schins, E. Hoffmann and E. Dopp (2009). "Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells." Part Fibre Toxicol **6**: 17.

Bian, S. W., I. A. Mudunkotuwa, T. Rupasinghe and V. H. Grassian (2011). "Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid." Langmuir **27**(10): 6059-6068.

Bielmyer-Fraser, G. K., T. A. Jarvis, H. S. Lenihan and R. J. Miller (2014). "Cellular partitioning of nanoparticulate versus dissolved metals in marine phytoplankton." Environmental Science & Technology **48**(22): 13443-13450.

Blinova, I., A. Ivask, M. Heinlaan, M. Mortimer and A. Kahru (2010). "Ecotoxicity of nanoparticles of CuO and ZnO in natural water." Environmental Pollution **158**(1): 41-47.

Bonner, J. C., R. M. Silva, A. J. Taylor, J. M. Brown, S. C. Hilderbrand, V. Castranova, D. Porter, A. Elder, G. Oberdorster, J. R. Harkema, L. A. Bramble, T. J. Kavanagh, D. Botta, A. Nel and K. E. Pinkerton (2013). "Interlaboratory evaluation of rodent pulmonary responses to engineered nanomaterials: the NIEHS Nano GO Consortium." Environ Health Perspect **121**(6): 676-682.

Botelho, M. C., C. Costa, S. Silva, S. Costa, A. Dhawan, P. A. Oliveira and J. P. Teixeira (2014). "Effects of titanium dioxide nanoparticles in human gastric epithelial cells in vitro." Biomed Pharmacother **68**(1): 59-64.

Botta, C., J. Labille, M. Auffan, D. Borschneck, H. Miche, M. Cabié, A. Masion, J. Rose and J.-Y. Bottero (2011). "TiO2-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: structures and quantities." Environmental Pollution **159**(6): 1543-1550.

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

Boxall, A. B., K. Tiede and Q. Chaudhry (2007). "Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?" Nanomedicine (Lond) **2**(6): 919-927.

Brayner, R., S. A. Dahoumane, C. Yéprémian, C. Djediat, M. Meyer, A. Couté and F. Fiévet (2010). "ZnO nanoparticles: synthesis, characterization, and ecotoxicological studies." Langmuir **26**(9): 6522-6528.

Brun, E., F. Barreau, G. Veronesi, B. Fayard, S. Sorieul, C. Chaneac, C. Carapito, T. Rabilloud, A. Mabondzo, N. Herlin-Boime and M. Carriere (2014). "Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia." Part Fibre Toxicol **11**: 13.

Buerki-Thurnherr, T., L. Xiao, L. Diener, O. Arslan, C. Hirsch, X. Maeder-Althaus, K. Grieder, B. Wampfler, S. Mathur, P. Wick and H. F. Krug (2013). "In vitro mechanistic study towards a better understanding of ZnO nanoparticle toxicity." Nanotoxicology **7**(4): 402-416.

Buffet, P., M. Richard, F. Caupos, A. Vergnoux, H. Perrein-Ettajani, A. Luna-Acosta, F. Akcha, J. Amiard, C. Amiard-Triquet, M. Guibbolini, C. Risso-De Faverney, H. Thomas-Guyon, P. Reip, A. Dybowska, D. Berhanu, E. Valsami-Jones and C. Mouneyrac (2013). "A mesocosm study of fate and effects of CuO nanoparticles on endobenthic species (Scrobicularia plana, Hediste diversicolor)." Environmental Science & Technology **47**(3): 1620-1628.

Carmona, E. R., C. Inostroza-Blancheteau, V. Obando, L. Rubio and R. Marcos (2015). "Genotoxicity of copper oxide nanoparticles in Drosophila melanogaster." Mutat Res Genet Toxicol Environ Mutagen **791**(Supplement C): 1-11.

Chang, H., C.-C. Ho, C. S. Yang, W.-H. Chang, M.-H. Tsai, H.-T. Tsai and P. Lin (2013). "Involvement of MyD88 in zinc oxide nanoparticle-induced lung inflammation." Experimental and Toxicologic Pathology **65**(6): 887-896.

Chapman, J., L. Le Nor, R. Brown, E. Kitteringham, S. Russell, T. Sullivan and F. Regan (2013). "Antifouling performances of macro-to micro-to nano-copper materials for the inhibition of biofouling in its early stages." J Mater Chem B **1**(45): 6194-6200.

Chen, B. T., A. Afshari, S. Stone, M. Jackson, D. Schwegler-Berry, D. G. Frazer, V. Castranova and T. A. Thomas (2010). "Nanoparticles-containing spray can aerosol: characterization, exposure assessment, and generator design." Inhalation Toxicology **22**(13): 1072-1082.

Chen, J. K., C. C. Ho, H. Chang, J. F. Lin, C. S. Yang, M. H. Tsai, H. T. Tsai and P. Lin (2015). "Particulate nature of inhaled zinc oxide nanoparticles determines systemic effects and mechanisms of pulmonary inflammation in mice." Nanotoxicology **9**(1): 43-53.

Chen, T., J. Yan and Y. Li (2014). "Genotoxicity of titanium dioxide nanoparticles." Journal of Food and Drug Analysis **22**(1): 95-104.

Chen, W. Y., Y. H. Cheng, N. H. Hsieh, B. C. Wu, W. C. Chou, C. C. Ho, J. K. Chen, C. M. Liao and P. Lin (2015). "Physiologically based pharmacokinetic modeling of zinc oxide nanoparticles and zinc nitrate in mice." International Journal of Nanomedicine **10**: 6277-6292.

Chen, Z., H. Meng, G. Xing, C. Chen, Y. Zhao, G. Jia, T. Wang, H. Yuan, C. Ye, F. Zhao, Z. Chai, C. Zhu, X. Fang, B. Ma and L. Wan (2006). "Acute toxicological effects of copper nanoparticles in vivo." Toxicology Letters **163**(2): 109-120.

Chen, Z., Y. Wang, T. Ba, Y. Li, J. Pu, T. Chen, Y. Song, Y. Gu, Q. Qian, J. Yang and G. Jia (2014). "Genotoxic evaluation of titanium dioxide nanoparticles in vivo and in vitro." Toxicology Letters **226**(3): 314-319.

Chio, C.-P., W.-Y. Chen, W.-C. Chou, N.-H. Hsieh, M.-P. Ling and C.-M. Liao (2012). "Assessing the potential risks to zebrafish posed by environmentally relevant copper and silver nanoparticles." Science of The Total Environment **420**: 111-118.

Cho, W. S., R. Duffin, C. A. Poland, A. Duschl, G. J. Oostingh, W. Macnee, M. Bradley, I. L. Megson and K. Donaldson (2012). "Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions in vitro and in vivo; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs." Nanotoxicology **6**(1): 22-35.

Cho, W. S., R. Duffin, C. A. Poland, S. E. Howie, W. MacNee, M. Bradley, I. L. Megson and K. Donaldson (2010). "Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing." Environ Health Perspect **118**(12): 1699-1706.

Chowdhury, I., D. M. Cwiertny and S. L. Walker (2012). "Combined factors influencing the aggregation and deposition of nano-TiO2 in the presence of humic acid and bacteria." Environ Sci Technol **46**(13): 6968-6976.

Christensen, F. M., H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger (2011). "Nano-TiO_2--feasibility and challenges for human health risk assessment based on open literature." Nanotoxicology **5**(2): 110-124.

Chuang, H. C., H. T. Juan, C. N. Chang, Y. H. Yan, T. H. Yuan, J. S. Wang, H. C. Chen, Y. H. Hwang, C. H. Lee and T. J. Cheng (2014). "Cardiopulmonary toxicity of pulmonary exposure to occupationally relevant zinc oxide nanoparticles." Nanotoxicology **8**(6): 593-604.

Chung, H. E., J. Yu, M. Baek, J. A. Lee, M. S. Kim, S. H. Kim, E. H. Maeng, J. K. Lee, J. Jeong and S. J. Choi (2013). Toxicokinetics of zinc oxide nanoparticles in rats. Nanosafe 2012: International Conferences on Safe Production and Use of Nanomaterials, IOP Publishing.

Chusuei, C. C., C. H. Wu, S. Mallavarapu, F. Y. S. Hou, C. M. Hsu, J. G. Winiarz, R. S. Aronstarn and Y. W. Huang (2013). "Cytotoxicity in the age of nano: the role of fourth period transition metal oxide nanoparticle physicochemical properties." Chemico-Biological Interactions **206**(2): 319-326.

Clemente, Z., V. L. Castro, L. O. Feitosa, R. Lima, C. M. Jonsson, A. H. Maia and L. F. Fraceto (2013). "Fish exposure to nano-TiO2 under different experimental conditions: methodological aspects for nanoecotoxicology investigations." Science of the Total Environment **463-464**: 647-656.

