Università Ca‘ Foscari Venezia
Dipartimento di Scienze Ambientali, Informatica e Statistica

Dottorato di Ricerca in SCIENZE AMBIENTALI, 25° ciclo
(A.A. 2009/2010 – A.A. 2011/2012)

DEVELOPMENT OF AN INTEGRATED FRAMEWORK FOR HUMAN HEALTH RISK ASSESSMENT OF ENGINEERED NANO-OBJECTS AND THEIR AGGREGATES AND AGGLOMERATES

SETTORE SCIENTIFICO-DISCIPLINARE DI AFFERENZA: Chimica dell’ambiente e dei beni culturali (CHIM/12)

Tesi di dottorato di DANAIL HRISTOZOV, matricola n° 955765

Coordinatore del dottorato
Prof. BRUNO PAVONI

Tutore del dottorato
Prof. ANTONIO MARCOMINI

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Summary

The production and use of nano-objects and their aggregates and agglomerates (NOAA) are addressed by the European REACH regulation № 1907/2006, which requires Risk assessment (RA) for each chemical substance produced or imported in quantities above 10 tons per year. The analysis of the feasibility of the RA for NOAA has identified substantial limitations, such as data deficits and methodological concerns with respect to their physico-chemical identity, toxicity, exposure pathways and fate. These issues have led to an increased global funding of projects aimed to facilitate nano risk analysis. One of these projects is the European Seventh Framework ENPRA, which funded this doctoral work.

Before ENPRA most scientific activities were focused on the production of experimental data for RA. However, filling the knowledge gaps will take decades, while risk analyses are urgently needed to trigger adequate regulatory response. The deficit of quantitative data has led to uncertain and ambiguous, largely qualitative risk estimations based on expert judgments, which have failed to inform proper Risk management actions. There is need for quantitative approaches, which effectively combine the currently available data to allow risk analysis and control in the foreseeable future.

In response to the above need, this thesis reports a tiered quantitative Weight of evidence (WoE) framework that utilizes Multi-criteria decision analysis methods for integrating physico-chemical, toxicological and exposure data with expert judgement to allow robust near-term risk analysis. For the first time, a WoE approach incorporates an explicit evaluation of data quality, while at the same time uses well-established methods such as the Margin of exposure and the Derived No-effect Level.

The framework was applied with exposure and effects data from the ENPRA project and the peer-reviewed literature that refer to a panel of commercially available NOAA (i.e. titanium dioxide, zinc oxide, silver and multi-walled carbon nanotubes) to rank and prioritise them for further testing (in lower tiers) and quantitatively estimate their occupational risks (in a higher tier). All uncertainties related to the input data, use of models and the application of the WoE aggregation procedures were probabilistically analysed using the Monte Carlo approach.

Sommario

La produzione e l'utilizzo di nano-oggetti e dei loro aggregati e agglomerati (NOAA) sono oggetto del regolamento europeo REACH № 1907/2006, che impone l'analisi dei rischi (AR) per ogni sostanza chimica prodotta o importate in quantità superiori a 10 tonnellate all'anno. L'analisi della fattibilità dell'AR per NOAA ha identificato limiti sostanziali, quali deficit di dati e carenze metodologiche relative alle loro caratteristiche fisico-chimiche e tossicologiche, e ai percorsi di esposizione e destino finale. Queste problematiche hanno portato ad un aumento dei finanziamenti di progetti finalizzati a rendere possibile l'analisi dei rischi dei nanomateriali. Uno dei progetti finanziati dalla Commissione Europea nell'ambito del settimo programma quadro è il progetto ENPRA, che ha finanziato questo lavoro di dottorato.

Prima di ENPRA, la maggioranza delle attività scientifiche si è concentrata sulla produzione di dati sperimentali utili per l'AR. Questo approccio, tuttavia, richiede decenni per colmare le attuali lacune di conoscenza, mentre le analisi di rischio sono urgentemente necessarie oggi per attivare e supportare la richiesta normativa. Il deficit di dati quantitativi ha portato a stime del rischio largamente qualitative e in gran parte basate su giudizi esperti, tali da non giustificare sufficientemente iniziative di gestione del rischio. C'è bisogno di approcci quantitativi, capaci di integrare i dati attualmente disponibili per consentire analisi e controllo del rischio in una prospettiva di breve termine.

In risposta a questa necessità, il lavoro di tesi qui riportato propone un approccio quantitativo basato sul peso delle evidenze (Weight of Evidence, WoE) basato su metodi di analisi decisionale multicriteriale per l'integrazione di dati fisico-chimici, tossicologici e di esposizione, e supportato da giudizio esperto per consentire una robusta analisi di rischio a breve termine. Per la prima volta, un approccio WoE incorpora una valutazione esplicita della qualità dei dati, e al tempo stesso utilizza metodi consoplidati, come il margine di esposizione (Margino of exposure) e la derivazione di livelli di non-effetto.

L'approccio proposto è stato applicato a dati di esposizione e di effetto ottenuti nell'ambito di ENPRA e a dati di letteratura peer-reviewed facenti riferimento a un gruppo di NOAA commercializzati (ad esempio, biossido di titanio, ossido di zinco, nano-argento, nanotubi di carbonio a pareti multiple) al fine di classificarli e prioritizzarli per ulteriori test (ai livelli di approfondimento più bassi) e di stimare quantitativamente i rischi occupazionali (ai livelli di approfondimento più alti). Tutte le incertezze relative ai dati di input, l'uso di modelli e l'applicazione di procedure di aggregazione basati sul WoE sono stati analizzati probabilisticamente utilizzando il metodo di Monte Carlo.

List of contributions

PUBLISHED/ACCEPTED ARTICLES AND REPORTS

Hristozov D, Gottardo S, Cinelli M, Isigonis P, Zabeo A, Critto A, Van Tongeren M, Tran L, Marcomini A. (2012). Application of a quantitative Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for titanium dioxide and carbon nanomaterials. Nanotoxicology (in press) **[Journal article]**

Hristozov D, Zabeo A, Foran C, Isigonis P, Critto A, Marcomini A, Linkov I. (2012). A Weight of evidence approach for hazard screening of engineered nanomaterials. Nanotoxicology (in press) **[Journal article]**

Hristozov D, Gottardo S, Critto A, Marcomini A. (2012). Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 6: 880–898. **[Journal article]**

Aschberger K, Boraschi D, Bos P, Byrne H, Dahmann D, Gottardo S, Fernandes T, **Hristozov D**, Hund-Rinke K, von der Kammer F et al. (2012). Nanosafety vision report: toxicity testing of engineered nanoparticles. Nanosafety Vision Group. **[Report]**

SUBMITTED ARTICLES AND REPORTS

Hristozov D, Gottardo S, Marcomini A. (2012). Chapter 5: Risk assessment and related approaches. In: Identification of knowledge gaps and strategic priorities for human and environmental hazard, exposure and Risk assessment of engineered nanomaterials. Heriot-Watt University: Edinburgh. **[Chapter in report]**

ARTICLES AND REPORTS IN PREPARATION

Hristozov D, Sayre P, Marcomini A. Regulatory Risk assessment of engineered nanomaterials (in preparation). **[Journal article]**

Hristozov D, Marcomini A. European and US regulatory approaches towards Risk assessment of engineered nanomaterials (in preparation). **[Journal article]**

Hristozov D, Moccia A, Oomen A, Scott-Fordsmand J, Landsiedel R, Marcomini A. Risk assessment of engineered nanomaterials: state of the art and reflection upon the MARINA framework (in preparation). **[Journal article]**

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EXTENDED ABSTRACTS

Hristozov D, Zabeo A, Linkov I, Critto A, Marcomini A. (2012). A Weight of evidence approach for hazard screening of engineered nanomaterials; Society for Risk Analysis in San Francisco, US, 9/12 December, 2012.

Hristozov D, Gottardo S, Critto A, Cinelli M, Zabeo A, Van Tongeren M, Tran L. A Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for Engineered Nanomaterials; SETAC World Meeting in Berlin, Germany, 21/25 May, 2012.

Hristozov D, Gottardo S, Critto A, Marcomini A. A Weight of evidence methodology for Risk assessment of engineered nanomaterials; SETAC 21 Annual Meeting in Milan, Italy, 15/19 May, 2011.

Hristozov D, Gottardo S, Critto A, Marcomini A. Health, safety and environment: assessment methods; Joint JRC Nano Event and 2nd ENPRA Stakeholders Workshop in Somma Lombardo, Italy, 10/12 May 2011.

ABSTRACTS

Hristozov D, Gottardo S, Critto A, Marcomini A. A tiered framework for Risk assessment of engineered nanomaterials; ENPRA project 36 Month Meeting in Edinburgh, UK, 25 May 2012.

Moccia A, **Hristozov D**, Critto A, Marcomini A. Moving towards quantitative ecological Risk assessment of engineered nanomaterials; SETAC World Meeting in Berlin, Germany, 21/25 May, 2012.

Hristozov D, Gottardo S, Critto A, Cinelli M, Zabeo A, Van Tongeren M, Tran L. A Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for Engineered Nanomaterials; SETAC World Meeting in Berlin, Germany, 21/25 May, 2012.

Hristozov D, Gottardo S, Critto A, Marcomini A. A tiered framework for Risk assessment of engineered nanomaterials; ENPRA project WP6 Meeting in Edinburgh, UK, 23 March 2012.

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Hristozov D, Gottardo S, Critto A, Marcomini A. A Weight of evidence approach for human health Risk assessment of engineered nanomaterials; ENPRA project WP6 Meeting in Bilthoven, The Netherlands, 15/16 September 2011.

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Zuin S, **Hristozov D**, Gottardo S, Critto A, Marcomini A. Approaches for the assessment of hazardousness and impacts posed by engineered nanoparticles; XII Congresso Nazionale di Chimica dell'Ambiente e dei Beni Culturali in Taormina, Italy, 26/30 September 2010.

Frequently used acronyms

AF	Assessment Factor
BMD(L)	Benchmark Dose (Lower Confidence Limit)
C&L	Classification and Labeling
CAA	Clean Air Act
CEN	European Standardisation Committee
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CPSA	Consumer Product Safety Act
CPSC	Consumer Product Safety Commission
CSA	Chemical Safety Assessment
CWA	Clean Water Act
DNEL	Derived No-effect Level
DRA	Dose-response Assessment
EA	Exposure Assessment
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EE	Expert Elicitation
EFSA	European Food Safety Authority
ES	Exposure Scenario
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HI	Hazard Identification
ISO	International Organization for Standardization
IVIVE	In vitro-in vivo extrapolation
LEV	Local Exhaust Ventilation
LOAEL	Lowest Observed Adverse Effect Level
LoE	Line of Evidence
LVE	Low-Volume Exemption
MCDA	Multi-criteria Decision Analysis
MoE	Margin of Exposure
NOAA	Nano-objects and their Aggregates and Agglomerates
NOAEL	No Observed Adverse Effect Level
OC	Operational Conditions
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limits
OSH	Occupational Safety and Health Act
OSHA	Occupational Safety and Health Administration
OWA	Ordered Weighted Average
PBPK	Physiologically-based Pharmacokinetic (modelling)
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
PoD	Point of Departure
QSAR	Quantitative Structure-activity Relationships
RA	Risk Assessment

RC	Risk Characterization
RCRA	Resource Conservation and Recovery Act
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RM	Risk Management
RMM	Risk Management Measures
RPE	Respiratory Protection Equipment
RR	Risk Ratio
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SME	Small and Medium Enterprises
SNUR	Significant New Use Rule
TSCA	Toxic Substances Control Act
WoE	Weight of Evidence
WS	Weighted Sum

CHAPTER 1

Introduction

1.1 Motivations and objectives

Nanotechnology is an emerging field in the area of Technology and Science, involving the design, production and use of structures at the nanoscale (i.e. from 1 to 100 nanometres) (British Standards Institution, 2007). In contrast to the small size of nanomaterials, the scale of their application is tremendous. Nanotechnology influences all industrial and public sectors including healthcare, agriculture, transport, energy, materials, information and communication technologies. It is well recognized as a key sector in Europe with a market size, steadily growing to estimated three trillion Euro (€) in 2015 (Lux Research, 2006).

Our understanding of the environmental and health risks associated with nanotechnology is still limited, which may result in stagnation of growth and innovation. There have been other technologies that revealed unexpected ecological and health effects many years after their broader market introduction. In the worst cases this caused tremendous costs for society and the enterprises in the form of lock-in effects, over-balancing regulations and demolished consumer acceptance (Koehler, 2008).

Many studies concluded that nano-objects and their aggregates and agglomerates (NOAA) (ISO, 2012) are biologically more active than their bulk counterparts, and toxicity has been observed in animals for carbon nanotubes (CNT) (Aschberger et al., 2010; Donaldson et al., 2010; Lam et al., 2004; Mercer et al., 2010; Poland et al., 2008; Shvedova et al., 2005; Takagi et al., 2008; Warheit et al., 2004), fullerenes (Chen et al., 1998; Fraser et al., 2010; Oberdörster et al., 2006; Ogami et al., 2011; Saitoh et al., 2010), metal (Christensen et al., 2010; Lansdown, 2007; Wijnhoven et al., 2009) and metal oxide (Christensen et al., 2011; Landsiedel et al., 2010; Simon et al., 2010; Warheit et al., 2009) nanoparticles. This has raised awareness of the need to assess the risks from NOAA in order to ensure high level of human and environmental protection.

Risk assessment (RA) is a central theme in the regulation of chemicals (European Parliament and the Council, 2006; US Congress, 1976). It is defined as "a process intended to calculate or estimate the risk to a given target organism, system or (sub) population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system" (OECD, 2003). One distinguishes between human health and ecological RA. This thesis is concerned solely with human health RA, which is a four-step process, consisting of Hazard identification (HI), Exposure assessment (EA), Dose-response assessment (DRA) and Risk characterization (RC) (US EPA, 1989; 1995).

The HI consists in gathering and evaluating relevant Health and Safety information. It often also involves the characterization of the behaviour of a chemical within the organism and its interactions with organs and cells, which includes the establishment of relationships between the observed biological responses and the physico-chemical properties of the substances (European Chemicals Agency, 2007). The principal aim of this step is to assess the intrinsic hazard of a chemical. It is the likelihood of impairment due to exposure that distinguishes risk from hazard. The EA is concerned with the estimation of the doses, which human populations are or may be exposed to. It starts with the formulation of one or more exposure scenarios (ES), describing how a substance is used by workers or consumers. It includes estimation of exposure by either direct measurements or by the application of models. This involves, for instance, the monitoring of indoor concentrations or the estimation of the amount of the substance coming into contact with the respiratory system, skin or intestinal tract. If significant exposure to a hazardous chemical is identified in the previous steps, it becomes relevant to perform DRA. It typically involves establishing the relationship between the exposure and the observed toxic effects and the derivation of a human effects threshold such as the Derived No-effect Level (DNEL). In the Risk characterization, the final step of the RA, the estimated exposure concentrations are typically compared to this effect level to calculate a risk quotient (RQ). Based on the RQ, it is possible to decide whether the risks for the target population are adequately controlled and, if needed, define a risk reduction strategy.

Although the RA framework is internationally recognized and employed by major actors, such as the World Health Organization (WHO) and the Organisation for Economic Cooperation and Development (OECD), the analysis of its feasibility for NOAA has identified substantial limitations (Hansen, 2009; Hristozov and Malsch, 2009; Stone et al., 2009). Multiple toxicity studies were performed with different nanomaterials, but most of them used non-standardised tests, producing non-reproducible and hardly comparable results, useless for univocal HI (Hristozov and Malsch, 2009). Deficient characterization data makes it difficult to identify which (combinations of) physico-chemical properties determine the effects documented in nano toxicity studies and to identify appropriate dose-exposure metrics (Oberdörster et al., 2007; Stoeger et al., 2005; Stoeger et al., 2007; Wittmaack, 2006; 2007). The EA of NOAA is constrained by uncertainties related to the numbers of exposed workers and consumers, market penetrations of nano-containing products, nanomaterial releases and concentrations in occupational and consumer settings (Bergamaschi, 2009; Gottschalk et al., 2010). Each step of the framework is hindered by serious uncertainties and the RC sums all of them (Hristozov et al., 2012). Filling the knowledge gaps will take decades (Grieger et al., 2010), while risk estimations are urgently needed to trigger adequate regulatory response (Hristozov et al., 2012).