Cohen, J. M., R. Derk, L. Wang, J. Godleski, L. Kobzik, J. Brain and P. Demokritou (2014). "Tracking translocation of industrially relevant engineered nanomaterials (ENMs) across alveolar epithelial monolayers in vitro." Nanotoxicology **8 Suppl 1**: 216-225.

Collins, D., T. Luxton, N. Kumar, S. Shah, V. K. Walker and V. Shah (2012). "Assessing the impact of copper and zinc oxide nanoparticles on soil: a field study." PLoS One **7**(8): e42663.

Conway, J. R., A. S. Adeleye, J. Gardea-Torresdey and A. A. Keller (2015). "Aggregation, dissolution, and transformation of copper nanoparticles in natural waters." Environmental science & technology **49**(5): 2749-2756.

Cushen, M., J. Kerry, M. Morris, M. Cruz-Romero and E. Cummins (2014). "Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment." Journal of Agricultural and Food Chemistry **62**(6): 1403-1411.

D'Agata, A., S. Fasulo, L. J. Dallas, A. S. Fisher, M. Maisano, J. W. Readman and A. N. Jha (2014). "Enhanced toxicity of 'bulk' titanium dioxide compared to 'fresh' and 'aged' nano-TiO2 in marine mussels (Mytilus galloprovincialis)." Nanotoxicology **8**(5): 549-558.

Dai, L., K. Syberg, G. T. Banta, H. Selck and V. E. Forbes (2013). "Effects, uptake, and depuration kinetics of silver oxide and copper oxide nanoparticles in a marine deposit feeder, Macoma balthica." ACS Sustainable Chemistry & Engineering **1**(7): 760-767.

Dale, A. L., G. V. Lowry and E. A. Casman (2015). "Stream dynamics and chemical transformations control the environmental fate of silver and zinc oxide nanoparticles in a watershed-scale model." Environ Sci Technol **49**(12): 7285-7293.

Demir, E., H. Akça, B. Kaya, D. Burgucu, O. Tokgün, F. Turna, S. Aksakal, G. Vales, A. Creus and R. Marcos (2014). "Zinc oxide nanoparticles: genotoxicity, interactions with UV-light and cell-transforming potential." J Hazard Mater **264**: 420-429.

Di Bucchianico, S., M. R. Fabbrizi, S. K. Misra, E. Valsami-Jones, D. Berhanu, P. Reip, E. Bergamaschi and L. Migliore (2013). "Multiple cytotoxic and genotoxic effects induced in vitro by differently shaped copper oxide nanomaterials." Mutagenesis **28**(3): 287-299.

Di Virgilio, A. L., M. Reigosa, P. M. Arnal and M. Fernández Lorenzo de Mele (2010). "Comparative study of the cytotoxic and genotoxic effects of titanium oxide and aluminium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells." J Hazard Mater **177**(1-3): 711-718.

Ding, X., M. B. Meneses, S. M. Albukhari, D. L. Richter, L. M. Matuana and P. A. Heiden (2013). "Comparing leaching of different copper oxide nanoparticles and ammoniacal copper salt from wood." Macromolecular Materials and Engineering **298**(12): 1335-1343.

Dobrzyńska, M. M., A. Gajowik, J. Radzikowska, A. Lankoff, M. Dušinská and M. Kruszewski (2014). "Genotoxicity of silver and titanium dioxide nanoparticles in bone marrow cells of rats in vivo." Toxicology **315**: 86-91.

EPA (2010). Nanomaterial case studies: nanoscale titanium dioxide in water treatment and in topical sunscreen.

EPA (2010). State of the science literature review: nano titanium dioxide environmental matters

Fabrega, J., R. Tantra, A. Amer, B. Stolpe, J. Tomkins, T. Fry, J. R. Lead, C. R. Tyler and T. S. Galloway (2011). "Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod Corophium volutator." Environmental science & technology **46**(2): 1128-1135.

Falck, G. C., H. K. Lindberg, S. Suhonen, M. Vippola, E. Vanhala, J. Catalán, K. Savolainen and H. Norppa (2009). "Genotoxic effects of nanosized and fine TiO2." Hum Exp Toxicol **28**(6-7): 339-352.

Farcal, L., F. T. Andon, L. Di Cristo, B. M. Rotoli, O. Bussolati, E. Bergamaschi, A. Mech, N. B. Hartmann, K. Rasmussen, J. Riego-Sintes, J. Ponti, A. Kinsner-Ovaskainen, F. Rossi, A. Oomen, P. Bos, R. Chen, R. Bai, C. Chen, L. Rocks, N. Fulton, B. Ross, G. Hutchison, L. Tran, S. Mues, R. Ossig, J. Schnekenburger, L. Campagnolo, L. Vecchione, A. Pietroiusti and B. Fadeel (2015). "Comprehensive in vitro toxicity testing of a panel of representative oxide nanomaterials: first steps towards an intelligent testing strategy." PLoS One **10**(5).

Franklin, N. M., N. J. Rogers, S. C. Apte, G. E. Batley, G. E. Gadd and P. S. Casey (2007). "Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): The importance of particle solubility." Environmental Science & Technology **41**(24): 8484-8490.

Fu, Y., Y. Zhang, X. Chang, Y. Zhang, S. Ma, J. Sui, L. Yin, Y. Pu and G. Liang (2014). "Systemic immune effects of titanium dioxide nanoparticles after repeated intratracheal instillation in rat." International journal of molecular sciences **15**(4): 6961-6973.

Fukui, H., M. Horie, S. Endoh, H. Kato, K. Fujita, K. Nishio, L. K. Komaba, J. Maru, A. Miyauhi, A. Nakamura, S. Kinugasa, Y. Yoshida, Y. Hagihara and H. Iwahashi (2012). "Association of zinc ion release and oxidative stress induced by intratracheal instillation of ZnO nanoparticles to rat lung." Chem Biol Interact **198**(1-3): 29-37.

Gajewicz, A., M. T. Cronin, B. Rasulev, J. Leszczynski and T. Puzyn (2015). "Novel approach for efficient predictions properties of large pool of nanomaterials based on limited set of species: nano-read-across." Nanotechnology **26**(1): 015701.

Gangwal, S., J. S. Brown, A. Wang, K. A. Houck, D. J. Dix, R. J. Kavlock and E. A. Hubal (2011). "Informing selection of nanomaterial concentrations for ToxCast in vitro testing based on occupational exposure potential." Environ Health Perspect **119**(11): 1539-1546.

Garner, K. L., S. Suh, H. S. Lenihan and A. A. Keller (2015). "Species sensitivity distributions for engineered nanomaterials." Environ Sci Technol **49**(9): 5753-5759.

Ge, Y., J. P. Schimel and P. A. Holden (2011). "Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities." Environmental Science & Technology **45**(4): 1659-1664.

Ghosh, M., M. Bandyopadhyay and A. Mukherjee (2010). "Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophic levels: plant and human lymphocytes." Chemosphere **81**(10): 1253-1262.

Gomes, S. I. L., S. C. Novais, J. J. Scott-Fordsmand, W. De Coen, A. M. V. M. Soares and M. J. B. Amorim (2012). "Effect of Cu-nanoparticles versus Cu-salt in Enchytraeus albidus (Oligochaeta): Differential gene expression through microarray analysis." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **155**(2): 219-227.

Gomes, T., O. Araújo, R. Pereira, A. C. Almeida, A. Cravo and M. J. Bebianno (2013). "Genotoxicity of copper oxide and silver nanoparticles in the mussel Mytilus galloprovincialis." Mar Environ Res **84**: 51-59.

Gomes, T., J. P. Pinheiro, I. Cancio, C. G. Pereira, C. Cardoso and M. J. Bebianno (2011). "Effects of copper nanoparticles exposure in the mussel Mytilus galloprovincialis." Environ Sci Technol **45**(21): 9356-9362.

Goncalves, D. M. and D. Girard (2014). "Zinc oxide nanoparticles delay human neutrophil apoptosis by a de novo protein synthesis-dependent and reactive oxygen species-independent mechanism." Toxicol In Vitro **28**(5): 926-931.

Gosens, I., F. R. Cassee, M. Zanella, L. Manodori, A. Brunelli, A. L. Costa, B. G. H. Bokkers, W. H. de Jong, D. Brown and D. Hristozov (2016). "Organ burden and pulmonary toxicity of nano-sized copper (II) oxide particles after short-term inhalation exposure." Nanotoxicology **10**(8): 1084-1095.

Gottschalk, F., C. Lassen, J. Kjoelholt, F. Christensen and B. Nowack (2015). "Modeling flows and concentrations of nine engineered nanomaterials in the Danish environment." International Journal of Environmental Research and Public Health **12**(5): 5581-5602.