Currently, most scientific activities in the nanosafety area are focused on the production of experimental data to facilitate risk analysis. Minor attention has been paid by the research community to developing new approaches that could complement the available toolset to enable quantitative RA in the near term. The

deficit of quantitative data has led to uncertain and ambiguous, largely qualitative risk estimations based on expert judgments, which have failed to support proper Risk management actions (Hristozov et al., 2012). For the first time the European Seventh Framework ENPRA (Engineered NanoParticles Risk Assessment) project aims to develop and implement a quantitative framework for regulatory RA of NOAA. This thesis aims at presenting the Ph.D. work carried out by the author concerning the design and application of the ENPRA approach to support industrial and regulatory decision making. Specifically, the main objectives of the work are:

- to critically discuss the state of the art in the emerging fields of nano RA and regulation in view of the available data and methods;
- to draft the conceptual structure of the ENPRA RA framework;
- to develop methodologies for hazard, exposure and risk analysis of NOAA;
- to implement the developed methodologies into the above framework;
- to apply the framework to a panel of commercially available NOAA (i.e. titanium dioxide, zinc oxide, silver and multi-walled carbon nanotubes) in order to rank and estimate their occupational risks.

The structure of the thesis is outlined in the next paragraph.

1.2 Thesis structure

This thesis starts with three theoretical chapters:

CHAPTER 2 critically discusses and compares the existing regulatory practices for nanomaterials in Europe and in the United States.

CHAPTER 3 describes the theory behind the Risk assessment and management paradigm, as well as some approaches useful in its context, such as Weight of evidence, Multi-criteria decision analysis, and Expert Elicitation;

CHAPTER 4 critically discusses the existing frameworks and tools for RA of nanomaterials in the context of the available Environmental, Health and Safety data.

The thesis continues with a methodological chapter:

CHAPTER 5 describes the tiered framework for RA of nanomaterials, which is the main scope of this work.

The results of applying the framework and their discussion are reported in CHAPTER 6, while the overall conclusions of the thesis are presented in CHAPTER 7, including final considerations on main findings and recommendations for further developments.

1.3 ENPRA project

The work presented in this thesis was developed within the European ENPRA project (NMP4-SL-2009-228789; www.enpra.eu). It is funded under the Seventh Framework Programme of the European Commission and led by the Institute of Occupational Medicine (IOM) in Edinburgh, Scotland. Three and half years long and worth €6.7 million, the project harnesses the knowledge and capabilities of 15 European and 6 US partners.

ENPRA is based on a multidisciplinary approach and aims to develop and implement a novel integrated approach for RA of nanomaterials. The main objectives of the project are:

- to obtain a bank of commercial nanomaterials with contrasting physico-chemical characteristics and measure them;
- to investigate the toxic effects of nanomaterials on five target systems (i.e. pulmonary, hepatic, renal, cardiovascular and developmental) and five endpoints (i.e. oxidative stress, inflammation; immune toxicity; fibrogenecity; genotoxicity) using *in vitro* animal/human models;
- to validate the *in vitro* findings with a small set of carefully chosen *in vivo* animal experiments;
- to construct mathematical models to extrapolate the exposure-dose-response relationship from *in vitro* to *in vivo* and to humans;
- to use Quantitative Structure-activity Relationship (QSAR)-like models to identify key nanomaterials characteristics driving adverse effects;
- to perform quantitative RA of nanomaterials using the Weight of evidence approach.

The rationale of the ENPRA project (Figure 1-1) is based on the following 4 main components:

1) **Hazard identification**

   A panel of 10 nanomaterials (i.e. titanium dioxide in five sizes; +/- charged zinc oxide; silver; bent and entangled multi-walled carbon nanotubes) were characterized using standardised experimental protocols. The measurement techniques included: Scanning Electron Microscopy, Transmission Electron Microscopy, X-Ray Diffraction, X-ray Absorption for Depth measurement, Nuclear Magnetic Resonance, Infra-Red spectroscopy, Elemental Analysis, Field Flow Fractionation, Dynamic Light Scattering, Gel Permeation Chromatography.

2) **Dose-response assessment**

   The biological effects of nanomaterials were studied using *in vitro* and *in vivo* test methods representing five body systems (i.e. pulmonary, cardio-vascular, hepatic, renal and developmental) and five endpoints (i.e. oxidative stress, inflammation, genotoxicity, fibrogenecity, and

developmental toxicity). Dose-response relationships were estimated for all nanomaterials across the five systems.

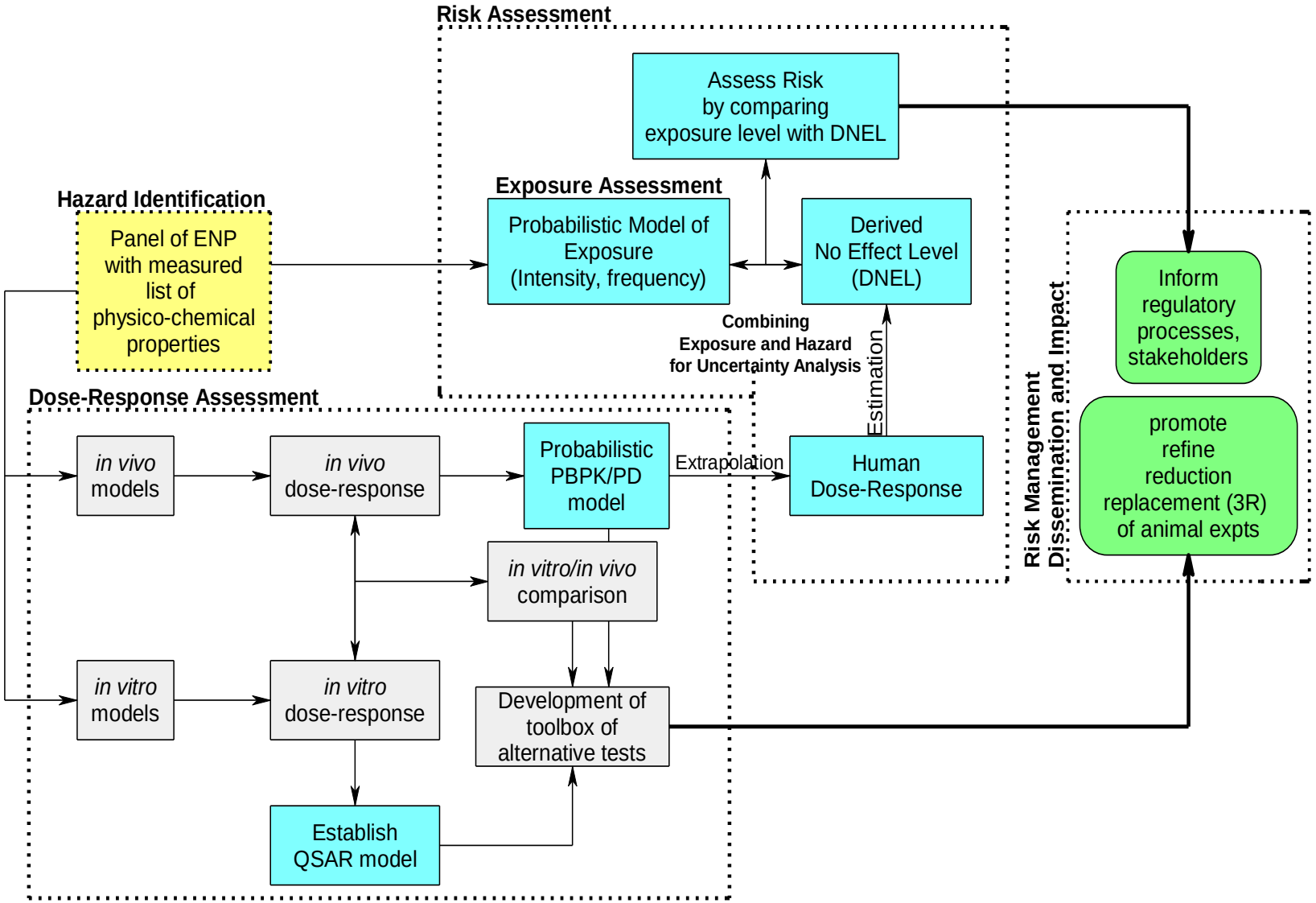
3) **Exposure assessment**

The NanoSafer exposure model was used to estimate workplace inhalation exposure.

4) **Risk assessment**

Dose-response relationships were derived from the *in vivo* and *in vitro* data by means of the PROAST model (http://www.rivm.nl/en/foodnutritionandwater/foodsafety/proast.jsp) and then compared using a mechanistic *in vitro-in vivo* extrapolation technique. A Physiologically based Pharmacokinetic/dynamic model was developed and used to derive nano-specific assessment factors (AF) to use for estimation of Derived No-effect Levels (DNEL). The DNEL were contrasted to exposure doses to calculate risk.

Figure 1-1: Rationale of ENPRA (Engineered NanoParticles Risk assessment) project



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CHAPTER 2

Regulatory frameworks for nanomaterials

The United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro (1992) represented a turning point in the way we look at production, consumption, population and our planet's life-supporting capacity. At the Earth Summit, world leaders adopted Agenda 21, an action plan for sustainable development in a number of policy areas (United Nations, 1992) that meets the needs of the poor and recognizes the limits of economic growth. Among other issues Agenda 21 emphasizes the need to strengthen national capacities towards assessment and regulation of chemical risks. This laid the basis for the World Summit on Sustainable Development (WSSD) in Johannesburg (2002), where the heads of states agreed to work towards minimizing adverse environmental and health effects of chemicals by 2020 (United Nations, 2002).

The new regulation for industrial substances called Registration, Evaluation, Authorization and Restriction of CHemicals (REACH) (European Parliament and the Council, 2006), moved Europe from words to deeds in meeting the WSSD goal. The main aim of the Regulation is to create a single system for safety evaluation of chemicals in order to ensure high level of health and environmental protection. In addition to REACH, a variety of directives and regulations for chemicals, occupational health, food and cosmetics were enforced in Europe, US, Canada and Japan (European Commission, 1967; European Council, 1991; 1993a; b; US Congress, 1976).

The main purpose of this chapter is to review and discuss the state of the art in the area of nano risk regulation. In particular the chapter seeks to identify key challenges and propose regulatory concepts and instruments as a roadmap to the safe production and use of NANOMATERIALS. Therefore the term "regulation" is understood not only in its narrow sense (as a legislative act), but also in its broader meaning involving secondary legislation and implementation instruments. Naturally, the discussion starts at the level of definition, a key aspect of legislation.

2.1 Definitions of "nanomaterial" for regulatory purposes

In response to the growing concerns about the risks from nanotechnologies, the European Commission (EC) issued the report "Nanoscience and nanotechnology: an action plan for Europe 2005-2009" (European Commission, 2005), which emphasized that nano-enabled products must comply with the Community's requirements for high level of public health, consumer, worker and environmental protection. In a regulatory review of this document from June 2008 [addressed to the European Parliament (EP)] (European Commission, 2008a), the EC stated that nanomaterials are in principle covered by the existing legislation. However, whenever the need for specific measures appears, the EC will consider changes to the legislation.

In April 2009, the EC review was followed by an EP resolution on nanomaterials by a very large majority (European Parliament, 2009), which expressed strong scepticism vis à vis the Commission's conclusion that nanomaterials are covered by the existing regulations, and requested a more in-depth review of legislation by June 2011. In particular, the EP requested that the review should address actual applications of NANOMATERIALS and include a market inventory. The EP stressed that "the current discussion about nanomaterials is characterized by a significant lack of knowledge and information, leading to disagreement and political struggles starting at the level of definitions". For this reason, the institution called on the EC to promote the adoption of a harmonized science-based definition of "nanomaterial" at the international level and to adapt the relevant European legislative framework accordingly (European Parliament, 2009).

Introducing a regulatory definition of "nanomaterial" is not a straightforward and easy task. Although a variety of interpretations have been already discussed and proposed by national authorities, there is neither a global nor an EU definition agreed yet that would fulfil the requirements for entering into legislation (Lövestam et al., 2010). For this reason, in this thesis we tend to substitute "nanomaterial" with the most generic term possible, i.e. nano-objects and their aggregates and agglomerates (NOAA), tailored by the Intentional Organisation for Standardisation (ISO, 2012). However, since nanomaterials are already manufactured, commercialized and used on the large scale, there is an immediate need for a common definition to use not only by regulators but also by all other stakeholders, including representatives from academia, industry and the general public.

A number of international and national organizations and authorities have published nano terminology standards and documents, including, among others, definitions for the term "nanomaterial". These definitions are reviewed below and are summarized in Annex 1. Many of the released documents are non-normative and have been published mainly to collect feedback from stakeholders (Lövestam et al., 2010). For this reason in most cases the proposed definitions are partially conflicting, which leads to a general consensus that further developments of nanotechnology terminology should no longer be pursued on a national or regional basis, but at a European or global level.

2.1.1 Definitions by international organizations

Within the International Organization for Standardisation (ISO) the body responsible for the harmonization work related to nanotechnologies is the Technical Committee (TC) 229. Together with a number of specific ISO working groups, ISO/TC 229 has established the Nanotechnologies Liaison Coordination Group to support the work of relevant ISO technical committees as well as other organizations, and to identify gaps and cross cutting opportunities. Nano "Terminology and Nomenclature" issues are particularly addressed by the JWG 1, which is a joint working group between ISO and the International Electrotechnical Commission (IEC).

Within the European Standardisation Committee (CEN), Technical Committee (TC) 352 is responsible for the standardization of nomenclature related to nanotechnologies. Because many of the members of CEN/TC 352 also participate in ISO/TC 229, it has been decided that CEN/TC 352 will not lead projects concerning nano-terminology, but instead will support the activities of the ISO/TC 229/JWG 1 (Lövestam et al., 2010). A number of nanomaterial-related definitions have already been published by ISO in Technical Specifications (TS), which can be accessed via the on-line ISO Concept Database (http://cdb.iso.org/). They are the following.

- CEN/ISO/TS 27687/2008 Nanotechnologies: Terminology and definitions for nano objects- nanoparticle, nanofibre and nanoplate (http://www.iso.org/iso/catalogue_detail? csnumber=44278) (ISO, 2008). After revision the number of the document will change to ISO TS 80004-2.
- ISO/TS 80004-1/2010 Nanotechnologies- Vocabulary- Part 1: Core Terms (http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=51240) *(*ISO, 2010). This document lists a number of core terms related to NOAA (e.g. nanomaterial, nanoobject, nanostructure).
- ISO/TS 80004-5 Nanotechnologies- Terminology and definitions- Part 5: Nanostructured materials. This document is still under preparation. It addresses the definition of the main categories of nano-structured materials, such as nanostructured powder, nanodispersion, nanolayer, nanocomposite and nanoporous material (Hatto, 2011).
- ISO/TC 229/2012: Nanotechnologies - Guidance on physico-chemical characterization of engineered nanoscale materials for toxicological assessment.

A comprehensive list of upcoming TS including nano-related definitions can be found on the ISO webpage: http://www.iso.org.

Table A1-1 of Annex 1 gives an overview of the definitions of “nanomaterial” and related terms (e.g. nanoobject, nanoparticle) proposed in the above TS. CEN/ISO/TS 27687/2008 defines “nanoscale” as referring to the size range from approximately 1 nm to 100 nm and “nanoobject” as a material with one or more external dimensions within this range. It also quotes an existing general definition for “particle” from the former ISO 14644-6/2007 (ISO, 2007) standard and introduces the terms “nanoparticle”, “agglomerate” and “aggregate” (Table A1-1). The TS names the agglomerates and aggregates “secondary” particles in order to distinguish them from the original individual particles, referred to as “primary” particles. CEN/ISO/TS 27687/2008 also defines the terminology for some types of nanoobjects including six distinct shapes: i.e. nanoparticle, nanoplate, nanofibre, nanotube, nanorod and nanowire; and includes an additional specific case, i.e. the quantum dot. ISO/TC 229/2012 introduces a new generic term, i.e. “nano-objects and their aggregates and agglomerates”, which was adopted and use in this thesis.

ISO/TS 80004-1/2010 goes a step further defining “nanomaterial” as material with any external dimension, internal or surface structure in the nanoscale. The latter definition is inclusive of two partly

overlapping subcategories: “nanoobject” and “nanostructured material” and it is quite generic, comprising also macroscopic materials such like nanocomposites.

Another international institution working on the definition the term “nanomaterial” is the Organisation for Economic Co-operation and Development (OECD), which established in 2006 the Working Party on Manufactured Nanomaterials (WPMN) under the OECD Joint Chemicals Programme. Some nano-related definitions of the WPMN were agreed upon in 2007 and they are reported in Table A1-1 (Annex 1). According to them a “manufactured nanomaterial” is an intentionally produced material, which has an internal or surface structure at the nanoscale to enable specific properties, as in this case “nanoscale” is defined as the size range between 1 and 100 nm (OECD, 2008). Here again the proposed definition is quite generic, encompassing also micro and macro- sized materials. However WPMN claims that the end products containing nanomaterials (e.g. electronic equipment) are not themselves nanomaterials, while the aggregates and agglomerates of the primary particles are “nanostructured materials”, which is in agreement with the definitions by ISO.

2.1.2 Definitions by European organizations

The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) is a scientific committee managed by the European Commission (EC), which gives advice to the EC on issues related to consumer safety, public health or the environment. In 2007 SCENIHR issued a report including an analysis of existing definitions and recommendations for their improvement (SCENIHR, 2007b). The definitions proposed by the Committee are reported in Table A1-2 (Annex 1). SCENIHR defines “nanomaterial” as “any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less”, which is in agreement with the OECD definition, except for the lack of a lower size bound specification. The SCENIHR definition is also inclusive of bulk materials.

In December 2007, the EU Scientific Committee on Consumer Products (SCCP) issued an opinion on “Safety of Nanomaterials in Cosmetic Products” (SCENIHR, 2007a), which included a glossary of nano-related terms (Table A1-2). According to SCCP the “nanoscale” is in the order of 100 nm or less, which is in agreement with the SCENIHR definition, while “nanoparticles” have one or more dimensions at the nanoscale and “nanomaterials” may also have an internal nanostructure, which could exhibit novel characteristics compared to the bulk form.

The new Regulation (EU) No 1169/2011 on Food Information (European parliament and Council, 2011), which came into force on 25 October 2011, defines “engineered nanomaterial” as “any intentionally produced material that has one or more dimensions of the order of 100 nm or less or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order

of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic to the nanoscale". This definition incorporates elements of both the SCENIHR and SCCP definitions and it is in line with the one of the Regulation (EC) No 258/97 on Novel Foods (European Commission, 2009a).

Similarly, along with the obligation to label nanomaterials in the list of ingredients, the recent European Cosmetic Products Regulation (European Commission, 2009b) defines "nanomaterial" as "an insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, at the scale from 1 to 100 nm", which is in agreement with the SCENIHR definition. However, in both regulations it is specified that the definition shall be revised according to the scientific progress in the nanosafety area.

In order to ensure harmonization among legislations, in October 2011 a recommendation for a common definition of "nanomaterial" was published by the European Commission (EC) (http://ec.europa.eu/environment/chemicals/nanotech/index.htm) based on the scientific advice from SCENIHR and the Joint Research Centre (JRC). EC defines nanomaterial is "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials". This definition is also in parts used in the new Regulation (EU) No 528/2012 on Biocide Products (European Parliament and of the Council, 2012), which came in force on 27 June 2012. Important aspects of this definition are that a size distribution threshold was introduced, size was selected as the main identifier of a nanomaterial, and no references were made to any other physico-chemical properties. There is a still on-going discussion whether size is sufficient to identify nanomaterials from regulatory perspective (JRC, 2011).

2.1.3 Definitions by national authorities

In the February 2009 edition of their Chemical Gazette the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (NICNAS, 2009) concludes that there is currently no agreed national or international definition of nanomaterials and proposes the following working version: "…industrial nanomaterials are those industrial materials intentionally produced, manufactured or engineered to have specific properties or specific composition, and one or more dimensions typically

between 1 nm and 100 nm. This size range refers to individual particle size, and does not take into account agglomeration of particles" (Table A1-3, Annex 1).

Similarly, in a policy statement Health Canada states that it "…considers any manufactured product, material, substance, ingredient, device, system or structure to be nanomaterial if: (a) it is at or within the nanoscale in at least one spatial dimension, or; (b) it is smaller or larger than the nanoscale in all spatial dimensions and exhibits one or more nanoscale phenomena" (Health Canada, 2009) (Table A1-3, Annex 1).

Although there is no officially accepted definition for "nanomaterial" in the United States (US), the US Environment Protection Agency (EPA) discusses this issue in their Concept Paper for the Nanoscale Materials Stewardship Program under the Toxic Substances Control Act (TSCA) (US EPA, 2010). EPA defines the term "engineered nanoscale material" as "any particle, substance, or material that has been engineered to have one or more dimensions in the nanoscale", where the term "engineered" means "purposefully designed", while "nanoscale" refers to the size range between the atomic/molecular state and the bulk/macro state of the materials, i.e. between approximately 1 and 100 nm (US EPA, 2010).

In Europe, the Danish Ministry of the Environment defines "nanomaterials" as materials shorter than 100 nanometres along their shortest side, which can also be built in larger materials. They can be produced from existing chemicals or completely new substances, and can be made from more than one compound. What distinguishes them is the small size of the materials resulting in special characteristics.

In order to establish definitions for "nanomaterial" and other related terms a voluntary reporting scheme for the period 2006-2008 was launched in the UK by the Department for Environment, Food and Rural Affairs (DEFRA, 2008). According to DEFRA "nanoscale materials are defined as having two or more dimensions up to 200 nm", as this definition will be reviewed according to the on-going work of ISO and CEN.

2.2 European legislation and requirements

In Europe one can distinguish between horizontal (e.g. environmental, worker protection and chemicals legislation) and specific sector regulations (e.g. cosmetic, food, biocide, plant protection, medicinal products and devices, aerosol dispensers, electronic or automotive industry). The regulations where specific actions were taken to address nanomaterials are the EU chemical REACH regulation (European Parliament and the Council, 2006), the European Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging (CLP) of Substances and Mixtures (European Commission, 2008d), the Cosmetic Products Regulation (European Commission, 2009b), the Food Information Regulation 1169/2011 (EC, 2011a), the Regulation (EC) No 1333/2008 on Novel Foods and in the new Biocides Regulation 528/2012 (EC, 2012). REACH is administered by the European Chemical Agency (ECHA), while the implementation of the rest is responsibility of the EU Member State national regulatory agencies.

REACH is the regulation of the European Parliament and Council on the Registration, Evaluation, Authorization and Restriction of Chemicals, which entered into force in June 2007. The purpose of REACH is to ensure high level of human health and the environmental protection and at the same time enhance the free circulation of substances on the internal EU market, while promoting competitiveness and innovation. Its provisions are underpinned by the Precautionary Principle and require that manufacturers, importers and downstream users ensure the health and environmental safety of chemical substances (European Parliament and the Council, 2006). There are no provisions in REACH specifically referring to NOAA. However, since the regulation applies to industrial substances in whatever size, shape or physical state, materials at the nanoscale are covered by it (European Commission, 2008b).

Under REACH, manufacturers and importers are obliged to submit a registration dossier for substances that they manufacture or import in annual quantities of above 1 ton. When an existing chemical substance, already placed on the market as bulk substance, is introduced on the market in a nanoform, the registration dossier has to be updated with additional information, including nano-specific classification and labelling of the nanoform as well nano-specific Risk management measures (RMM). Industries importing or producing nanomaterials in quantities above 10 tons per year are obliged to perform Chemical Safety Assessment (CSA). For high-concern substances, special authorization is required for their placement on the market (European Commission, 2008a).

The European Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging (CLP) of Substances and Mixtures (European Commission, 2008d) came into force on 20 January 2009 to implement the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (ECOSOC, 2003). Registrants must submit information on classification and labelling of substances and mixtures and communicate instructions for a safe handling along the supply chain via the Safety Data Sheet (SDS), which should reflect the current state of knowledge on chemical safety. Because the majority of NOAA are currently not classifiable as hazardous substances there would be no obligation to label them as such or even prepare an SDS (Alessandrelli and Polci ML, 2011). In order to find a solution for this issue, a number of recommendations on how to integrate nano-specific information into the SDS chapters of the CLP have been provided by the Swiss Federal Office for Public Health (FOAG, 2010) and they are reported in Annex 2.

The EU regulatory requirements concerning the safety of cosmetic products are currently laid down in Directive 76/768/EEC (European Commission, 1976), which was adopted in 1976. Although Directive 76/768/EEC is still in force it will be soon repealed by Regulation (EC) No 1223/2009 on cosmetic products, whose provisions will become effective in 2013 (European Commission, 2009b). The new statute will be more stringent and will implement more rigorous market surveillance (European Commission, 2009b; NanoKommission, 2010). For the first time, Regulation (EC) No 1223/2009 includes specific provisions on nanomaterials. Article 2 contains a definition of “nanomaterial”, while Article 16 imposes an obligation to industries to notify the responsible national regulatory agency 6 months prior to placing a cosmetic product

on the market. The notification should include information on the physico-chemical and toxicological profile of the material, production volume and market penetration (European Commission, 2009b). Based on these data the EC makes a decision if particular RMM should be implemented. All nanoscale particulate ingredients present in cosmetic products must be indicated clearly using the appropriate International Nomenclature of Cosmetic Ingredients (INCI), followed by the word “nano” in brackets.

In the EU, food safety and packaging are controlled by the Food Law Regulation (EC) No 178/200235 (European Commission, 2002), the Novel Foods Regulation EC 258/97 (European Commission, 2009a), the Regulation (EC) No 1333/2008 on Food Additives (European Parliament and Council, 2008) and the new Regulation (EU) No 1169/2011 on Food Information. Among them only the latter specifically addresses nanomaterials and includes a specific definition of "nanomaterial", which is different from the one recommended by the EC. Nano-labelling is explicitly required: i.e. all ingredients present in the form of nanomaterials have to be indicated in the list of ingredients and their names followed by the word "nano" in brackets.

Currently, Regulation (EC) No 1333/2008 is under revision and the adaptation of its provisions to nanotechnologies is a spotlight issue. In the revised Novel Foods Regulation it is expected that a definition of “nanomaterial” will be included and the scope of forthcoming nano-specific provisions will be explicitly set out (European Commission, 2008b).

The new Regulation (EU) No 528/2012 on the use and placing on the market of biocide products (European Parliament and of the Council, 2012) was published on 27 June 2012 to repeal the current Biocide Directive (European Council, 1993). It includes a definition of "nanomaterial" and requires nano-specific labelling, which means that all nanoforms should be indicated in the list of ingredients and their names should be followed by the word "nano". Where nanomaterials are used in the product, their environmental and health risks should be assessed separately.

2.3 US legislation and requirements

In the United States, regulation of nanomaterials and products involves several federal agencies. These include the EPA, the Food and Drug Administration (FDA), the Occupational Health and Safety Administration (OSHA), and the Consumer Product Safety Commission (CPSC). Each agency is responsible for enforcing regulations to control risks from specific types or uses of substances, including NOAA. Table 2-1 lists these regulations and the responsible federal agencies.

Table 2-1: Federal Environmental, Health and Safety regulations and agencies responsible for their implementation.

Regulation	Agency
Toxic Substances Control Act (TSCA)	Environmental Protection Agency (EPA)
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)	EPA
Federal Food, Drug, and Cosmetic Act (FFDCA) Consumer Product Safety Act (CPSA)	Food and Drug Administration (FDA) Consumer Product Safety Commission (CPSC)
Occupational Safety and Health Act (OSH)	Occupational Safety and Health Administration (OSHA)
Resource Conservation and Recovery Act (RCRA)	EPA
Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)	EPA
Clean Water Act (CWA)	EPA
Clean Air Act (CAA)	EPA

Chemical substances and pesticides are regulated by EPA under TSCA (US Congress, 1976b) and FIFRA (US Congress, 1996). Food additives and drugs are regulated under the FFDCA (US Congress, 1938), which is administered by the FDA. TSCA and FIFRA are applied mainly through a "pre-market" registration, risk analysis and management approach, where decisions are made before a product is released to the market. FFDCA applies to drugs, food additives, dietary supplements and cosmetics. All other consumer products are regulated under the CPSA (US Congress, 2011). Provisions under FFDCA and CPSA operate through "post-market" mechanisms whereby producers are responsible ensuring the safety of products, and regulatory agencies have the authority to remove unsafe products from the market. RCRA (US Congress, 1976a) ensures that hazardous wastes are handled and disposed of safely, while the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (US Congress, 1980) deals with accidental releases into the environment that are not otherwise controlled under RCRA. The Occupational Safety and Health (OSH) Act was enacted to "assure safe and healthful working conditions for working men and women" (US Congress, 1970). Enforcement and administration of the OSH Act is handled primarily by OSHA. Safety and health standards related to field sanitation and temporary labor camps in the agriculture industry are administered by the US Department of Labour Employment Standards Administration's Wage and Hour Division.

The regulations where specific actions were taken to address nanomaterials are the TSCA and FIFRA as discussed below.

EPA has the statutory authority to regulate nanomaterials during various stages of their lifecycle through TSCA. The agency can regulate the sale, distribution, and use of pesticide products containing NOAA through FIFRA.

EPA's management of nanomaterials under TSCA has evolved from a participatory to a regulatory approach (US EPA, 2011b). In January 2008, EPA started collecting Environmental, Health and Safety (EHS) data from manufacturers through the voluntary Nanoscale Materials Stewardship Program (NMSP) (US EPA, 2009). 29 companies provided data for 123 nanomaterials, but only 4 of them were willing to sponsor the development of test data for use by EPA. Due to the limited support of the program, EPA discontinued the NMSP in December 2009 and made provisions under Sections 5 and 8(a) of TSCA to require nano-specific data from manufacturers.

Similarly, EPA is working on new data reporting requirements under FIFRA. In July 2010, the agency submitted a proposal for a FIFRA revision to the Office of Management and Budget (OMB) seeking to clarify the ability of the regulation to adequately collect information by including a description of nanomaterials, which would require manufacturers to submit more nano-specific data (US EPA, 2011b).

Then, on 17 June 2011, EPA issued a Federal Register notice (76 FR 35383) seeking comments on how to use FIFRA Section 6(a)(2) or FIFRA Section 3(c)(2)(B) to gain information on nanoscale materials in pesticide products, and proposing their classification as "new" active or inert ingredients (US EPA, 2011b).