Gottschalk, F., R. W. Scholz and B. Nowack (2010). "Probabilistic material flow modeling for assessing the environmental exposure to compounds: methodology and an application to engineered nano-TiO2 particles." Environmental Modelling & Software **25**(3): 320-332.

Gottschalk, F., T. Sonderer, R. W. Scholz and B. Nowack (2009). "Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for different regions." Environmental Science & Technology **43**(24): 9216-9222.

Griffitt, R. J., J. Luo, J. Gao, J.-C. Bonzongo and D. S. Barber (2008). "Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms." Environmental Toxicology and Chemistry **27**(9): 1972-1978.

Griffitt, R. J., R. Weil, K. A. Hyndman, N. D. Denslow, K. Powers, D. Taylor and D. S. Barber (2007). "Exposure to Copper Nanoparticles Causes Gill Injury and Acute Lethality in Zebrafish (Danio rerio)." Environmental Science & Technology **41**(23): 8178-8186.

Guichard, Y., J. Schmit, C. Darne, L. Gaté, M. Goutet, D. Rousset, O. Rastoix, R. Wrobel, O. Witschger, A. Martin, V. Fierro and S. Binet (2012). "Cytotoxicity and genotoxicity of nanosized and microsized titanium dioxide and iron oxide particles in Syrian hamster embryo cells." Ann Occup Hyg **56**(5): 631-644.

Gunawan, C., W. Y. Teoh, C. P. Marquis and R. Amal (2011). "Cytotoxic origin of copper(II) oxide nanoparticles: comparative studies with micron-sized particles, leachate, and metal salts." ACS Nano **5**(9): 7214-7225.

Guo, D., H. Bi, B. Liu, Q. Wu, D. Wang and Y. Cui (2013). "Reactive oxygen species-induced cytotoxic effects of zinc oxide nanoparticles in rat retinal ganglion cells." Toxicol In Vitro **27**(2): 731-738.

Hackenberg, S., G. Friehs, K. Froelich, C. Ginzkey, C. Koehler, A. Scherzed, M. Burghartz, R. Hagen and N. Kleinsasser (2010). "Intracellular distribution, geno- and cytotoxic effects of nanosized titanium dioxide particles in the anatase crystal phase on human nasal mucosa cells." Toxicology Letters **195**(1): 9-14.

Hackenberg, S., G. Friehs, M. Kessler, K. Froelich, C. Ginzkey, C. Koehler, A. Scherzed, M. Burghartz and N. Kleinsasser (2011). "Nanosized titanium dioxide particles do not induce DNA damage in human peripheral blood lymphocytes." Environ Mol Mutagen **52**(4): 264-268.

Hackenberg, S., A. Scherzed, A. Technau, M. Kessler, K. Froelich, C. Ginzkey, C. Koehler, M. Burghartz, R. Hagen and N. Kleinsasser (2011). "Cytotoxic, genotoxic and pro-inflammatory effects of zinc oxide nanoparticles in human nasal mucosa cells in vitro." Toxicol In Vitro **25**(3): 657-663.

Hackenberg, S., F. Z. Zimmermann, A. Scherzed, G. Friehs, K. Froelich, C. Ginzkey, C. Koehler, M. Burghartz, R. Hagen and N. Kleinsasser (2011). "Repetitive exposure to zinc oxide nanoparticles induces dna damage in human nasal mucosa mini organ cultures." Environ Mol Mutagen **52**(7): 582-589.

Ham, S., C. Yoon, E. Lee, K. Lee, D. Park, E. Chung, P. Kim and B. Lee (2012). "Task-based exposure assessment of nanoparticles in the workplace." Journal of Nanoparticle Research **14**(9): 1126.

Hamzeh, M. and G. I. Sunahara (2013). "In vitro cytotoxicity and genotoxicity studies of titanium dioxide (TiO2) nanoparticles in Chinese hamster lung fibroblast cells." Toxicol In Vitro **27**(2): 864-873.

Hanna, K. S., J. R. Miller and S. H. Lenihan (2014). "Accumulation and toxicity of copper oxide engineered nanoparticles in a marine mussel." Nanomaterials **4**(3): 535-547.

Hanna, S. K., R. J. Miller, D. Zhou, A. A. Keller and H. S. Lenihan (2013). "Accumulation and toxicity of metal oxide nanoparticles in a soft-sediment estuarine amphipod." Aquat Toxicol **142-143**: 441-446.

Hannon, J. C., J. P. Kerry, M. Cruz-Romero, S. Azlin-Hasim, M. Morris and E. Cummins (2016). "Human exposure assessment of silver and copper migrating from an antimicrobial nanocoated packaging material into an acidic food simulant." Food and Chemical Toxicology **95**(Supplement C): 128-136.

Hansen, S. F., K. A. Jensen and A. Baun (2014). "NanoRiskCat: a conceptual tool for categorization and communication of exposure potentials and hazards of nanomaterials in consumer products." Journal of Nanoparticle Research **16**(1): 1-25.

Hedberg, J., H. L. Karlsson, Y. Hedberg, E. Blomberg and I. Odnevall Wallinder (2016). "The importance of extracellular speciation and corrosion of copper nanoparticles on lung cell membrane integrity." Colloids and Surfaces B: Biointerfaces **141**(Supplement C): 291-300.

Heinlaan, M., A. Ivask, I. Blinova, H. C. Dubourguier and A. Kahru (2008). "Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus." Chemosphere **71**(7): 1308-1316.

Ho, M., K. Y. Wu, H. M. Chein, L. C. Chen and T. J. Cheng (2011). "Pulmonary toxicity of inhaled nanoscale and fine zinc oxide particles: mass and surface area as an exposure metric." Inhalation Toxicology **23**(14): 947-956.

Holbrook, D., D. Motabar, O. Quinones, B. Stanford, B. Vanderford and D. Moss (2013). "Titanium distribution in swimming pool water is dominated by dissolved species." Environ Pollut **181**: 68-74.

Hong, F., J. Hong, L. Wang, Y. Zhou, D. Liu, B. Xu, X. Yu and L. Sheng (2015). "Chronic exposure to nanoparticulate TiO2 causes renal fibrosis involving activation of the Wnt pathway in mouse kidney." J Agric Food Chem **63**(5): 1639-1647.

Hong, F., W. Si, X. Zhao, L. Wang, Y. Zhou, M. Chen, Y. Ge, Q. Zhang, Y. Wang and J. Zhang (2015). "TiO2 nanoparticle exposure decreases spermatogenesis via biochemical dysfunctions in the testis of male mice." Journal of Agricultural and Food Chemistry **63**(31): 7084-7092.

Hong, T.-K., N. Tripathy, H.-J. Son, K.-T. Ha, H.-S. Jeong and Y.-B. Hahn (2013). "A comprehensive in vitro and in vivo study of ZnO nanoparticles toxicity." J Mater Chem B **1**(23): 2985-2992.

Hsiao, I. L. and Y. J. Huang (2013). "Effects of serum on cytotoxicity of nano- and micro-sized ZnO particles." Journal of Nanoparticle Research **15**: 1829.

Hsu, L.-Y. and H.-M. Chein (2007). "Evaluation of nanoparticle emission for TiO2 nanopowder coating materials." Journal of Nanoparticle Research **9**(1): 157-163.

Hu, C. W., M. Li, Y. B. Cui, D. S. Li, J. Chen and L. Y. Yang (2010). "Toxicological effects of TiO2 and ZnO nanoparticles in soil on earthworm Eisenia fetida." Soil Biology and Biochemistry **42**(4): 586-591.

Hu, W., S. Culloty, G. Darmody, S. Lynch, J. Davenport, S. Ramirez-Garcia, K. A. Dawson, I. Lynch, J. Blasco and D. Sheehan (2014). "Toxicity of copper oxide nanoparticles in the blue mussel, Mytilus edulis: A redox proteomic investigation." Chemosphere **108**: 289-299.

Hu, X., S. Cook, P. Wang and H. M. Hwang (2009). "In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles." Science of the Total Environment **407**(8): 3070-3072.

Isani, G., M. L. Falcioni, G. Barucca, D. Sekar, G. Andreani, E. Carpenè and G. Falcioni (2013). "Comparative toxicity of CuO nanoparticles and CuSO4 in rainbow trout." Ecotoxicol Environ Saf **97**: 40-46.

Ivask, A., O. Bondarenko, N. Jepihhina and A. Kahru (2010). "Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO(2), silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: differentiating the impact of particles and solubilised metals." Analytical and Bioanalytical Chemistry **398**(2): 701-716.