In October 2010, EPA submitted a proposed TSCA revision to OMB, according to which any chemical substance in the 1-100 nm range is subject to TSCA's Significant New Use Rule (SNUR). The SNUR identifies existing uses of nanoscale materials based on information submitted under the NMSP. It requires persons or companies who intend to manufacture, import, or process new nanoscale materials to submit a Significant New Use Notice (SNUN) to EPA at least 90 days prior to commencing activity (US EPA, 2011a). SNUN are intended to provide EPA with a basic set of information, such as physico-chemical properties, commercial uses, production volumes, exposure, fate and toxicity data. This information would help the agency to evaluate the risks of NOAA and (if needed) take preventive action (US EPA, 2011a).

The on-going activities of EPA intended to strengthen the regulation of NOAA under TSCA and FIFRA are reported in Table 2-2.

Table 2-2: On-going activities of EPA intended to strengthen the regulation of NOAA under TSCA and FIFRA (US EPA, 2011).

Provision	Description	Status
TSCA 5(a)(2) Nanomaterial SNUR	This change under TSCA would require that production of certain new nanoscale materials would constitute a significant new use of a chemical substance. Manufacturers must notify EPA at least 90 days before starting production to provide EPA the opportunity to evaluate the intended use and, if necessary, to prohibit or limit its use.	Awaiting OMB approval
TSCA 8(a) Information Gathering	This change under TSCA would require that persons who manufacture nanomaterials already in commerce notify EPA of information including production volume, methods of manufacture and processing, exposure and release information, and available health and safety data.	Awaiting OMB approval
TSCA Section 5 New Chemical Review	OCSPP stated that since 2005 it has received and reviewed over 120 new chemical notices under TSCA for nanoscale materials, including carbon nanotubes. The Agency has taken a number of actions to control and limit exposures to these chemicals utilizing its authority under TSCA Sections 5(e) and 5(a) (2).	On-going
FIFRA 6(a)(2) or 3(c)(2)(b)	EPA issued a Federal Register notice on June 17, 2011, seeking comments on how the Agency could use FIFRA Section 6(a)(2) or FIFRA Section 3(c)(2)(B) to gain information on what nanoscale materials are in pesticide products. The notice also proposed a policy of classifying any application for registration of a pesticide product containing nanoscale material as an application for a "new" active or inert ingredient.	Proposal currently undergoing revisions by EPA

2.4 Assessing the limitations in EU and US regulations

In theory, the existing regulations cover NOAA. In practice, however, there are ways that nanomaterials can escape comprehensive risk analysis and management. Both EU and US regulations are complex and nuanced, and assessments of their feasibility for NOAA need to account for this complexity. This section does not aim at such a detailed analysis, but instead gives a basic insight into existing challenges that may prevent adequate regulation of nanomaterials.

2.4.1 Thresholds and exemptions

In the EU REACH regulation the production/import volume of substances is important because the associated data requirements for CSA are dependent on tonnage triggers. Currently, for each substance, produced or imported in quantities below 1 ton/year, no safety testing is required, while quantities of above 1000 tons/year imply full CSA (European Commission, 2008; Pronk et al., 2009). Several CARACAL CASG Nano (Competent Authorities for REACH and CLP subgroup on nanomaterials) stakeholders have raised the issue that the 1 ton/year threshold may prevent the registration of many substances at the

nanoscale since they are typically manufactured/imported in lower volumes (European Commission, 2008; Pronk et al., 2009). Moreover, since the nanoforms can differ significantly from their bulk counterparts in terms of physico-chemical properties and biological effects, the REACH tonnage bands and the CSA data requirements should be revised.

If a substance exists both in the bulk and nanoform, a "sameness" analysis should be carried out by the REACH registrant to determine if the nanomaterial can be considered as a specific physical form of the bulk substance or as a different substance (European Commission, 2008; Pronk et al., 2009). If it is identified as a nanoform of the bulk substance, obligations for data sharing and joint registration will apply, while the registration of new substances should be addressed separately (European Commission, 2008; Pronk et al., 2009). However, "current state of development is not mature enough to include guidance on the identification of substances in the nanoform" (European Commission, 2008). Moreover, there are still no agreed criteria to evaluate "sameness". REACH does not strictly require nano-specific testing prior to registration and therefore, even if a nanomaterial exhibits novel properties and biological responses, those may remain obscured prior to registration, which would lead to the material being identified as a "nanoform of a bulk substance". In those cases reduced data requirements would apply and the substance may escape CSA.

Several US statutes include applicability thresholds or exemptions based mass, volumes, or categories that may allow some NOAA to escape federal oversight. Under TSCA, EPA distinguishes between "existing substances" [i.e. those previously added to the Chemical Substances Inventory (CSI)] and "new substances". "Existing substances" are considered safe and are authorized for use, while "new substances" undergo risk analysis prior to being added to the CSI. EPA currently considers NOAA with the "same molecular identity" like substances already in the CSI as "existing substances" and exempts them from RA obligation (US EPA, 2011c) despite the solid evidence that nanoscale materials with the same chemical composition may have different toxicity (Donaldson et al., 2010; Poland et al., 2008).

Another potential issue is the TSCA Low-Volume Exemption (LVE) for substances manufactured/imported in annual amounts of less than 10 tons (US Congress, 1976c). The LVE assumes that the overall risk from substances produced in lower volumes is lower and therefore exempts those substances from a full 90-day risk review (Beaudrie et al., 2012). However, due to their high surface area, NOAA tend to be more reactive than their bulk counterparts and therefore they may be more toxic and pose significant risks even at low volumes (Daniel and Astruc, 2004; Jiang et al., 2008; Oberdörster et al., 2005). Therefore the 10 ton threshold applied to conventional chemicals may be too high for NOAA and may need to be revised.

FIFRA only applies to materials that claim to be pesticides (US Congress, 2003). Manufacturers may use NOAA in products for their pesticide properties (e.g. nano-Ag as antimicrobial agent), but not claim

those as pesticides in order to avoid RA under FIFRA. In order to avoid this, EPA released a fact sheet to clarify what types of claims trigger requirements for pesticide registration under FIFRA (US EPA, 2012b).

According to a category-based exemption for food additives under FFDCA a premarket approval is not required for "generally recognized as safe" (GRAS) additives, which is a consideration left to the manufacturer (Beaudrie et al., 2012). Therefore, if producers claim NOAA as GRAS, they may avoid risk regulation. Alternatively, manufacturers can escape premarket review if they submit a food contact notification (FCN) for substances present in food in very small amounts. However, in this case the FDA has 120 days for reviewing the FCN.

Hazardous substances are subject to regulation under RCRA. However RCRA exempts manufacturers producing NOAA in annual volumes of less than 100 kg from reporting to EPA their activities or waste-storage plans (Powell et al., 2008). Although this is a low production volume for bulk materials, it may be significant for highly reactive nanomaterials. Another issue is that the RCRA regulation does not apply to household hazardous wastes (Breggin and Pendergrass, 2007). Although small volumes of household waste may not be hazardous, when aggregated in collection centres, recycling facilities or landfills it may pose significant risks for workers and for the environment (Beaudrie et al., 2012).

2.4.2 Uncertainty and the burden of proof

Under the European REACH, Cosmetics and Novel Food regulations industries are liable for assessing the risks from their chemical products and provide appropriate safety information to their users (European Commission, 2009a; b; European Parliament and the Council, 2006). In contrast, rather than placing responsibility on the manufacturer, US federal statutes typically require that regulatory agencies carry the burden of proof. Federal agencies generally operate under the "safe until proven harmful" principle, which significantly limits regulators' options under conditions of high uncertainty (Beaudrie et al., 2012) and makes it difficult to anticipate and model risks (Beaudrie et al., 2012; US EPA, 2011b).

Under TSCA, chemical manufacturers are responsible to submit substance-specific data to EPA through a Pre-Manufacture Notice (PMN) or a SNUN to allow for the assessment of risks. However, manufacturers are not required by TSCA to test new chemicals and companies generally do not perform voluntary testing (Jeffords et al., 2005). Considering the paucity of substance specific toxicity data, in order to estimate risk EPA must either rely solely on *in silico* or *read across* methods (still unavailable for NOAA) (Morris et al., 2011) or they must propose a test rule (Beaudrie et al., 2012; US Congress, 1988). Moreover, while the SNUR is a flexible mechanism, it is a burdensome process for the agency and the typical time for issuing a SNUR is two years (US EPA, 2011a). The expected significant increase of nano-products on the market will likely make it impossible for the EPA to employ the SNUR approach for evaluating risks from nanoscale "existing" substances on case-by-case basis (Beaudrie et al., 2012).

Food supplements and cosmetics are regulated under FFDCA by FDA, which can remove them from the market if they "present a significant or unreasonable risk of illness or injury" (US Congress, 1938). However, industries are not obliged to specifically report use of nano-formulations in their products (US FDA, 2007) and therefore the agency must solely rely on less effective voluntary reporting (Beaudrie et al., 2012), which results in difficulties to perform robust RA and justify potential removal of products from the market (US FDA, 2007).

The RCRA requires that before being "listed" a nanomaterial should be first identified as a hazardous substance (Hester, 2006; Mandel, 2008; US Congress, 1976a). Similarly, the Clean Water and Clean Air acts require "sufficient" data before NOAA can be classified as "pollutants" (US Congress, 1972c). The paucity of data for hazard analysis will likely prevent effective management of risks under the above regulations (Beaudrie et al., 2012).

OSHA has the authority to issue substance-specific standards such as Permissible Exposure Limits if significant potential harm from NOAA in occupational settings can be demonstrated (Balbus et al., 2007). However, with the available data, it would be very difficult to meet the statutory thresholds for regulation under the OSH Act (Lin, 2007).

2.4.3 Risk assessment and management challenges

Depending on the identity of a substance, different legal provisions apply under REACH, which has a direct effect on its deadline for submitting a registration dossier. For instance, information on those nanomaterials that are "phase-in" substances will be provided late by registrants due to "staggered registration deadlines". Of course this will impact their RA in the near term. In order to solve this issue, it has been suggested that all nanoscale materials should be registered, regardless of the volume in which they are manufactured or imported, as reduced information requirements should be applied, similar to those for the "exemptions" in the Process Orientated Research and Development scheme.

Products subject to "pre-market" risk analysis may also be subject to iterative re-assessment in the "post-market" phase given the emergence of new information and analytical techniques. FIFRA particularly requires that pesticides are repeatedly registered every 15 years to stimulate iterative risk reviews (US Congress, 1972b). In contrast, TSCA requires a single risk analysis performed within 90-days after submission of a Pre-Manufacture Notice (US Congress, 1976). The same holds true for the EU Novel Food and Cosmetic regulations, which require Product Safety Report only prior to market authorization. However, since nanoscale products are often novel, there are little or no real-world data on their use and end-of-life stages. Even when adequate data and tools become available, there are no automatic mechanisms I place to encourage iterative risk analysis.

While FIFRA and TSCA enable federal agencies to perform risk reviews along the entire life cycle, CPSA and FFDCA do not explicitly address the end-of-life stage (Beaudrie et al., 2012; US Congress, 1972a; Wu and Janssen, 2010). The existing US regulations rely heavily upon CAA, CWA and RCRA to manage potential environmental and end-of-life risks from consumer products, drugs, food additives, supplements, and cosmetics. These environmental laws are expected to provide adequate regulation of NOAA in cases where the risks can be clearly demonstrated (Hester, 2006). However, as noted above, they face challenges identifying NOAA as a pollutants or hazardous substance, and in many instances they may not be triggered at all.

2.4.4 Confidential Business Information and voluntary reporting

Under TSCA, manufacturers can claim data as "confidential business information" (CBI) in order to restrict them to review only by EPA (Jeffords et al., 2005). While designed to protect proprietary information, CBI claims are largely overused (Breggin et al., 2009; Jeffords et al., 2005; US EPA, 2011), severely limiting the availability of exiting information to other stakeholders, which constrains the development of new methods, analytics, and decision support tools for assessment and management nano risks (Morris et al., 2011).

REACH poses no explicit registration requirements for nano-specific tests or an obligation to declare substances in the nanoform. Instead, industries are expected to report this on voluntary basis. A preliminary evaluation by ECHA of the dossiers submitted to date reveals that some but not many nanoforms have been registered (NanoKommission, 2010; Quinn, 2011). Out of more than 26000 REACH registrations and 3.2 million CLP notifications (submitted before April 2011) for more than 4700 and 109000 respective substances, there are only 3 REACH registration dossiers and 14 CLP notifications for nanomaterials (Quinn, 2011). In this context, the REACH provisions appear inadequate to ensure consistent identification of NOAA and their uses (NanoKommission, 2010). Despite that it is currently being debated at the European level within the REACH Implementation Project on Nano (RIP-oN) 1 (JRC, 2011), this issue has not been resolved yet.

2.5 Solving the limitations in EU and US regulations

Several policies to reinforce the regulation of NOAA have been discussed in the last five years (Balbus et al., 2007; European Commission, 2004; 2008; US EPA, 2007) ranging from a ''laissez-faire'' attitude to an absolute moratorium on nanotechnology research, development and commercialization (Hansen, 2009). A few options are available to decision makers, including implementation of nano-specific regulations, voluntary programs and the "incremental" approach (Hansen, 2009).

Currently, implementing nano-specific regulations is deemed unfeasible in European context due to the difficulty to establish links between exiting pieces of EU and national legislation, which is a challenge for a sensible regulatory process (European Commission, 2004). Some governments started voluntary programs such as the Voluntary Reporting Scheme for Nanomaterials (VRSN) in UK and the NMSP in US (DEFRA, 2006a; b; 2008; Hansen and Tickner, 2007). Both programs engaged industry to submit existing information on nano production, hazard, exposure and risk (DEFRA, 2006a; US EPA, 2009), but did not ask for developing new data. VRSN received only eleven submissions by DEFRA, two from academia and nine from industry (DEFRA 2008). Similarly only 29 companies provided data for 123 nanomaterials under NMSP, and only 4 companies were willing to sponsor testing for production of new data. The question to what extend voluntary measures will be sufficient to deliver the information needed to perform regulatory risk analysis remains open. Key elements of a successful voluntary scheme include incentives for stakeholder participation, guidance and technical assistance, signed commitments and periodical reporting, quality of information, and transparency in design, reporting and evaluation (Hansen, 2009). Many of these aspects were not fully addressed in voluntary schemes applied to nanomaterials. Hansen and Tickner (2007) concluded that relying solely on the voluntary reporting schemes will not be sufficient to ensure the gathering and production of sufficient information for informed and proactive nano Risk management. The authors recommend that an increased effort is made by regulators to provide guidance on reporting for public recognition, and that any voluntary program on nanomaterials should be made mandatory after no more than three years, which would allow companies to adapt and develop methodologies for collecting and producing data, while increasing information exchange with regulators (Hansen and Tickner, 2007).

EU has adopted the "incremental" approach proposing nano-specific changes to the existing Cosmetic Products, the Food Information, the Novel Foods and Biocides regulations (section 2.2). The EC is working on adapting also the REACH and CLP regulations to nanoscale materials based on recommendations from the REACH Implementation Projects on Nano (RIP-oN) (Aitken et al., 2011; Hankin et al., 2011; JRC, 2011). In US, nano-specific legislation was proposed to reform TSCA (Lautenberg, 2011; Rush and Waxman, 2010), which aims to address many of the shortcomings described above, including minimum data requirements, strengthening authority to request more data, limiting CBI claims, improving stakeholder communication (Beaudrie et al., 2012). There are also proposals for defining risk-relevant physico-chemical properties (e.g. size distribution, shape and surface properties) as "special substance characteristics" (Lautenberg, 2011), which would give EPA greater latitude in defining whether a nanoform of an "existing" substance on the TSCA inventory constitutes a "new" substance and is therefore subject to a more profound risk review. Until changes to TSCA are adopted, the EPA has focused on developing an additional SNUR to require pre-marketing notification and a test rule to require post-marketing testing for NOAA (US EPA, 2012). Similarly, FFDCA reforms aim at full ingredient disclosure and improved data sharing between agencies when it comes to toxic materials (Schakowsky, 2011).

Based on the above analysis further recommendations on additional improvements can be proposed. One important aspect is to address challenges in environmental and end-of-life regulation. This would require development of new technologies for air and water monitoring of NOAA (55), and the adoption of adequate risk reduction approaches to handling nano wastes. Since environmental and end-of-life regulations currently do not sufficiently address nano risks, these stages must be anticipated and managed upstream at the premarket stage under high uncertainty (Beaudrie et al., 2012). However, the dynamic behaviour and fate of NOAA are very difficult to predict at early stage when real-world data is scarce. This significant challenge will require regulators to move away from a traditional "adversarial model" to a collaborative one in interactions with manufacturers, recyclers, and waste disposal companies in managing NOAA risks (Beaudrie et al., 2012). While enhancing current efforts to engage industry, governmental agencies should provide guidance for managing NOAA risks in the workplace and minimizing environmental releases to avoid harmful implications for human and environmental health. In addition, more risk-relevant data should be disclosed by industries to assist stakeholders in managing risks while enabling researchers to develop new analytical tools and decision support methodologies (Beaudrie et al., 2012).

Regulatory agencies and research-funding institutions should promote NOAA product stewardship and encourage proactive *top-down* Risk management. There is no doubt that bottom-up approaches are a key to nanotechnology governance. However, their integration for safety evaluation and decision making is difficult to achieve without well-structured top-down coordination. Top-down approaches provide clear and transparent methodology for combining information from disparate sources and the ability to clearly explain and quantify technical judgment and values. They help to generate and map empirical data and/or individual judgments into organized structures that can be linked with technical tools from risk analysis, modelling, monitoring, and cost estimation. Such an integration of bottom-up and top-down approaches results in an integrated decision-making framework, underpinned by the concept of *shared responsibility* in the oversight of health and environmental risks.

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CHAPTER 3

Theoretical foundations of human health Risk assessment and management

The Risk assessment (RA) is a central theme in the regulation of chemicals and their safety for human health and the environment and it is an important component in the scientific foundations of many national, European and international regulatory guidelines. The scope of a RA can range widely, depending on its intended purpose as well as the available data resources (Patton, 1993). Some assessments are retrospective, focusing on the effects of pollution incidents, while others seek to anticipate or predict probability of future harm to human health or the environment. Originally, analyses were primarily focused on human health risks; however accidents like the Sandoz disaster in Switzerland increased social and regulatory awareness of the environmental implications of large-scale contamination, which stimulated developments in the ecological RA field.

Because pollution does not recognize political borders the Risk management (RM) of chemicals has become an important issue on the international agenda. Chapter 19 of the United Nations Conference on Environment and Development (UNCED) Agenda 21 recommends the adoption of an international approach towards the governance of chemical risks, which requires mutual acceptance of RA methodologies. Following the implementation of Agenda 21, chemical risks were again a highlight of the 2002 World Summit on Sustainable Development in Johannesburg (United Nations, 2002). This has led to considerable activity in the RA/M area over the last few decades.

RA/M activities have mainly taken place in international bodies such as the Organization for Economic Co-operation and Development (OECD) (OECD, 1992a; b; 2010), the World Health Organization (WHO) [e.g. in the context of its International Programme on Chemical Safety (IPCS)], the European and Mediterranean Plant Protection Organization (EMPPO), the Council of Europe (EMPPO and Council of Europe, 1993), and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1992; 1993). A number of directives and regulations, where the RA plays an important role were released by the European Community (European Commission, 1976; 2008; European Parliament and the Council, 2006), the US Environmental Protection Agency (US Congress, 1976), and the Japanese Ministry of Health, Labour and Welfare (JMHLW, 1972).

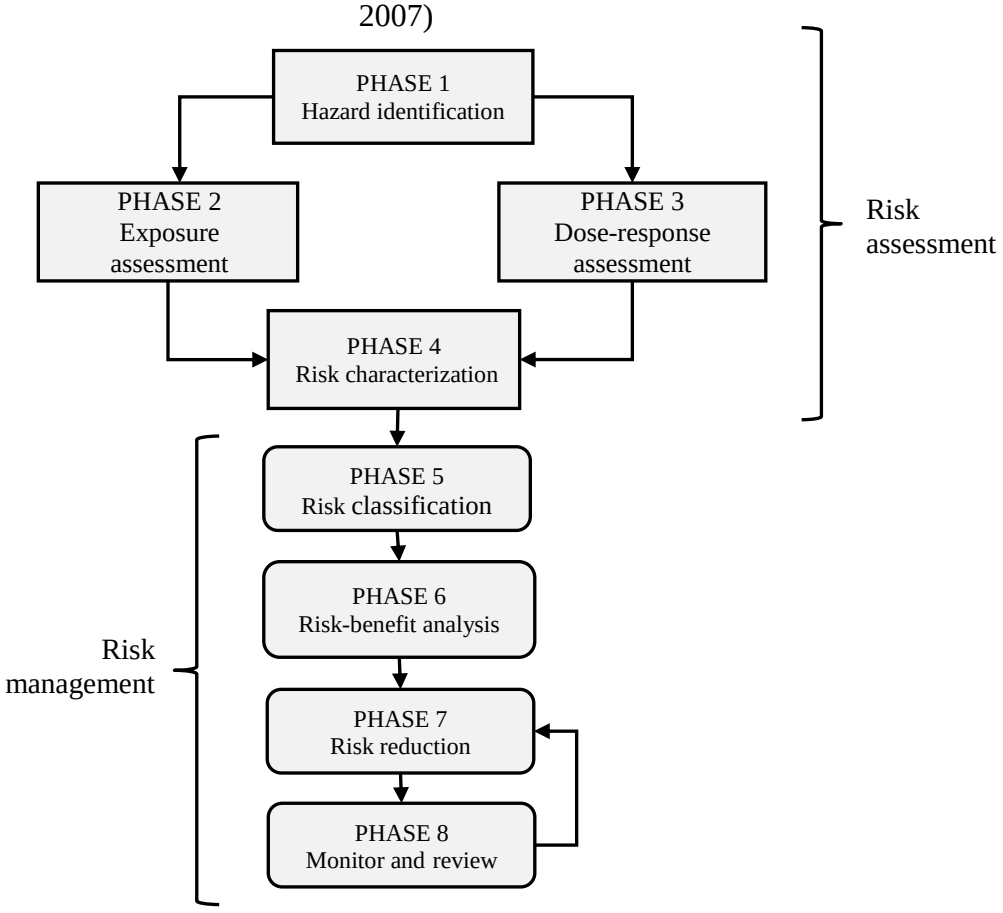
3.1 Risk management framework

This chapter is a general introduction to the Human Health Risk assessment and management framework, which aims to reflect the current regulatory practices in most countries with special focus on the European REACH (Registration, Evaluation, Authorization and restriction of Chemicals) Chemical Safety Assessment (CSA). The paradigm encompasses eight steps equally divided into two different, but closely

related processes: i.e. RA and RM (Figure 3-1). The RA can be seen as the objective, scientific component, which systematically combines scientific and regulatory principles to describe the health hazard associated with the human exposure to a chemical substance. RA is generally an iterative tiered approach, moving from screening assessments based on assumptions to more realistic and data-intensive conclusions. RM is a decision-making process based on the results of the RA, but also considering legal, political and socio-economic aspects to establish a risk reduction strategy (Van Leeuwen, 2007).

The following sections illustrate the eight steps of the framework on the basis of several documents, including: REACH Guidance on Information Requirements and Chemical Safety Assessment (European Chemicals Agency, 2007a; b; 2008a); US EPA's Risk assessment Guidelines of 1986 (US EPA, 1987); US EPA's Guidelines for Exposure assessment (US EPA, 1992); and US EPA's Risk assessment Guidance for Superfund (US EPA, 1989).

Figure 3-1: Human health Risk assessment and management framework (modified after Van Leeuwen, 2007)



3.1.1 Hazard identification

The first step is the Hazard identification (HI), which consists in gathering and evaluating information about the effects that a substance can cause and the exposure scenarios that can lead to injury or disease. HI often involves the characterization of the behaviour of a chemical within the organism and its interactions

with organs and cells, which includes the establishment of relationships between the observed biological responses and the physico-chemical properties of the substances (European Chemicals Agency, 2007a).

The principal question HI tries to answer is whether the existing evidence base suggests a potential risk for the human health. The hazard is the intrinsic property of a chemical to cause harm. It is the likelihood of impairment due to exposure that distinguishes risk from hazard. A toxic chemical that is hazardous to health does not constitute risk unless humans are exposed to it. Therefore *hazard* can be seen as *potential risk*. Once it has been identified, a number of other steps become important, i.e. Exposure Assessment, Dose-response assessment and Risk characterisation.

The HI should generally start with collection of all available physico-chemical and toxicological information that is relevant for the RA (US EPA, 1987), as the assessor should take into account what specific information is required by the regulator on a given endpoint. These data should include both human (i.e. epidemiological or clinical trial) and non-human (i.e. *in vivo* and *in vitro*) results or, alternatively, information from *in silico* (e.g. QSAR) or *read-across* studies. Specific tonnage-based information requirements are provided in Annexes VII-X of the EU REACH Regulation, while under the US Toxic Substances Control Act (TSCA), data requirements are reported in Section 8(e) (US Congress, 1976). Data may be obtained from a variety of sources, including on-line databases, open-source literature or industry surveys.

The collected dataset should then be evaluated with regard to quality and completeness. Data quality criteria include *adequacy*, *reliability* and *relevance*. These terms were defined by Klimisch et al. (1997) along the following lines:

- **Reliability** - evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings;
- **Relevance** - covering the extent to which data and tests are appropriate for a particular Hazard identification or Risk characterisation; and
- **Adequacy** - defining the usefulness of data for Hazard/Risk assessment purposes.

The reliability of the data is a key consideration, which can be done relatively quickly to filter out unreliable studies (OECD, 2004). Two approaches for assessing data reliability have been proposed. One approach is that developed by Klimisch et al. (1997) as a scoring system, which is particularly tailored to (eco)toxicity studies, but may be extended also to physico-chemical and environmental fate studies. The other approach was developed in 1998 as part of the US EPA High Production Volume (HPV) Challenge Programme (http://www.epa.gov/hpv/index.htm). In fact, the data reliability criteria presented by Klimisch et al. (1997) and by EPA (in Tier 1) are quite similar. The main difference between the two approaches is in how the criteria are used.

REACH recommends using the Klimisch scoring system, which evaluates the quality of toxicological data, considering the applied test methods, the clarity and comprehensiveness of their description and the plausibility results (European Chemicals Agency, 2007a). On this basis a reliability category is assigned to each datum: i.e. (1) reliable without restriction, (2) reliable with restrictions, (3) not reliable, and (4) not assignable (Klimisch et al., 1997).

The studies that have passed the initial reliability screen should be evaluated in terms of relevance and adequacy. In this step the use of sound scientific judgment is essential (OECD, 2004). Guidance of how to evaluate these two criteria is provided in the REACH Guidelines and in the EPA's Guidance for Developing Robust Study Summaries for SIDS Dossiers (http://www.epa.gov/hpv/pubs/general/robsumgd.htm).

The REACH guidelines recommend using a Weight of evidence (WoE) approach to assess the quality of the available dataset (European Chemicals Agency, 2007a). The WoE concept is not a scientifically well-defined or agreed term. It is discussed in more detail in the following section 3.2, where a classification framework of WoE methods is provided. In the context of data evaluation the WoE involves assessing the relevance, reliability and adequacy of each datum in order to reach a conclusion on its intrinsic hazard. This process always involves expert judgment and it is important to document its application in a robust and transparent manner.

The outcomes form the HI can be used for Classification and Labelling (C&L) as recommended by REACH. In fact, the C&L can be seen as a Risk management approach, which directly uses the results of the HI. Once all the physico-chemical and (eco)toxicological characteristics that may pose risk during normal handling of a substance have been identified, the preparation must be labelled (European Commission, 1967; 2008). The C&L should be based on a set of well-defined criteria, which can be seen as guidelines intended to supplement, but not substitute expert knowledge, sound clinical judgement or previous experience with the compound (Vermeire et al., 2007). Some examples of international C&L systems are:

- The Global Harmonised System (GHS) for C&L (United Nations, 2003);
- The EC classification, packaging and labelling requirements for substances and mixtures (European Commission, 2008);
- The WHO guidelines to classification of pesticides (World Health Organization, 2005).

All differences among the systems are solely due to variations in evaluation criteria.

3.1.2 Exposure assessment

The HI is typically followed by Exposure assessment (EA), which is concerned with the estimation of the doses, which human populations are or may be exposed to. An EA would start with the formulation of one or more exposure scenarios (ES), describing how a substance is used during its lifecycle by workers

and/or consumers. It is important to highlight that there are two fundamentally different definitions of the term *exposure scenario*: one by OECD/IPCS and one used in REACH. According to OECD/IPCS an ES is a combination of facts, inferences and assumptions that define a discrete situation where potential for exposure may arise (OECD, 2003). In contrast, the REACH definition encompasses an integral risk reduction strategy, including recommended Operational Conditions (OC) and Risk management measures (RMM) under which the risks arising from the uses of the compound in the ES are fully controlled (European Chemicals Agency, 2007). In both cases, the ES formulation is followed by (or includes) estimation of exposure by either direct measurements or by the application of models. This involves for instance monitoring of indoor concentrations or the estimation of the amount of the substance coming into contact with the respiratory system, skin or intestinal tract, which is referred to as external exposure. The internal exposure or uptake is the quantity of a substance that has passed the above receptors and entered the systemic circulation. It can be estimated by means of physiologically-based pharmacokinetic-dynamic (PBPK-PD) models designed to assess the fraction of the external dose which has been absorbed, or in other words the bioavailability of the chemical.

3.1.3 Dose-response assessment

The next step is the Dose-response assessment (DRA), which is intended to quantitatively characterize the relationship between the dose of a substance and the incidence of adverse health effects in the exposed population. Data are typically obtained from (quantitative) structure-activity relationships [(Q)SAR], read-across, *in vitro*, *in vivo* and/or epidemiologic studies (European Chemicals Agency, 2008a). Different exposure routes (e.g. inhalation, dermal contact and ingestion) and dose levels are considered, and the behaviour of the material in the target organs is analysed. The outcome of the DRA is the identification of a safe dose under which adverse effects are not likely to occur in test animals. This dose is typically called a Point of departure (PoD) or a Reference point (RP). Generally, one can discriminate between threshold toxic effects, which are not expressed below a certain dose threshold, and non-threshold effects.

Dose-response evaluation for threshold effects

Historically, the NOAEL (No Observed Adverse Effect Level) approach has been the standard for threshold dose-response modelling. It is defined as the highest dose at which no (adverse) effects were observed in the test animals (US EPA, 1989), while the lowest dose that statistically significantly differs from the negative control is the LOAEL (Lowest Observed Adverse Effect Level). For each endpoint, the dose below the LOAEL is the endpoint-specific NOAEL. The lowest NOAEL over all endpoints in the study is the overall NOAEL for that study, while the lowest of the NOAEL of the available studies is the chemical-specific NOAEL. The endpoint and study associated with the NOAEL for the chemical are referred to as *critical study* and *critical endpoint*, respectively (Vermeire et al., 2007).

The NOAEL can be anywhere between zero and the detectable effect size. However, in practice, this point is typically overlooked, and the NOAEL is simply considered as a dose where the effect has been shown to be zero. This is a major disadvantage of the approach, since in some cases the detectable effect size is not negligible, and biologically significant effects cannot be excluded (Vermeire et al., 2007). Some other important flaws in the NOAEL method are briefly summarized below.