Jeong, S. H., H. J. Kim, H. J. Ryu, W. I. Ryu, Y. H. Park, H. C. Bae, Y. S. Jang and S. W. Son (2013). "ZnO nanoparticles induce TNF-α expression via ROS-ERK-Egr-1 pathway in human keratinocytes." J Dermatol Sci **72**(3): 263-273.

Jo, H. J., J. W. Choi, S. H. Lee and S. W. Hong (2012). "Acute toxicity of Ag and CuO nanoparticle suspensions against Daphnia magna: the importance of their dissolved fraction varying with preparation methods." J Hazard Mater **227-228**: 301-308.

Jose, G. P., S. Santra, S. K. Mandal and T. K. Sengupta (2011). "Singlet oxygen mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells." J Nanobiotechnol **9**(1): 9.

Juang, Y. M., B. H. Lai, H. J. Chien, M. Ho, T. J. Cheng and C. C. Lai (2014). "Changes in protein expression in rat bronchoalveolar lavage fluid after exposure to zinc oxide nanoparticles: an iTRAQ proteomic approach." Rapid communications in mass spectrometry **28**(8): 974-980.

Jugan, M. L., S. Barillet, A. Simon-Deckers, N. Herlin-Boime, S. Sauvaigo, T. Douki and M. Carriere (2012). "Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells." Nanotoxicology **6**(5): 501-513.

Kaegi, R., A. Ulrich, B. Sinnet, R. Vonbank, A. Wichser, S. Zuleeg, H. Simmler, S. Brunner, H. Vonmont, M. Burkhardt and M. Boller (2008). "Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment." Environ Pollut **156**(2): 233-239.

Karlsson, H. L., P. Cronholm, J. Gustafsson and L. Möller (2008). "Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes." Chemical Research in Toxicology **21**(9): 1726-1732.

Karlsson, H. L., P. Cronholm, Y. Hedberg, M. Tornberg, L. De Battice, S. Svedhem and I. O. Wallinder (2013). "Cell membrane damage and protein interaction induced by copper containing nanoparticles—Importance of the metal release process." Toxicology **313**(1): 59-69.

Karlsson, H. L., J. Gustafsson, P. Cronholm and L. Möller (2009). "Size-dependent toxicity of metal oxide particles--a comparison between nano- and micrometer size." Toxicology Letters **188**(2): 112-118.

Kasemets, K., A. Ivask, H. C. Dubourguier and A. Kahru (2009). "Toxicity of nanoparticles of ZnO, CuO and TiO2 to yeast Saccharomyces cerevisiae." Toxicol In Vitro **23**(6): 1116-1122.

Kaweeteerawat, C., A. Ivask, R. Liu, H. Zhang, C. H. Chang, C. Low-Kam, H. Fischer, Z. Ji, S. Pokhrel, Y. Cohen, D. Telesca, J. Zink, L. Mädler, P. A. Holden, A. Nel and H. Godwin (2015). "Toxicity of metal oxide nanoparticles in Escherichia coli correlates with conduction band and hydration energies." Environ Sci Technol **49**(2): 1105-1112.

Keller, A. A., S. McFerran, A. Lazareva and S. Suh (2013). "Global life cycle releases of engineered nanomaterials." Journal of Nanoparticle Research **15**(6).

Keller, A. A., W. Vosti, H. Wang and A. Lazareva (2014). "Release of engineered nanomaterials from personal care products throughout their life cycle." Journal of Nanoparticle Research **16**(7): 2489.

Keller, A. A., H. Wang, D. Zhou, H. S. Lenihan, G. Cherr, B. J. Cardinale, R. Miller and Z. Ji (2010). "Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices." Environ. Sci. Technol **44**(6): 1962-1967.

Kermanizadeh, A., B. K. Gaiser, G. R. Hutchison and V. Stone (2012). "An in vitro liver model - assessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanomaterials." Particle and Fibre Toxicology **9**(1): 28.

Kim, Y. H., F. Fazlollahi, I. M. Kennedy, N. R. Yacobi, S. F. Hamm-Alvarez, Z. Borok, K. J. Kim and E. D. Crandall (2010). "Alveolar epithelial cell injury due to zinc oxide nanoparticle exposure." Am J Respir Crit Care Med **182**(11): 1398-1409.

Kobayashi, N., M. Naya, S. Endoh, J. Maru, K. Yamamoto and J. Nakanishi (2009). "Comparative pulmonary toxicity study of nano-TiO2 particles of different sizes and agglomerations in rats: different short-and long-term post-instillation results." Toxicology **264**(1-2): 110-118.

Koivisto, A. J., J. Lyyränen, A. Auvinen, E. Vanhala, K. Hämeri, T. Tuomi and J. Jokiniemi (2012). "Industrial worker exposure to airborne particles during the packing of pigment and nanoscale titanium dioxide." Inhalation Toxicology **24**(12): 839-849.

Komatsu, T., M. Tabata, M. Kubo-Irie, T. Shimizu, K. Suzuki, Y. Nihei and K. Takeda (2008). "The effects of nanoparticles on mouse testis Leydig cells in vitro." Toxicol In Vitro **22**(8): 1825-1831.

Kumari, M., S. S. Khan, S. Pakrashi, A. Mukherjee and N. Chandrasekaran (2011). "Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa." J Hazard Mater **190**(1-3): 613-621.

Kwon, J. Y., S. Y. Lee, P. Koedrith, J. Y. Lee, K.-M. Kim, J.-M. Oh, S. I. Yang, M.-K. Kim, J. K. Lee and J. Jeong (2014). "Lack of genotoxic potential of ZnO nanoparticles in in vitro and in vivo tests." Mutat Res Genet Toxicol Environ Mutagen **761**: 1-9.

Lazareva, A. and A. A. Keller (2014). "Estimating potential life cycle releases of engineered nanomaterials from wastewater treatment plants." ACS Sustainable Chemistry & Engineering **2**(7): 1656-1665.

Lee, J. H., M. Kwon, J. H. Ji, C. S. Kang, K. H. Ahn, J. H. Han and I. J. Yu (2011). "Exposure assessment of workplaces manufacturing nanosized TiO2 and silver." Inhalation Toxicology **23**(4): 226-236.

Lee, S.-W., S.-M. Kim and J. Choi (2009). "Genotoxicity and ecotoxicity assays using the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius to

screen the ecological risks of nanoparticle exposure." Environmental Toxicology and Pharmacology **28**(1): 86-91.

Li, C. H., C. C. Shen, Y. W. Cheng, S. H. Huang, C. C. Wu, C. C. Kao, J. W. Liao and J. J. Kang (2012). "Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice." Nanotoxicology **6**(7): 746-756.

Li, S., L. K. Wallis, S. A. Diamond, H. Ma and D. J. Hoff (2014). "Species sensitivity and dependence on exposure conditions impacting the phototoxicity of TiO2 nanoparticles to benthic organisms." Environmental Toxicology and Chemistry **33**(7): 1563-1569.

Liao, C.-M., Y.-H. Chiang and C.-P. Chio (2008). "Model-based assessment for human inhalation exposure risk to airborne nano/fine titanium dioxide particles." Science of The Total Environment **407**(1): 165-177.

Lim, J. H., P. Sisco, T. K. Mudalige, G. Sánchez-Pomales, P. C. Howard and S. W. Linder (2015). "Detection and characterization of SiO2 and TiO2 nanostructures in dietary supplements." Journal of Agricultural and Food Chemistry **63**(12): 3144-3152.

Lin, Q. B., H. Li, H. N. Zhong, Q. Zhao, D. H. Xiao and Z. W. Wang (2014). "Migration of Ti from nano-TiO_2-polyethylene composite packaging into food simulants." Food Addit Contam Part A Chem Anal Control Expo Risk Assess **31**(7): 1284-1290.

Lin, S., A. A. Taylor, Z. Ji, C. H. Chang, N. M. Kinsinger, W. Ueng, S. L. Walker and A. E. Nel (2015). "Understanding the transformation, speciation, and hazard potential of copper particles in a model septic tank system using zebrafish to monitor the effluent." ACS nano **9**(2): 2038-2048.

Lin, W., Y. Xu, C.-C. Huang, Y. Ma, K. B. Shannon, D.-R. Chen and Y.-W. Huang (2009). "Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells." Journal of Nanoparticle Research **11**(1): 25-39.

Lindberg, H. K., G. C. M. Falck, J. Catalán, A. J. Koivisto, S. Suhonen, H. Järventaus, E. M. Rossi, H. Nykäsenoja, Y. Peltonen, C. Moreno, H. Alenius, T. Tuomi, K. M. Savolainen and H. Norppa (2012). "Genotoxicity of inhaled nanosized TiO2 in mice." Mutat Res Genet Toxicol Environ Mutagen **745**(1–2): 58-64.