The detectable effect size of a study depends on the number of used test animals, which also influences the value of the NOAEL. In practice the NOAEL tends to be higher when fewer animals are used, which is controversial, since less data points would add uncertainty, which should normally be paired with a conservative approach (Hoffman and Hammonds, 1994). Furthermore, the NOAEL can only be one of the applied doses, which implies that the NOAEL strongly depends on the choice of dose levels and number of animals per dose. Therefore, by changing the study design the value of the NOAEL is likely to change as well. The uncertainty in each NOAEL value may be large, but it cannot be assessed, which is another disadvantage of the approach (Hoffman and Hammonds, 1994).

Given the disadvantages of the NOAEL approach, an alternative Benchmark Dose (BMD) method for deriving a PoD has been proposed by Crump (1984). The BMD is defined as a dose level that is associated with a pre-defined change in response (i.e. benchmark response) compared with the control (Crump, 1984). The BMD is estimated from toxicity data by fitting a dose-response model to the observations. To take the uncertainties arising from experimental errors into account, the lower confidence limit of the BMD (i.e. the BMDL) is normally used as the PoD. While the BMD approach was originally intended to substitute the NOAEL only for threshold effects, it can be applied equally well for non-threshold effects as explained below.

Dose-response evaluation for non-threshold effects

For some endpoints non-threshold effects are assumed. For instance it is considered that carcinogens can act by a genotoxic mechanism, which implies that, theoretically, each molecule of a potentially carcinogenic substance could give rise to a malignant cell. This shows that the onset of tumours is stochastic in nature and it cannot be predicted. In this case decreasing the dose will simply decrease probability of tumour occurrence. However a dose-threshold below which tumours would not appear at all is implausible.

Due to the lack of a dose-threshold the NOAEL approach cannot be used for genotoxic carcinogens. Because it is assumed that the risk in this case would never be zero at any dose, a RA of genotoxic carcinogens typically determines a dose where the probability of impairment is acceptably small, e.g. one in a million (10-6) over a lifetime (Vermeire et al., 2007). A critical problem of defining this level is that most carcinogenicity data originate from animal studies, where relatively small groups of test animals were used, e.g. 10-100 animals per dose. Therefore, the acceptable risk level is far below the range of observation, which in animal studies would typically be in the order one in ten (10-1), which is five orders of magnitude

higher than an acceptable risk of 10^{-6}. Establish a dose associated with a risk five orders of magnitude lower that the range of observation requires the so called *low-dose* extrapolation.

The low-dose extrapolation is addressed differently in different countries. Some practitioners consider this type of extrapolation impossible and instead they apply the "as low as reasonably achievable" (ALARA) principle (Environmental Services Group, 2009). A major weakness of the ALARA approach is that it treats all genotoxic carcinogens as exactly the same, even when there is evidence that some compounds are more concerning than others. Another approach is the US virtually safe dose (VSD), which fits a linearized multi-stage (LMS) dose-response model to the tumour incidence data, and uses the fitted curve to estimate the dose associated with a default low risk level (typically 10-6) (Gaylor and Gold, 1995). The VSD approach is now increasingly recognized as an unwarranted extrapolation method (Vermeire et al., 2007). Currently, there is a global tendency towards applying the BMD approach for dose-response modelling of non-threshold effects.

The BMD approach is used to fit a dose-response model to the dataset, which is then used to estimate a dose associated with a response (typically 10%) within the observation range (i.e. the so called the BMD10), and its lower confidence limit (i.e. the BMDL10). Of course, a 10% cancer incidence is far above the acceptable level for humans. For this reason, in this case the BMDL10 is considered as a "PoD for further evaluation". In current practice there are two ways to proceed with further evaluation (Vermeire et al., 2007):

- Linear extrapolation, assuming that the tumour probability is proportional to the dose in the low-dose region.
- The Margin of exposure (MoE) approach, where the estimated human exposure is divided by the PoD to obtain the interval between the actual exposure concentration and the dose with known effect level.

The MoE can be used for relative risk ranking of compounds, as the higher the MoE is the lower the concern is (O'Brien et al., 2006). Important considerations about the MoE approach are the following.

- MoE values are not absolute measures of risk. Therefore, it should be communicated in terms of *concern* rather than risk.
- MoE values are not necessarily directly comparable. Incomparable PoD and/or exposure estimates can lead to incomparable MoE.
- The application of the approach should always involve characterization of the uncertainties in the PoD and the exposure estimates used to derive the MoE.

Deriving a human health-based limit value

Obtaining a NOAEL or a BMDL for a particular substance is the starting point in the process of deriving a human health-based limit value. An example of such a value is the REACH Derived No-effect Level (DNEL), which is defined as the exposure level above which humans should not be exposed (European Parliament and the Council, 2006). PoD themselves do not account for the uncertainty and variability associated with differences in sensitivity between laboratory animals and humans, exposure routes and intraspecies variations. They need to be *corrected* and extrapolated by applying assessment (or uncertainty) factors (AF), usually in the 10-10000 range (European Chemicals Agency, 2008a; European Commission, 2003).

Correcting the PoD means converting it into a *starting point*, directly comparable with exposure data for RA (European Chemicals Agency, 2008a). The next step is extrapolation to the human situation.

The most important aspects to consider in the extrapolation process are:

- intraspecies differences;
- interspecies differences;
- differences in exposure duration;
- quality of the database.

For each of the above uncertainties an AF is defined, which is either default or based on experimental data. All AF are multiplied to obtain an overall AF, which is then multiplied by the "corrected" PoD to obtain a DNEL.

The DNEL should generally be expressed as external exposure values to be easily comparable with exposure concentrations for RA purposes; and because local effects per definition cannot be expressed as internal values (European Chemicals Agency, 2008a). In fact, the DNEL may also be expressed as internal biomarker doses, but this only applies to the limited number of substances where bio-monitoring data are available and have been reliably associated with effects (European Chemicals Agency, 2008a).

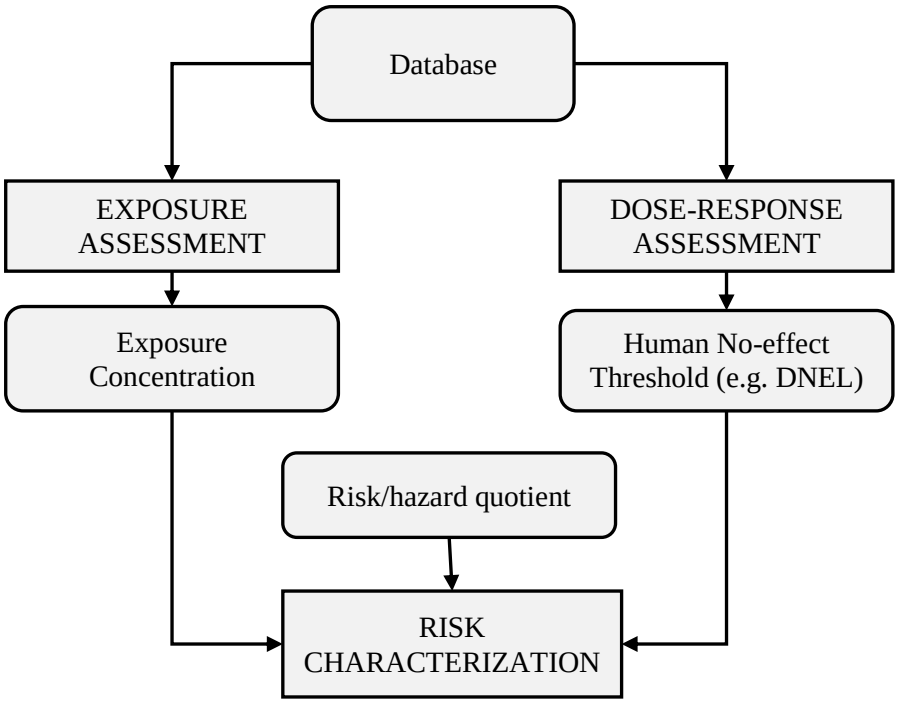
3.1.4 Risk characterization

Risk characterization (RC) is the final step of the RA (Figure 3-2), where Hazard Quotient (HQ) is calculated. HQ is the ratio of the exposure estimate to a human effect threshold value (e.g. DNEL). HQs may range from 0 to infinity, with values less than 1 considered indicative of acceptable risk.

The HQ approach is generally employed to assess risks from non-carcinogenic contaminants (i.e. threshold-effects), while for carcinogens (i.e. non threshold-effects) slope factors (SF) are typically used. A SF is the 95% upper bound of the increase in cancer risk from a lifetime exposure via inhalation or ingestion (Committee on Risk Assessment of Hazardous Air Pollutants et al., 1994; US EPA, 1987). SF are usually

expressed in units of proportion (of a population) affected per mg of substance per kilogram of body weight per day. They are generally derived from the low-dose region of the dose-response relationship, i.e. for exposures corresponding to risks of less than 1 in 100 (Committee on Risk Assessment of Hazardous Air Pollutants et al., 1994).

Figure 3-2: A schematic illustration of the Risk characterization procedure. DNEL=Derived No-Effect Level.



3.1.5 Risk classification

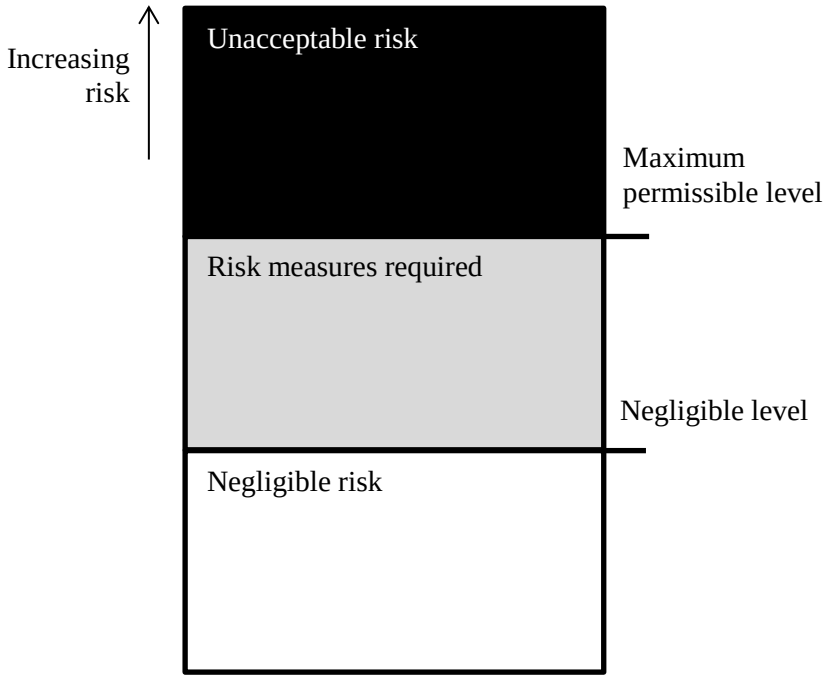
The RC is followed by the Risk Classification (RC*), which is the first step of the RM process, which consists in the valuation of risks in order to decide if risk reduction measures are required. It is a complex process of determining the significance or value of the estimated risks to those concerned with or affected by the decision (Van Leeuwen and Vermeire, 2007). Risks cannot be evaluated only from scientific perspective, since scientists cannot alone decide what the socially acceptable risk level is. Decisions about RC* are strongly linked to "risk acceptance" and must always be taken by policymakers, who should consider the opinion of all involved stakeholders (Bro-Rasmussen, 1988). Although defining acceptable risk levels requires scientific knowledge, it also needs an appreciation of the limits of that knowledge, good understanding of the context of the risk and willingness for an open and transparent discussion. Risk acceptance varies with time and place. What was acceptable in the past may not be acceptable in the future. What may be acceptable in one country or culture may be completely unacceptable in another. Cultural differences have significant impact on Risk management approaches in national regulatory frameworks. In short, since risk classification is related to risk acceptability, it becomes a technical, social, cultural, political, educational and economic issue (Van Leeuwen, 2007).

Over the past years there has been a debate on risk acceptability, which led to defining two widely agreed risk levels:

- Maximum Permissible Level (MPL); and
- Negligible Level (NL).

These two limits map three risk zones: a black (high risk), a grey (medium risk) and a white (low risk) zone as represented by Figure 3-3 (Jorgensen, 2010). Risks in the black zone above the MPL are unacceptable and further RMM are necessary. In the grey zone, risk reduction is required on the basis of the ALARA principle, which is a powerful Risk management framework (Jorgensen, 2010; Van Leeuwen, 2007). In this case risk managers are asked to reduce risks up to a limit they can justify to the regulatory authorities. This limit should generally balance the cost of the RMM and the expected benefits. Because risks in the white zone (below the NL) are negligible no further RMM are strictly required (Jorgensen, 2010).

Figure 3-3: Risk limits and risk reduction (Van Leeuwen, 2007).



3.1.6 Risk-benefit analysis

Once RC* has identified the need for RMM, the next step is to select the most suitable options for risk reduction. Such options span from slight adaptations of the manufacturing process or the use of the chemical to a complete ban and removal of the substance from the market. In order to identify the best risk reduction strategy, a risk-benefit analysis is carried out that looks at the trade-off between the respective risks and benefits of a given set of measures as compared to the situation of not imposing any measures at all.

The Risk-Benefit Analysis (RBA) is considered the most difficult step in the RM process, where the risk manager has to consider many important aspects, such as the technical feasibility, social and economic implications, ethical and cultural values, legislative and political factors, as well as the scientific aspects of the proposed measures (Van Leeuwen, 2007).

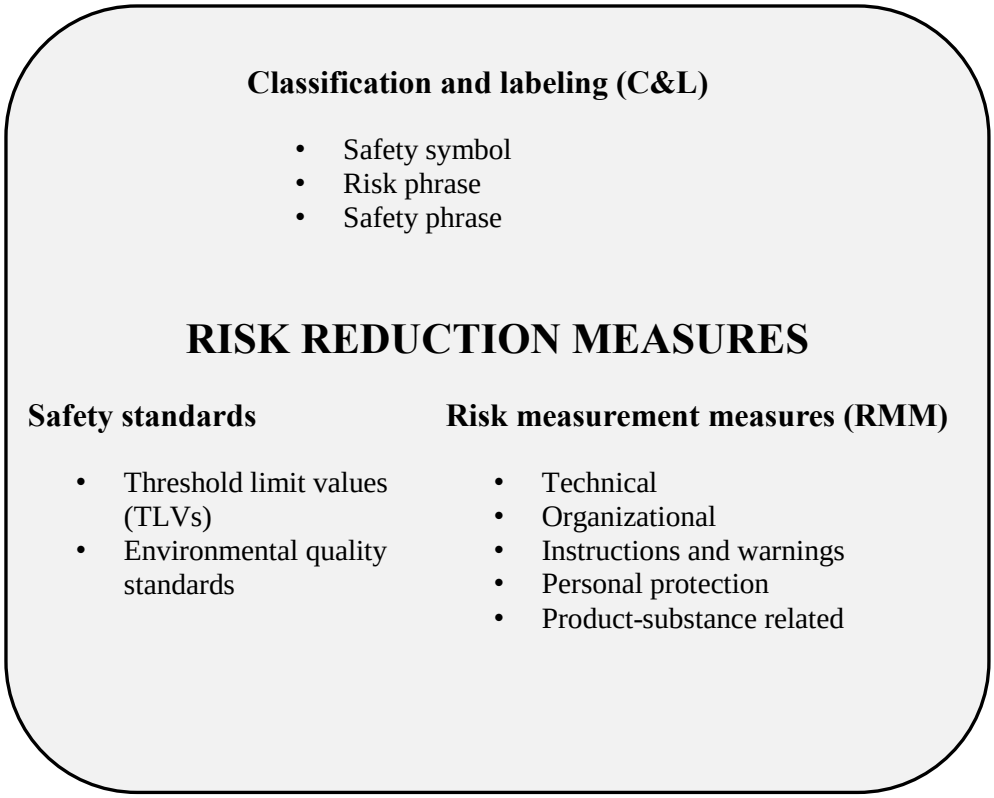
In selecting RMM discussions about risk acceptability are still relevant, that would focus on the anticipated consequences of risk reduction measures. This discussion requires risk communication, i.e. the process by which all involved stakeholders discuss the potential consequences with one another. Because different stakeholders often perceive risks differently, a delicate approach towards risk communication is often required, which would stimulate a genuine dialogue (US EPA, 2007).