Liou, S. H., C. S. Tsai, D. Pelclova, M. K. Schubauer-Berigan and P. A. Schulte (2015). "Assessing the first wave of epidemiological studies of nanomaterial workers." Journal of Nanoparticle Research **17**: 413.

Liu, H. H., M. Bilal, A. Lazareva, A. Keller and Y. Cohen (2015). "Simulation tool for assessing the release and environmental distribution of nanomaterials." Beilstein journal of nanotechnology **6**: 938.

Liu, H. H. and Y. Cohen (2014). "Multimedia environmental distribution of engineered nanomaterials." Environ Sci Technol **48**(6): 3281-3292.

Liu, R., H. Y. Zhang, Z. X. Ji, R. Rallo, T. Xia, C. H. Chang, A. Nel and Y. Cohen (2013). "Development of structure-activity relationship for metal oxide nanoparticles." Nanoscale **5**(12): 5644-5653.

Lorenz, C., H. Hagendorfer, N. von Goetz, R. Kaegi, R. Gehrig, A. Ulrich, M. Scheringer and K. Hungerbühler (2011). "Nanosized aerosols from consumer sprays: experimental analysis and exposure modeling for four commercial products." Journal of Nanoparticle Research **13**(8): 3377-3391.

Lorenz, C., N. Von Goetz, M. Scheringer, M. Wormuth and K. Hungerbuehler (2011). "Potential exposure of German consumers to engineered nanoparticles in cosmetics and personal care products." Nanotoxicology **5**(1): 12-29.

Luo, M., C. Shen, B. N. Feltis, L. L. Martin, A. E. Hughes, P. F. A. Wright and T. W. Turney (2014). "Reducing ZnO nanoparticle cytotoxicity by surface modification." Nanoscale **6**(11): 5791-5798.

Lv, J., S. Zhang, L. Luo, J. Zhang, K. Yang and P. Christie (2015). "Accumulation, speciation and uptake pathway of ZnO nanoparticles in maize." Environmental Science: Nano **2**(1): 68-77.

Manusadžianas, L., C. Caillet, L. Fachetti, B. Gylytė, R. Grigutytė, S. Jurkonienė, R. Karitonas, K. Sadauskas, F. Thomas, R. Vitkus and J. F. Férard (2012). "Toxicity of copper oxide nanoparticle suspensions to aquatic biota." Environ Toxicol Chem **31**(1): 108-114.

Manzo, S., M. L. Miglietta, G. Rametta, S. Buono and G. Di Francia (2013). "Toxic effects of ZnO nanoparticles towards marine algae Dunaliella tertiolecta." Science of the Total Environment **445-446**: 371-376.

Markus, A. A., J. R. Parsons, E. W. M. Roex, P. De Voogt and R. Laane (2016). "Modelling the transport of engineered metallic nanoparticles in the river Rhine." Water research **91**: 214-224.

Miao, A. J., X. Y. Zhang, Z. Luo, C. S. Chen, W. C. Chin, P. H. Santschi and A. Quigg (2010). "Zinc oxide-engineered nanoparticles: dissolution and toxicity to marine phytoplankton." Environ Toxicol Chem **29**(12): 2814-2822.

Miao, L., C. Wang, J. Hou, P. Wang, Y. Ao, Y. Li, N. Geng, Y. Yao, B. Lv, Y. Yang, G. You and Y. Xu (2016). "Aggregation and removal of copper oxide (CuO) nanoparticles in wastewater environment and their effects on the microbial activities of wastewater biofilms." Bioresour Technol **216**: 537-544.

Midander, K., P. Cronholm, H. L. Karlsson, K. Elihn, L. Möller, C. Leygraf and I. O. Wallinder (2009). "Surface characteristics, copper release, and toxicity of nano- and micrometer-sized copper and copper(II) oxide particles: a cross-disciplinary study." Small **5**(3): 389-399.

Mihai, C., W. B. Chrisler, Y. Xie, D. Hu, C. J. Szymanski, A. Tolic, J. A. Klein, J. N. Smith, B. J. Tarasevich and G. Orr (2015). "Intracellular accumulation dynamics and fate of zinc ions in alveolar epithelial cells exposed to airborne ZnO nanoparticles at the air-liquid interface." Nanotoxicology **9**(1): 9-22.

Minetto, D., G. Libralato and A. Volpi Ghirardini (2014). "Ecotoxicity of engineered TiO2 nanoparticles to saltwater organisms: an overview." Environ Int **66**: 18-27.

Misra, S. K., S. Nuseibeh, A. Dybowska, D. Berhanu, T. D. Tetley and E. Valsami-Jones (2014). "Comparative study using spheres, rods and spindle-shaped nanoplatelets on dispersion stability, dissolution and toxicity of CuO nanomaterials." Nanotoxicology **8**(4): 422-432.

Monteiller, C., L. Tran, W. MacNee, S. Faux, A. Jones, B. Miller and K. Donaldson (2007). "The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area." Occupational and Environmental Medicine **64**(9): 609-615.

Mortimer, M., K. Kasemets, M. Vodovnik, R. Marinsek-Logar and A. Kahru (2011). "Exposure to CuO nanoparticles changes the fatty acid composition of protozoa Tetrahymena thermophila." Environ Sci Technol **45**(15): 6617-6624.

Mueller, N. C. and B. Nowack (2008). "Exposure modeling of engineered nanoparticles in the environment." Environmental Science & Technology **42**(12): 4447-4453.

Navarro, E., A. Baun, R. Behra, N. B. Hartmann, J. Filser, A.-J. Miao, A. Quigg, P. H. Santschi and L. Sigg (2008). "Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi." Ecotoxicology **17**(5): 372-386.

Navratilova, J., A. Praetorius, A. Gondikas, W. Fabienke, F. von der Kammer and T. Hofmann (2015). "Detection of engineered copper nanoparticles in soil using single particle ICP-MS." International Journal of Environmental Research and Public Health **12**(12).

Naya, M., N. Kobayashi, M. Ema, S. Kasamoto, M. Fukumuro, S. Takami, M. Nakajima, M. Hayashi and J. Nakanishi (2012). "In vivo genotoxicity study of titanium dioxide nanoparticles using comet assay following intratracheal instillation in rats." Regulatory Toxicology and Pharmacology **62**(1): 1-6.

Nazarenko, Y., T. W. Han, P. J. Lioy and G. Mainelis (2011). "Potential for exposure to engineered nanoparticles from nanotechnology-based consumer spray products." J Expo Sci Environ Epidemiol **21**(5): 515-528.

Nohynek, G. J. and E. K. Dufour (2012). "Nano-sized cosmetic formulations or solid nanoparticles in sunscreens: a risk to human health?" Arch Toxicol **86**(7): 1063-1075.

Notter, D. A., D. M. Mitrano and B. Nowack (2014). "Are nanosized or dissolved metals more toxic in the environment? A meta-analysis." Environ Toxicol Chem **33**(12): 2733-2739.

Nowack, B., J. F. Ranville, S. Diamond, J. A. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A. A. Koelmans and S. J. Klaine (2012). "Potential scenarios for nanomaterial release and subsequent alteration in the environment." Environmental Toxicology and Chemistry **31**(1): 50-59.

O'Brien, N. and E. Cummins (2010). "Nano-scale pollutants: fate in Irish surface and drinking water regulatory systems." Human and Ecological Risk Assessment **16**(4): 847-872.

O'Brien, N. J. and E. J. Cummins (2011). "A risk assessment framework for assessing metallic nanomaterials of environmental concern: aquatic exposure and behavior." Risk Analysis **31**(5): 706-726.

Ogura, I., H. Sakurai and M. Gamo (2011). "Onsite aerosol measurements for various engineered nanomaterials at industrial manufacturing plants." J Phys Conf Ser **304**(1): 012004.

Osman, I. F., A. Baumgartner, E. Cemeli, J. N. Fletcher and D. Anderson (2010). "Genotoxicity and cytotoxicity of zinc oxide and titanium dioxide in HEp-2 cells." Nanomedicine (Lond) **5**(8): 1193-1203.

Osmond-McLeod, M. J., R. I. Osmond, Y. Oytam, M. J. McCall, B. Feltis, A. Mackay-Sim, S. A. Wood and A. L. Cook (2013). "Surface coatings of ZnO nanoparticles mitigate differentially a host of transcriptional, protein and signalling responses in primary human olfactory cells." Part Fibre Toxicol **10**(1): 54.

Osmond, M. J. and M. J. McCall (2010). "Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard." Nanotoxicology **4**(1): 15-41.

Pakrashi, S., N. Jain, S. Dalai, J. Jayakumar, P. T. Chandrasekaran, A. M. Raichur, N. Chandrasekaran and A. Mukherjee (2014). "In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations." PLoS One **9**(2): e87789.