The RBA often involves a cost-benefit analysis, where the net benefits and the net costs to society of applying the proposed risk reduction measures are estimated. In order to assess benefit in absolute terms, it is necessary to assign a numerical value to the avoided risk (e.g. saved human lives, extended lifetime). The general philosophy is that the magnitude of the risk is proportional to the incentive to reduce it. RBA looks at cost-effectiveness, which refers in this context to the selection of actions, which would maximize the level of risk reduction per unit cost (Kopp et al., 1997). However, human health and environmental risks are very difficult to quantify in monetary terms. Therefore one can conclude that even though cost-benefit analyses are useful in prioritizing risk reduction investments in terms of cost-effectiveness, this approach can only be a guideline, and simply another input into a decision (Viamonte et al., 2006).

3.1.7 Risk reduction

The purpose of risk reduction measures is to protect humans from the identified risks. In addition to the above considerations, a number of factors should be taken into account before a Risk management decision can be taken. They include, but are not restricted to: effectiveness, practicality, monitorability, equity, administrative simplicity, consistency, and public acceptability (Van Leeuwen, 2007). There are a wide variety of options, which can be classified in three groups, i.e. C&L, Safety standards, and RMM (Figure 3-4). In this section they are only briefly summarized.

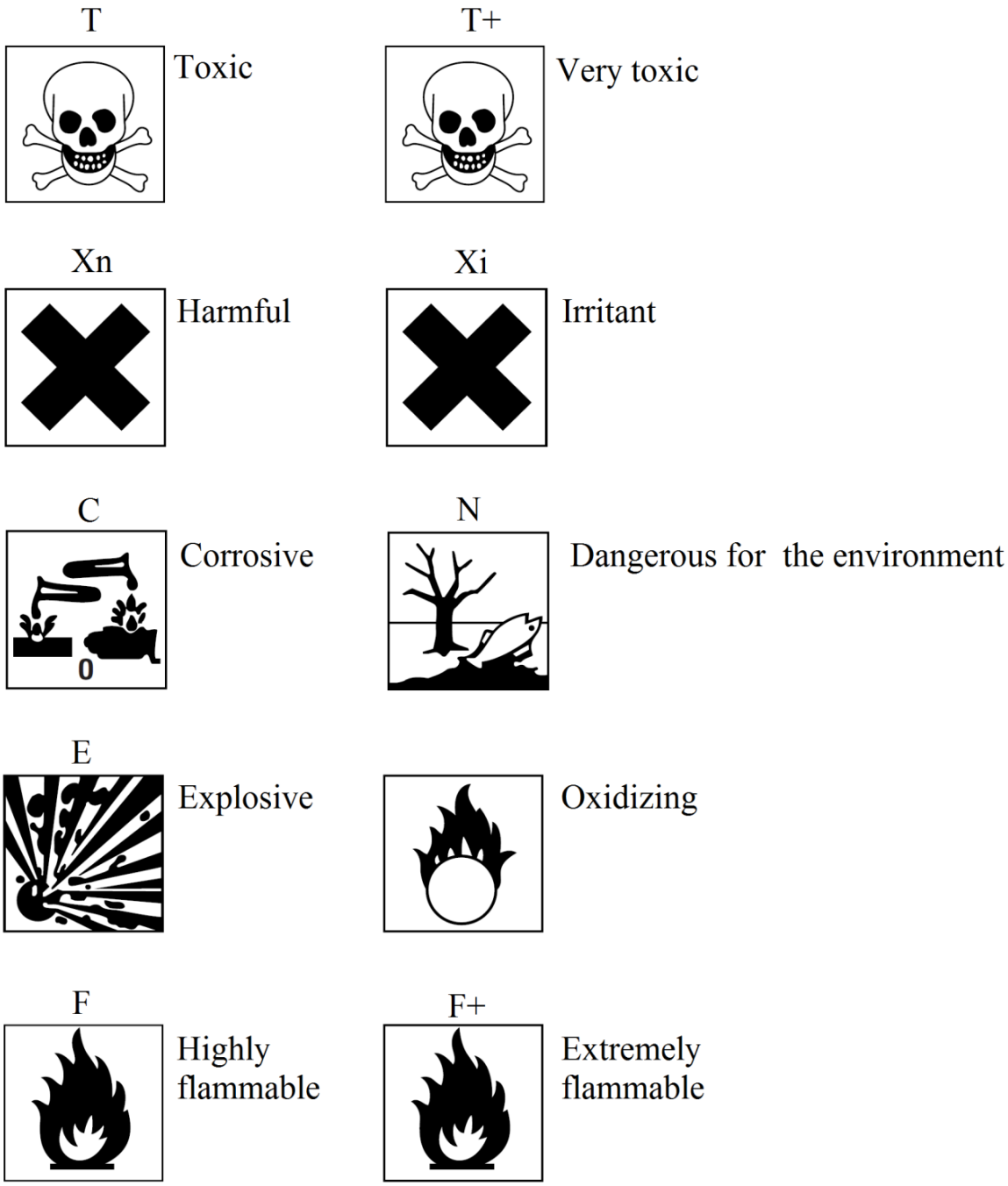
Figure 3-4: Classification of risk reduction measures.



Classification and labelling (C&L)

A more detailed description of the C&L concept and approach is given in section 3.1.1. In summary, a notification system is required to provisionally classify and label dangerous substances on the basis of their intrinsic properties. Decisions on how to classify and label a chemical are taken on the basis of a series of criteria which are based on the results of standard laboratory tests. The C&L includes assigning a Symbol, a Risk phrase and a Safety phrase (Figure 3-5) (European Commission, 1992; 2008; United Nations, 2005).

Figure 3-5: Some symbols, risk and safety phrases for Classification and Labelling.



Safety standards

Safety or quality standards are an important approach to control chemicals, thus protecting human health and the environment. Standards are fixed upper exposure limits that are laid down in enforceable laws or regulations. Therefore, they are legislative provisions, which are no more suggestive, but are legally binding.

Examples of such standards are the environmental (i.e. air, soil and water) quality standards, the Threshold Limit Values for workplace airborne concentrations as well as the Acceptable Daily Intake (ADI) and the Occupational Exposure Limits. All of them indicate the control levels at which exposure leads to

acceptable risk levels. Chemical safety standards are derived from criteria, often by applying safety factors. The ADI, for instance, is derived by applying an uncertainty factor to no observed effect levels (NOELs) derived from toxicological studies. It refers to the daily exposure dose that is unlikely to cause any adverse effects even if the individual is exposed to a chemical over his/her lifetime.

Risk management measures

RMM may include (European Chemicals Agency, 2005; 2008) the following.

- Technical measures such as use closed systems, exhaust ventilation, clarification techniques, physical, chemical and biological treatment, redesign of production and use processes.
- Organizational measures such as controlling exposure duration and frequency, training, monitoring and surveillance, prohibiting eating, drinking and smoking at the site of activity.
- Safe use instructions, information and warnings, including for instance C&L.
- Restrictions and/or instructions to limit the use of a substance or product by limiting certain applications and uses.
- Personal protection equipment such as filter masks, gloves, goggles and protective clothing.
- Product-substance related measures such as limiting the concentration of a substance in a formulation or article.

RMM normally target at reducing or eliminating exposure. The effectiveness of RMM can markedly vary depending on the expertise of the RMM user to install and apply technical measures (European Chemicals Agency, 2008). Organizational measures, such as management systems, training schemes, operating practices and monitoring, and Risk management equipment can contribute to higher effectiveness of the RMM setup.

3.1.8 Monitoring and review

Monitoring and review is the final step in the RM process. Monitoring is the process of repetitive observation of one or more physical, chemical or biological parameters according to a pre-determined plan, and preferably using standardised methods producing comparable results (Van Leeuwen, 2007).

Monitoring has a control function, ensuring that safety standards are being met. In addition to this, it serves the purposes listed below (De Zwart, 1994).

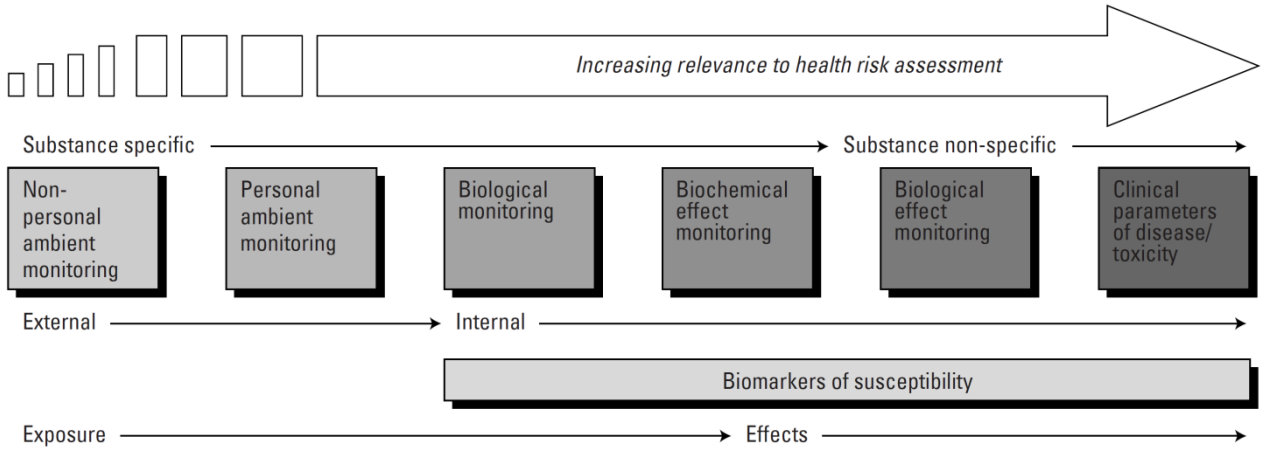
- Verify the effectiveness of risk reduction (control) strategies and check for compliance.
- Detect sudden (adverse) changes in the human health or the environment.
- Enable the prediction of future developments based on time series analysis.

- Help in the recognition and clarification of underlying processes.

In health RM biomonitoring is part of the exposure-disease continuum depicted in Figure 3-6 (ECETOC, 2005). Biological and biochemical effect monitoring are important to understand the toxicokinetics of chemicals and their potential adverse health effects. Both methods give a measure of the total actual exposure, regardless of the exposure route, and in this sense they should be regarded as exposure monitoring tools with high substance specificity (ECETOC, 2005). Typical examples of biological monitoring are the determination of chemicals or their metabolites in blood or urine or volatile compounds in exhaled breath (Van Leeuwen, 2007). Biochemical effect monitoring includes for instance the determination or increased or decreased levels of specific enzyme activities (Van Leeuwen, 2007).

Besides monitoring, some other ways to review health management measures include: audits and inspections, market investigations, product registers, technology assessments, performance measurements and indicators for human health and sustainable development (European Environment Agency, 1999; US EPA, 2004; United Nations Environment Programme, 1995; World Health Organization, 2004). All these tools are important to ensure sustainable production, use and disposal of chemicals.

Figure 3-6: Monitoring techniques as part of the ECETOC exposure-disease continuum (ECETOC, 2005).



3.1.9 Estimation of uncertainty and variability

Risk management decisions should be informed not only by the available scientific knowledge, but also by information about uncertainties and lacunae both in the knowledge base and in the models and tools used for RA. One should distinguish between uncertainty and variability. Uncertainty is often based on knowledge or data gaps and it can be reduced by obtaining or generating more information. Variability, often named “aleatory uncertainty”, is a natural phenomenon, referring to the quality and the degree of being variable or changeable. It cannot be reduced, however it influences the assessment results and therefore should be accounted for. Uncertainty can lead to inaccurate or biased estimates, whereas variability can affect the precision of the estimates and the degree to which they can be generalized (US EPA, 1997).

Uncertainty propagates through the whole RA process and may result from each of its steps. It can be associated with insufficient knowledge about relevant mechanisms of toxicity or exposure scenarios, as well as to the structure of a model and its variables. Epistemic uncertainties are the most common type and they are related to ignorance, which can be divided into reducible and irreducible. Reducible ignorance may be resolved by conducting further research to facilitate a better understanding. Irreducible ignorance (often called "indeterminacy") applies when research cannot produce sufficient knowledge about essential relationships (Walker et al., 2005). The nature and extent of the characterization of uncertainties normally depends on the objective of the Risk assessment and the form of its output.

In the context of human health RA, US EPA proposes three broad categories for classifying uncertainty: (1) parameter, (2) model, and (3) scenario uncertainty (US EPA, 1997; 2001).

- Parameter is the uncertainty in the estimate of an input variable to the risk model. It may stem from imprecise or biased measurements, sampling errors, natural variability or use of surrogate data.
- Model uncertainty refers to the structure of a model and its application in a specific context. The simplification of reality, which is inherent in the modelling process, can lead to errors. Inadequacies of models include lack of knowledge about underlying mechanisms, failure to extrapolate beyond the range of observation, or instability of parameter estimates. In this context, two related types of uncertainties can be spelled out: quantifiable (the "known unknowns") and undefined (the "unknown unknowns" that cannot be described or quantified) (Van Leeuwen, 2007).
- Scenario uncertainty relates to missing or incomplete information, necessary to fully characterize exposure and dose (US EPA, 1997). Its typical sources include descriptive errors (concerning the magnitude and extent of chemical exposure or toxicity), aggregation errors derived by approximations (e.g. homogeneous population, steady-state conditions), errors in expert judgment or incomplete analysis (e.g. missing exposure pathways).

Uncertainty can be characterized in either qualitative or quantitative manner. The qualitative approach typically addresses unknowns in the analytical outcomes and conclusions of the Risk assessment (WHO, 2006). Often a scale ranging from "low" to "high" can be used to assess the sensitivity of the Risk assessment outputs to parameter or model uncertainties. A good example of a "low" level of uncertainty is the measurement uncertainty associated with parameters, stemming from the fact that a measurement can practically never precisely represent the "true" value of what is being measured.

Quantitative Uncertainty characterization includes deterministic and probabilistic approaches.

Deterministic approaches

RA usually uses point dose or exposure estimates based on "worst case" scenarios (US-EPA, 2001). The worst-case paradigm is aimed to ensure that even the most sensitive component of the population is

protected. Approaches commonly used are the 90th percentile or maximum of concentration data, the selection of worst-case consumption level, the use of safety factors for incorporating uncertainty due to extrapolations (Sioen et al., 2007).

Probabilistic approaches

Probabilistic approaches use probability density functions to characterize uncertainty and variability in input data (US EPA, 2001). They propagate variability and uncertainty through the RA process and represent the outputs as probability distributions, which gives more complete information compared to point estimates. Commonly used statistical approaches are the Monte Carlo and Latin Hypercube sampling (WHO, 2006). They can be used to estimate risk for different percentiles of exposed populations and quantify variability only, uncertainty only, variability and uncertainty together, or variability and uncertainty distinguished (WHO, 2006).