Papa, E., J. P. Doucet and A. Doucet-Panaye (2015). "Linear and non-linear modelling of the cytotoxicity of TiO2 and ZnO nanoparticles by empirical descriptors." SAR QSAR Environ Res **26**(7-9): 647-665.

Park, E. J., J. Yoon, K. Choi, J. Yi and K. Park (2009). "Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation." Toxicology **260**(1-3): 37-46.

Pasupuleti, S., S. Alapati, S. Ganapathy, G. Anumolu, N. R. Pully and B. M. Prakhya (2012). "Toxicity of zinc oxide nanoparticles through oral route." Toxicology and industrial health **28**(8): 675-686.

Pathakoti, K., M. J. Huang, J. D. Watts, X. He and H. M. Hwang (2014). "Using experimental data of Escherichia coli to develop a QSAR model for predicting the photo-induced cytotoxicity of metal oxide nanoparticles." J Photochem Photobiol B **130**: 234-240.

Pelclova, D., H. Barosova, J. Kukutschova, V. Zdimal, T. Navratil, Z. Fenclova, S. Vlckova, J. Schwarz, N. Zikova, P. Kacer, M. Komarc, J. Belacek and S. Zakharov (2015). "Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO 2 particles: a cross-sectional study." Journal of Breath Research **9**(3): 036008.

Peng, X., S. Palma, N. S. Fisher and S. S. Wong (2011). "Effect of morphology of ZnO nanostructures on their toxicity to marine algae." Aquat Toxicol **102**(3-4): 186-196.

Perreault, F., S. Pedroso Melegari, C. Henning da Costa, A. L. de Oliveira Franco Rossetto, R. Popovic and W. Gerson Matias (2012). "Genotoxic effects of copper oxide nanoparticles in Neuro 2A cell cultures." Science of the Total Environment **441**: 117-124.

Petković, J., T. Küzma, K. Rade, S. Novak and M. Filipič (2011). "Pre-irradiation of anatase TiO2 particles with UV enhances their cytotoxic and genotoxic potential in human hepatoma HepG2 cells." Journal of Hazardous Materials **196**: 145-152.

Petković, J., B. Zegura, M. Stevanović, N. Drnovšek, D. Uskoković, S. Novak and M. Filipič (2011). "DNA damage and alterations in expression of DNA damage responsive genes induced by TiO2 nanoparticles in human hepatoma HepG2 cells." Nanotoxicology **5**(3): 341-353.

Pettibone, J. M., A. Adamcakova-Dodd, P. S. Thorne, P. T. O'Shaughnessy, J. A. Weydert and V. H. Grassian (2008). "Inflammatory response of mice following inhalation exposure to iron and copper nanoparticles." Nanotoxicology **2**(4): 189-204.

Pini, M., B. Salieri, A. M. Ferrari, B. Nowack and R. Hischier (2016). "Human health characterization factors of nano-TiO2 for indoor and outdoor environments." Int J Life Cycle Assess **21**(10): 1452-1462.

Pirela, S. V., G. A. Sotiriou, D. Bello, M. Shafer, K. L. Bunker, V. Castranova, T. Thomas and P. Demokritou (2015). "Consumer exposures to laser printer-emitted engineered nanoparticles: a case study of life-cycle implications from nano-enabled products." Nanotoxicology **9**(6): 760-768.

Prach, M., V. Stone and L. Proudfoot (2013). "Zinc oxide nanoparticles and monocytes: impact of size, charge and solubility on activation status." Toxicology and applied pharmacology **266**(1): 19-26.

Pradhan, A., S. Seena, C. Pascoal and F. Cássio (2012). "Copper oxide nanoparticles can induce toxicity to the freshwater shredder Allogamus ligonifer." Chemosphere **89**(9): 1142-1150.

Praetorius, A., M. Scheringer and K. Hungerbuehler (2012). "Development of environmental fate models for engineered nanoparticles-a case study of TiO2 nanoparticles in the Rhine river." Environmental Science & Technology **46**(12): 6705-6713.

Prasad, R. Y., K. Wallace, K. M. Daniel, A. H. Tennant, R. M. Zucker, J. Strickland, K. Dreher, A. D. Kligerman, C. F. Blackman and D. M. Demarini (2013). "Effect of treatment media on the agglomeration of titanium dioxide nanoparticles: impact on genotoxicity, cellular interaction, and cell cycle." ACS Nano **7**(3): 1929-1942.

Pu, Y., F. Tang, P.-M. Adam, B. Laratte and R. E. Ionescu (2016). "Fate and characterization factors of nanoparticles in seventeen subcontinental freshwaters: A case study on copper nanoparticles." Environmental science & technology **50**(17): 9370-9379.

Puzyn, T., B. Rasulev, A. Gajewicz, X. Hu, T. P. Dasari, A. Michalkova, H.-M. Hwang, A. Toropov, D. Leszczynska and J. Leszczynski (2011). "Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles." Nature Nanotechnology **6**(3): 175-178.

Raemy, D. O., R. N. Grass, W. J. Stark, C. M. Schumacher, M. J. Clift, P. Gehr and B. Rothen-Rutishauser (2012). "Effects of flame made zinc oxide particles in human lung cells - a comparison of aerosol and suspension exposures." Part Fibre Toxicol **9**: 33.

Ren, G., D. Hu, E. W. C. Cheng, M. A. Vargas-Reus, P. Reip and R. P. Allaker (2009). "Characterisation of copper oxide nanoparticles for antimicrobial applications." International Journal of Antimicrobial Agents **33**(6): 587-590.

Roszak, J., M. Stępnik, M. Nocuń, M. Ferlińska, A. Smok-Pieniążek, J. Grobelny, E. Tomaszewska, W. Wąsowicz and M. Cieślak (2013). "A strategy for in vitro safety testing of nanotitania-modified textile products." J Hazard Mater **256-257**: 67-75.

Roy, R., V. Parashar, L. K. Chauhan, R. Shanker, M. Das, A. Tripathi and P. D. Dwivedi (2014). "Mechanism of uptake of ZnO nanoparticles and inflammatory responses in macrophages require PI3K mediated MAPKs signaling." Toxicol In Vitro **28**(3): 457-467.

Roy, R., S. K. Singh, M. Das, A. Tripathi and P. D. Dwivedi (2014). "Toll-like receptor 6 mediated inflammatory and functional responses of zinc oxide nanoparticles primed macrophages." Immunology **142**(3): 453-464.

Saber, A. T., N. R. Jacobsen, A. Mortensen, J. Szarek, P. Jackson, A. M. Madsen, K. A. Jensen, I. K. Koponen, G. Brunborg and K. B. Gützkow (2012). "Nanotitanium dioxide toxicity in mouse lung is reduced in sanding dust from paint." Particle and fibre toxicology **9**(1): 1-15.

Sahu, D., G. M. Kannan and R. Vijayaraghavan (2014). "Size-dependent effect of zinc oxide on toxicity and inflammatory potential of human monocytes." J Toxicol Environ Health A **77**(4): 177-191.

Sahu, D., G. M. Kannan, R. Vijayaraghavan, T. Anand and F. Khanum (2013). "Nanosized zinc oxide induces toxicity in human lung cells." ISRN Toxicology **2013**: 316075.

Sang, X., L. Zheng, Q. Sun, N. Li, Y. Cui, R. Hu, G. Gao, Z. Cheng, J. Cheng, S. Gui, H. Liu, Z. Zhang and F. Hong (2012). "The chronic spleen injury of mice following long-term exposure to titanium dioxide nanoparticles." J Biomed Mater Res A **100**(4): 894-902.

Saquib, Q., A. A. Al-Khedhairy, M. A. Siddiqui, F. M. Abou-Tarboush, A. Azam and J. Musarrat (2012). "Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells." Toxicol In Vitro **26**(2): 351-361.

Sarkar, J., M. Ghosh, A. Mukherjee, D. Chattopadhyay and K. Acharya (2014). "Biosynthesis and safety evaluation of ZnO nanoparticles." Bioprocess Biosyst Eng **37**(2): 165-171.

Sayes, C. M., P. A. Smith and I. V. Ivanov (2013). "A framework for grouping nanoparticles based on their measurable characteristics." Int J Nanomedicine **8 Suppl 1**: 45-56.

Schilling, K., B. Bradford, D. Castelli, E. Dufour, J. F. Nash, W. Pape, S. Schulte, I. Tooley, J. van den Bosch and F. Schellauf (2010). "Human safety review of "nano" titanium dioxide and zinc oxide." Photochemical & Photobiological Sciences **9**(4): 495-509.

Sekar, D., M. L. Falcioni, G. Barucca and G. Falcioni (2014). "DNA damage and repair following In vitro exposure to two different forms of titanium dioxide nanoparticles on trout erythrocyte." Environ Toxicol **29**(1): 117-127.

Semisch, A., J. Ohle, B. Witt and A. Hartwig (2014). "Cytotoxicity and genotoxicity of nano - and microparticulate copper oxide: role of solubility and intracellular bioavailability." Particle and Fibre Toxicology **11**.

Setyawati, M. I., C. Y. Tay, S. L. Chia, S. L. Goh, W. Fang, M. J. Neo, H. C. Chong, S. M. Tan, S. C. Loo, K. W. Ng, J. P. Xie, C. N. Ong, N. S. Tan and D. T. Leong (2013). "Titanium dioxide nanomaterials cause endothelial cell leakiness by disrupting the homophilic interaction of VE-cadherin." Nat Commun **4**: 1673.

Shandilya, N., O. Le Bihan, C. Bressot and M. Morgeneyer (2015). "Emission of titanium dioxide nanoparticles from building materials to the environment by wear and weather." Environmental Science & Technology **49**(4): 2163-2170.

Sharma, V., D. Anderson and A. Dhawan (2012). "Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2)." Apoptosis **17**(8): 852-870.

Sharma, V., R. K. Shukla, N. Saxena, D. Parmar, M. Das and A. Dhawan (2009). "DNA damaging potential of zinc oxide nanoparticles in human epidermal cells." Toxicology Letters **185**(3): 211-218.

Sharma, V., P. Singh, A. K. Pandey and A. Dhawan (2012). "Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles." Mutat Res **745**(1-2): 84-91.

Sharma, V., S. K. Singh, D. Anderson, D. J. Tobin and A. Dhawan (2011). "Zinc oxide nanoparticle induced genotoxicity in primary human epidermal keratinocytes." J Nanosci Nanotechnol **11**(5): 3782-3788.

Sharma, V. K. (2009). "Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment--a review." J Environ Sci Health A Tox Hazard Subst Environ Eng **44**(14): 1485-1495.

Shen, C., S. A. James, M. D. de Jonge, T. W. Turney, P. F. Wright and B. N. Feltis (2013). "Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells." Toxicological Sciences **136**(1): 120-130.

Shen, C., T. W. Turney, T. J. Piva, B. N. Feltis and P. F. Wright (2014). "Comparison of UVA-induced ROS and sunscreen nanoparticle-generated ROS in human immune cells." Photochemical & Photobiological Sciences **13**(5): 781-788.

Shi, J., H. L. Karlsson, K. Johansson, V. Gogvadze, L. Xiao, J. Li, T. Burks, A. Garcia-Bennett, A. Uheida, M. Muhammed, S. Mathur, R. Morgenstern, V. E. Kagan and B. Fadeel (2012). "Microsomal glutathione transferase 1 protects against toxicity induced by silica nanoparticles but not by zinc oxide nanoparticles." ACS Nano **6**(3): 1925-1938.

Shi, L. E., Z. H. Li, W. Zheng, Y. F. Zhao, Y. F. Jin and Z. X. Tang (2014). "Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: a review." Food Addit Contam Part A Chem Anal Control Expo Risk Assess **31**(2): 173-186.

Shimizu, M., H. Tainaka, T. Oba, K. Mizuo, M. Umezawa and K. Takeda (2009). "Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse." Part Fibre Toxicol **6**: 20.

Shrivastava, R., S. Raza, A. Yadav, P. Kushwaha and S. J. Flora (2014). "Effects of sub-acute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain." Drug Chem Toxicol **37**(3): 336-347.

Shukla, R. K., A. Kumar, D. Gurbani, A. K. Pandey, S. Singh and A. Dhawan (2013). "TiO(2) nanoparticles induce oxidative DNA damage and apoptosis in human liver cells." Nanotoxicology **7**(1): 48-60.

Shukla, R. K., V. Sharma, A. K. Pandey, S. Singh, S. Sultana and A. Dhawan (2011). "ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells." Toxicol In Vitro **25**(1): 231-241.

Sizochenko, N., B. Rasulev, A. Gajewicz, V. Kuz'min, T. Puzyn and J. Leszczynski (2014). "From basic physics to mechanisms of toxicity: the "liquid drop" approach applied to develop predictive classification models for toxicity of metal oxide nanoparticles." Nanoscale **6**(22): 13986-13993.

Spinazzè, A., A. Cattaneo, M. Limonta, V. Bollati, P. A. Bertazzi and D. M. Cavallo (2016). "Titanium dioxide nanoparticles: occupational exposure assessment in the photocatalytic paving production." Journal of Nanoparticle Research **18**(6): 1-12.

Srivastava, R. K., Q. Rahman, M. P. Kashyap, M. Lohani and A. B. Pant (2011). "Ameliorative effects of dimetylthiourea and n-acetylcysteine on nanoparticles induced cyto-genotoxicity in human lung cancer cells-A549." PLoS One **6**(9): e25767.

Studer, A. M., L. K. Limbach, L. Van Duc, F. Krumeich, E. K. Athanassiou, L. C. Gerber, H. Moch and W. J. Stark (2010). "Nanoparticle cytotoxicity depends on intracellular solubility: comparison of stabilized copper metal and degradable copper oxide nanoparticles." Toxicology Letters **197**(3): 169-174.

Sun, Q., D. Tan, Y. Ze, X. Sang, X. Liu, S. Gui, Z. Cheng, J. Cheng, R. Hu and G. Gao (2012). "Pulmotoxicological effects caused by long-term titanium dioxide nanoparticles exposure in mice." Journal of hazardous materials **235**: 47-53.

Sun, T. Y., F. Gottschalk, K. Hungerbühler and B. Nowack (2014). "Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials." Environmental pollution **185**: 69-76.

Sycheva, L. P., V. S. Zhurkov, V. V. Iurchenko, N. O. Daugel-Dauge, M. A. Kovalenko, E. K. Krivtsova and A. D. Durnev (2011). "Investigation of genotoxic and cytotoxic effects of micro- and nanosized titanium dioxide in six organs of mice in vivo." Mutat Res Genet Toxicol Environ Mutagen **726**(1): 8-14.

Takahashi, Y., K. Mizuo, Y. Shinkai, S. Oshio and K. Takeda (2010). "Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice." J Toxicol Sci **35**(5): 749-756.

Tamayo, L. A., P. A. Zapata, N. D. Vejar, M. I. Azócar, M. A. Gulppi, X. Zhou, G. E. Thompson, F. M. Rabagliati and M. A. Páez (2014). "Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into Listeria monocytogenes." Mater Sci Eng Proc Conf **40**(Supplement C): 24-31.

Tavares, A. M., H. Louro, S. Antunes, S. Quarre, S. Simar, P. J. De Temmerman, E. Verleysen, J. Mast, K. A. Jensen, H. Norppa, F. Nesslany and M. J. Silva (2014). "Genotoxicity evaluation of nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes." Toxicology in Vitro **28**(1): 60-69.

Taylor, A. A. and S. L. Walker (2016). "Effects of copper particles on a model septic system's function and microbial community." Water Research **91**: 350-360.

Toropova, A. P. and A. A. Toropov (2013). "Optimal descriptor as a translator of eclectic information into the prediction of membrane damage by means of various TiO(2) nanoparticles." Chemosphere **93**(10): 2650-2655.

Trevisan, R., G. Delapedra, D. F. Mello, M. Arl, É. C. Schmidt, F. Meder, M. Monopoli, E. Cargnin-Ferreira, Z. L. Bouzon and A. S. Fisher (2014). "Gills are an initial target of zinc oxide nanoparticles in oysters Crassostrea gigas, leading to mitochondrial disruption and oxidative stress." Aquatic toxicology **153**: 27-38.

Trouiller, B., R. Reliene, A. Westbrook, P. Solaimani and R. H. Schiestl (2009). "Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice." Cancer Res **69**(22): 8784-8789.

Unrine, J. M., O. V. Tsyusko, S. E. Hunyadi, J. D. Judy and P. M. Bertsch (2010). "Effects of particle size on chemical speciation and bioavailability of copper to earthworms (Eisenia fetida) exposed to copper nanoparticles." Journal of environmental quality **39**(6): 1942-1953.

Valdiglesias, V., C. Costa, G. Kiliç, S. Costa, E. Pásaro, B. Laffon and J. P. Teixeira (2013). "Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles." Environ Int **55**: 92-100.

Vaquero, C., N. Gelarza, J. L. L. de Ipiña, C. Gutierrez-Cañas, I. Múgica, G. Aragón, M. Jaen, R. Pina, I. Larraza and A. Esteban-Cubillo (2015). "Occupational exposure to nano-TiO2 in the life cycle steps of new depollutant mortars used in construction." J Phys Conf Ser **617**: 012006.

Vevers, W. F. and A. N. Jha (2008). "Genotoxic and cytotoxic potential of titanium dioxide (TiO2) nanoparticles on fish cells in vitro." Ecotoxicology **17**(5): 410-420.

Vicario-Pares, U., L. Castanaga, J. Maria Lacave, M. Oron, P. Reip, D. Berhanu, E. Valsami-Jones, M. P. Cajaraville and A. Orbea (2014). "Comparative toxicity of metal oxide nanoparticles (CuO, ZnO and TiO2) to developing zebrafish embryos." Journal of Nanoparticle Research **16**(8).

Vílchez, A., E. Fernández-Rosas, D. González-Gálvez and S. Vázquez-Campos (2015). Nanomaterials release from nano-enabled products. Indoor and Outdoor Nanoparticles, Springer**:** 127-158.

Von Goetz, N., C. Lorenz, L. Windler, B. Nowack, M. Heuberger and K. Hungerbuhler (2013). "Migration of Ag-and TiO2-(Nano) particles from textiles into artificial sweat under physical stress: experiments and exposure modeling." Environmental science & technology **47**(17): 9979-9987.

Wan, R., Y. Mo, L. Feng, S. Chien, D. J. Tollerud and Q. Zhang (2012). "DNA damage caused by metal nanoparticles: involvement of oxidative stress and activation of ATM." Chem Res Toxicol **25**(7): 1402-1411.

Wang, J., X. Deng, F. Zhang, D. Chen and W. Ding (2014). "ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes." Nanoscale Res Lett **9**(1): 117.

Wang, L.-F., N. Habibul, D.-Q. He, W.-W. Li, X. Zhang, H. Jiang and H.-Q. Yu (2015). "Copper release from copper nanoparticles in the presence of natural organic matter." Water research **68**: 12-23.

Wang, X. Z., Y. Yang, R. Li, C. McGuinnes, J. Adamson, I. L. Megson and K. Donaldson (2014). "Principal component and causal analysis of structural and acute in vitro toxicity data for nanoparticles." Nanotoxicology **8**(5): 465-476.

Wang, Z., N. Li, J. Zhao, J. C. White, P. Qu and B. Xing (2012). "CuO nanoparticle interaction with human epithelial cells: cellular uptake, location, export, and genotoxicity." Chem Res Toxicol **25**(7): 1512-1521.

Wang, Z., A. von dem Bussche, P. K. Kabadi, A. B. Kane and R. H. Hurt (2013). "Biological and Environmental Transformations of Copper-Based Nanomaterials." ACS Nano **7**(10): 8715-8727.

Wang, Z., A. von dem Bussche, P. K. Kabadi, A. B. Kane and R. H. Hurt (2013). "Biological and environmental transformations of copper-based nanomaterials." ACS Nano **7**(10): 8715-8727.

Weir, A., P. Westerhoff, L. Fabricius, K. Hristovski and N. von Goetz (2012). "Titanium dioxide nanoparticles in food and personal care products." Environmental Science & Technology **46**(4): 2242-2250.

Wilhelmi, V., U. Fischer, H. Weighardt, K. Schulze-Osthoff, C. Nickel, B. Stahlmecke, T. A. Kuhlbusch, A. M. Scherbart, C. Esser, R. P. Schins and C. Albrecht (2013). "Zinc oxide nanoparticles induce necrosis and apoptosis in macrophages in a p47phox- and Nrf2-independent manner." PLoS One **8**(6): e65704.

Wilson, W. (2013, February, 2013). "The Project on Emerging Nanotechnologies." from http://www.nanotechproject.org/inventories/consumer/.

Windler, L., C. Lorenz, N. Von Goetz, K. Hungerbuhler, M. Amberg, M. Heuberger and B. Nowack (2012). "Release of titanium dioxide from textiles during washing." Environmental science & technology **46**(15): 8181-8188.

Wong, S. W., P. T. Leung, A. B. Djurisic and K. M. Leung (2010). "Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility." Anal Bioanal Chem **396**(2): 609-618.

Wongrakpanich, A., I. A. Mudunkotuwa, S. M. Geary, A. S. Morris, K. A. Mapuskar, D. R. Spitz, V. H. Grassian and A. K. Salem (2016). "Size-dependent cytotoxicity of copper oxide nanoparticles in lung epithelial cells." Environ Sci Nano **3**(2): 365-374.

Woodruff, R. S., Y. Li, J. Yan, M. Bishop, M. Y. Jones, F. Watanabe, A. S. Biris, P. Rice, T. Zhou and T. Chen (2012). "Genotoxicity evaluation of titanium dioxide nanoparticles using the Ames test and comet assay." Journal of Applied Toxicology **32**(11): 934-943.

Wu, B., C. Torres-Duarte, B. J. Cole and G. N. Cherr (2015). "Copper oxide and zinc oxide nanomaterials act as inhibitors of multidrug resistance transport in sea urchin embryos: their role as chemosensitizers." Environ Sci Technol **49**(9): 5760-5770.

Xia, T., R. F. Hamilton, J. C. Bonner, E. D. Crandall, A. Elder, F. Fazlollahi, T. A. Girtsman, K. Kim, S. Mitra, S. A. Ntim, G. Orr, M. Tagmount, A. J. Taylor, D. Telesca, A. Tolic, C. D. Vulpe, A. J. Walker, X. Wang, F. A. Witzmann, N. Q. Wu, Y. M. Xie, J. I. Zink, A. Nel and A. Holian (2013). "Interlaboratory evaluation of in vitro cytotoxicity and inflammatory responses to

engineered nanomaterials: the NIEHS Nano GO consortium." Environmental Health Perspectives **121**(6): 683-690.

Xiong, D., T. Fang, L. Yu, X. Sima and W. Zhu (2011). "Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage." Science of the Total Environment **409**(8): 1444-1452.

Yang J-B, Xu D-L (2002) Nonlinear information aggregation via evidential reasoning in multiattribute decision analysis under uncertainty. IEEE Trans Syst Man Cybern A Syst Hum 32:376-393 doi:10.1109/TSMCA.2002.802809

Yang, Y., P. Mao, C. I. Xu, S.-w. Chen, J.-h. Zhang and Z.-p. Wang (2011). "Distribution characteristics of nano-TiO2 aerosol in the workplace." Aerosol Air Qual Res **11**: 466-472.

Yu, K. N., T. J. Yoon, A. Minai-Tehrani, J. E. Kim, S. J. Park, M. S. Jeong, S. W. Ha, J. K. Lee, J. S. Kim and M. H. Cho (2013). "Zinc oxide nanoparticle induced autophagic cell death and mitochondrial damage via reactive oxygen species generation." Toxicol In Vitro **27**(4): 1187-1195.

Yung, M., C. Mouneyrac and K. Leung (2014). Ecotoxicity of zinc oxide nanoparticles in the marine environment. Encyclopedia of Nanotechnology. B. Bhushan. Netherlands, Springer**:** 1-17.

Zhang, D., T. Hua, F. Xiao, C. Chen, R. M. Gersberg, Y. Liu, W. J. Ng and S. K. Tan (2014). "Uptake and accumulation of CuO nanoparticles and CdS/ZnS quantum dot nanoparticles by Schoenoplectus tabernaemontani in hydroponic mesocosms." Ecological Engineering **70**: 114-123.

Zhang, Y., Y. R. Leu, R. J. Aitken and M. Riediker (2015). "Inventory of engineered nanoparticle-containing consumer products available in the Singapore retail market and likelihood of release into the aquatic environment." International Journal of Environmental Research and Public Health **12**(8): 8717-8743.

Zhang, Y., K. C. Nguyen, D. E. Lefebvre, P. S. Shwed, J. Crosthwait, G. S. Bondy and A. F. Tayabali (2014). "Critical experimental parameters related to the cytotoxicity of zinc oxide nanoparticles." Journal of Nanoparticle Research **16**: 2440.

Zhu, X., Y. Chang and Y. Chen (2010). "Toxicity and bioaccumulation of TiO2 nanoparticle aggregates in Daphnia magna." Chemosphere **78**(3): 209-215.

Zimmer, R., R. Hertel and G. F. Böl (2010). BfR-Delphi Study on Nanotechnology: Expert Survey of the Use of Nanomaterials in Food and Consumer Products, Federal Institute for Risk Assessment.

Zúñiga Rojas, R., X. Blamey Benavides, E. Mosquera and L. Ahumada Bolton (2013). "Estudio exploratorio de higiene industrial en ambientes de trabajo donde se producen o utilizan nanopartículas." Ciencia & trabajo **15**(48): 124-130.

Zuverza-Mena, N., I. A. Medina-Velo, A. C. Barrios, W. Tan, J. R. Peralta-Videa and J. L. Gardea-Torresdey (2015). "Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (Coriandrum sativum)." Environ Sci Processes Impacts **17**(10): 1783-1793.