3.2 Weight of evidence

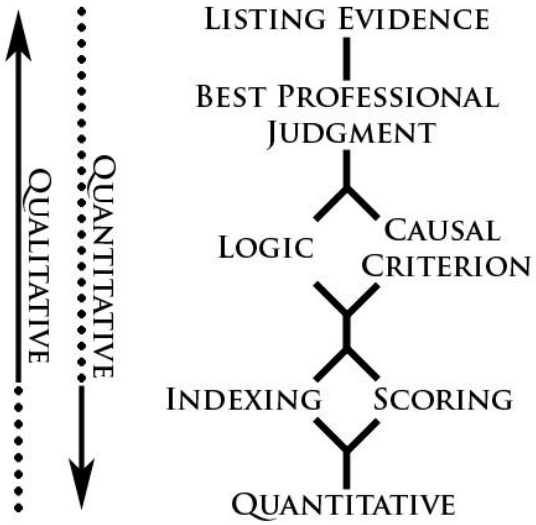
One approach commonly used in Health and Safety decision making is the Weight of evidence (WoE). The Massachusetts Weight of evidence Workgroup (1995) defined it as a "…process by which measurement endpoints are related to an assessment endpoint to evaluate whether a significant risk of harm is posed…" (MWW, 1995). An assessment endpoint is an environmental or health value, which needs to be protected, while the measurement endpoints are Lines of Evidence (LoE) used to evaluate the assessment endpoint. LoE are sets of comparable information that pertain to a significant environmental or health aspects (Smith et al., 2002). WoE can be seen as a decision framework to systematically combine individual (qualitative or quantitative) LoE into objective conclusions.

The WoE framework has been applied to a variety of health-related problems, including selection of Risk management criteria, benchmarks, and permit levels (Linkov et al., 2011). Health risk analyses used WoE to estimate carcinogenicity, neurotoxicity, and general health hazards associated with exposure to chemical or biological stressors (Linkov et al., 2011). In a few cases, a policy guidance exists that describes a specific WoE process for LoE integration (US EPA, 1998), but in most instances there is latitude for incorporating relevant information. Hence, a diverse set of WoE techniques have been developed on a case-to-case basis with WoE methods applied only to a single particular application, without a generalization of the methodology across multiple fields (Linkov et al., 2011). In this context it should be stressed that there is no standard approach or a general guideline describing how a WoE process should be conducted (Burton et al., 2002).

Linkov et al (2009) proposed a conceptual framework for categorization of WoE methodologies into quantitative and qualitative ones (Figure 3-7) (Linkov et al., 2009; Linkov et al., 2011). As one moves from Listing Evidence to Quantitative the attributes of the previous methods are incorporated into the next. The

most basic qualitative integration occurs through Listing Evidence where all evidence is made explicit and readers are allowed to make their personal judgments (King and Richardson, 2003). The Best Professional Judgment goes a step further by providing, in addition to the evidence, also its informed interpretation (Hawkyard and Koerner, 2007; Staples et al., 2004). Logic methods place LoE in structured frameworks to reach dichotomous conclusions (Chapman, 2007; Roberts et al., 2002). Causal Criterion follows a similar method, but seeks to identify cause-effect relationships (Burkhardt-Holm and Scheurer, 2007). Scoring and Indexing normalize LoE to numerical values for interpretation (Coo and Aronson, 2004; Feron et al., 2004; Hertzberg and Teuschler, 2002; Landis et al., 2004). Fully Quantitative methods characterize problems numerically with statistical tools or Multi-criteria decision analysis (MCDA) (Good, 1991; Kiker et al., 2005; Linkov et al., 2009).

Figure 3-7: Categorization of Weight of evidence methods (Linkov et al., 2009).



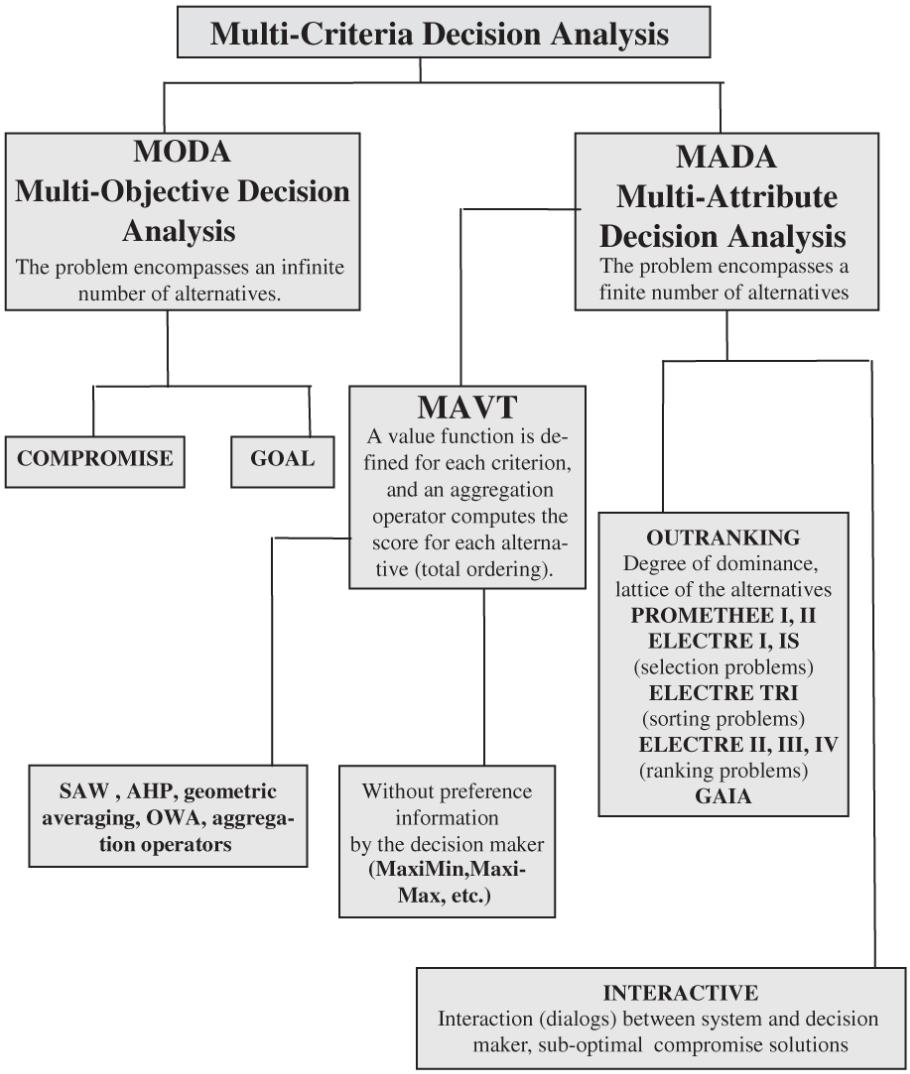
Qualitative methods are typically applied in situations where evidence is very limited and the assessment relies largely on expert judgment. Quantitative methods are useful when systems are complex and there are many types of data to consider. A typical example of quantitative WoE approaches is the family of MCDA methods.

3.3 Multi-criteria decision analysis

MCDA involves a large group of methods (Giove et al., 2009), designed to ensure that the synthesis of multiple sources of information is documented and directed towards a pre-defined goal (Linkov et al., 2011). Some important examples are MAUT/MAVT (Multi-Attribute Utility/Value Theory), Outranking, Interactive, Goal aspiration, AHP (Analytic Hierarchy Process), ELECTRE (Elimination and Choice Expressing Reality), PROMETHEE (Preference Ranking Organization Method for Enrichment Evaluations) and TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) (Giove et al., 2009; Linkov et al., 2011). A framework for categorization of MCDA methods is shown on Figure 3-8. The application of

these quantitative methods yields multiple benefits over qualitative approaches such as the ability to incorporate conflicting information, to facilitate trade-offs among competing alternatives, and to propagate uncertainty.

Figure 3-8: Categorization of Multi-criteria decision analysis methods (Giove et al., 2009).



Quantitative MCDA can also be built on other WoE methods, as the best combination depends on what is required by a particular application. For instance, MCDA can strengthen the Logic and Causal Criterion frameworks (Linkov et al., 2009). This creates synergy, which increases the standardization of logic criteria while preserving the expert judgment.

Indeed, the MCDA methods have the property to integrate the opinions of multiple experts, thus decreasing the bias of subjective judgments (Linkov et al., 2007; Linkov et al., 2011). In this context MCDA can be classified as single or multiple-person. The latter type is based on the Group Decision Theory and involves multiple experts or decision makers providing various perspectives on the decision problem to reach objective conclusions. In this case the MCDA algorithms have to include consensus measures showing how much the group of decision makers agree or disagree on the results (Carlsson et al., 1992).

3.4 Expert elicitation

Risk assessors are often required to make decisions in the presence of uncertainties. Because relevant data are frequently unavailable to characterize the uncertainties decisions often rely on expert judgment through informal or formal processes. Expert elicitation (EE) provides a formal process to obtain expert judgment. It represents the synthesis of opinions of experts on subjects, where insufficient or conflicting data prevent adequate decision making (Morgan, 2005). The goal of EE is to collect expert beliefs about relationships, quantities, events, or parameters of interest (US EPA, 2009) and represent them in a way to facilitate their interpretation (often probabilistically).

Subjectivity is inherent to collection and interpretation of data and may influence conclusions. EE is not different in this regard. However, because EE findings contain knowledge from data combined with probability judgments about that knowledge, the subjectivity is more obvious (US EPA, 2009). Therefore it is generally believed that formal EE processes are more objective than informal expert inquiries.

The way experts are selected and their judgments are elicited is very important and should be carefully designed. Several EE approaches have evolved such as Elicitation Protocol Design, Nominal Group Technique, Collective Judgement, Delphi methods, Team Building, and Decision Conferencing. While group processes have the advantage that they can often obtain consensus, they are potentially biased by the influence of group dynamics (e.g., strong and controlling personalities) (US EPA, 2009). Therefore, if group techniques are used, the effect of group dynamics must be accounted for in addition to other types of bias.

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CHAPTER 4

Risk assessment of engineered nano-objects and their aggregates and agglomerates: state of the art

Contents partially included in:

Aschberger K, Boraschi D, Bos P, Byrne H, Dahmann D, Gottardo S, Fernandes T, **Hristozov D**, Hund-Rinke K, von der Kammer F et al. (2012). Toxicity testing of engineered nanoparticles. Nanosafety Vision Group.

Hristozov D, Gottardo S, Marcomini A. (2012). Chapter 5: Risk assessment and related approaches. In: Identification of knowledge gaps and strategic priorities for human and environmental hazard, exposure and Risk assessment of engineered nanomaterials. Heriot-Watt University: Edinburgh.

and

Hristozov D, Gottardo S, Critto A, Marcomini A. (2012). Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 6: 880–898.

4.1 Limitations and uncertainties

Although the Risk assessment (RA) is a powerful approach, the analysis of its feasibility for nano-objects and their aggregates and agglomerates (NOAA) has identified substantial limitations and uncertainties (Hansen, 2009; Hristozov and Malsch, 2009; Stone et al., 2009), which are shortly summarized below.

Occupational and consumer exposure

Knowledge about the presence of nanoparticles in consumer products is growing (Nowack et al., 2012; Pasricha et al., 2012; Weir et al., 2012; Windler et al., 2012), and new studies have investigated consumer exposure to nanomaterials (Hansen et al., 2008; Nazarenko et al., 2012), but no empirical data on actual consumer exposure measurements are available. Empirical data on workplace exposure has been collected (e.g. in the European NANOSH and the French Nano-INNOV projects) and currently data are slowly emerging on emission characteristics and source strengths for different exposure scenarios in the production stage, such as powder handling (Schneider and Jensen, 2009), simulated sanding (Koponen et al., 2010; Vorbau et al., 2009), drilling and cutting of nanocomposites (Bello et al., 2009; Bello et al., 2010).

Most measurement devices are unable to discriminate NOAA from background nano aerosols (Aitken et al., 2011) or among different NOAA types (Ono-Ogasawara et al., 2009). Distinguishing background concentrations often requires combinations of several techniques, involving time-integrated sampling and

offline analysis such as near- to far- field or before- to after- task comparisons or calculations based on intrusion factors (Brouwer et al., 2011; Brouwer et al., 2009; Kuhlbusch et al., 2011); all of them time consuming and unable to integrate in portable personal devices for real-time surveillance.

Another critical aspect is the exposure sampling design. Regulations typically require personal full-shift measurements since most OEL[1] are based on 8-hour time-weighted average concentrations. In contrast, modelling requires activity-specific measurements in order to calculate daily exposure. Moreover, to facilitate effective modelling more contextual information on process and use rates, duration of activity, room-size and ventilation in needed.

REACH suggests a tiered Exposure assessment (EA) framework. Low-tier occupational exposure models are EASE (http://www.hse.gov.uk/research/rrpdf/rr136.pdf), Stoffenmanager 4.0 (https://www.stoffenmanager.nl/), and ECETOC TRA (http://www.ecetoc.org/tra). A low-tier consumer exposure model is CONSEXPO (http://www.rivm.nl/en/healthanddisease/productsafety/ConsExpo.jsp). In case that the above models provide unrealistic results, higher-tier modelling is required using for instance the occupational Advanced Reach Tool (http://www.advancedreachtool.com/). The Seventh Framework Programme (FP7) NANEX project (http://nanex-project.eu/) evaluated the feasibility of applying the above models to nanomaterials and concluded that in their current form all are improperly calibrated (Clark et al., 2010).

Early nano-specific models for consumer (Hansen et al., 2011) and occupational exposure (Duuren-Stuurman et al., 2011; Genaidy et al., 2009; Giacobbe et al., 2009; Jensen et al., in peparation; Paik et al., 2008) are based on qualitative Weight of evidence evaluation. Only two models, the recent Stoffenmanager Nano (Duuren-Stuurman et al., 2011) and NanoSafer (Jensen et al., in preparation) consider personal exposure, while only NanoSafer bases its assessment on a time-resolved exposure potential estimate, which allows evaluation of both acute and chronic exposure. Schneider et al. (2011) developed a conceptual model for prediction of occupational exposure considering several important aerosol dynamic processes. Aerosol dynamic modelling is a major step forward in the NOAA Exposure Assessment, but strongly constrained by the sparse data on source strengths, workplace measurements and contextual information (Schneider et al., 2011). In most models, the focus has been set on inhalation exposure, nevertheless dermal exposure and oral intake should not be neglected in a comprehensive approach. There is pressing need to further develop models and to build up databases for exposure, process-related emission potentials, and efficiency of engineered controls and Personal Protection Equipment (PPE).

It has been recognized that there is necessity for pooling the existing exposure data for nanomaterials in view of future formulation of exposure scenarios (ES) (Brouwer et al., 2011). For this reason the NANEX

[1] OEL for Carbon Nanotubes and nano-TiO_2 were proposed by the National Institute for Occupational Safety and Health (NIOSH, 2010; 2011), the Organization for Economic Co-operation and Development (OECD, 2008), and by national regulators in Germany (BAuA, 2008; 2009; IFA, 2009) and UK (BSI, 2007).

project released a public catalogue of 107 occupational and 24 consumer ES for a number of nanomaterials. Although the NANEX database represents the state of the art in the field of nano occupational and consumer Exposure Assessment, most of the ES are characterized by scarce data and hardly generalizable exposure estimations (Clark et al., 2010), which makes it difficult to use *read-across* and fill the data gaps. In result, the data in the NANEX ES are not sufficient to use for high-tier exposure modelling or quantitative RA (Clark et al., 2010). It has been acknowledged that data produced in the future should be compatible to use for multiple purposes including exposure modelling and meta-analysis for RA or epidemiology studies (Brouwer et al., 2011). Therefore data should be collected in a harmonized format to be easily shared through databases such as the NECID-repository developed by PEROSH (www.perosh.eu).

Environmental release, behaviour, fate and exposure

The behaviour and fate of pristine (i.e. produced specifically for testing) nanomaterials have been studied to a great extent and are currently being addressed in F7 ENNSATOX, MARINA and many other projects, and a lot of knowledge has been acquired. However, almost nothing is known about the quantity and physico-chemical identity of NOAA released from actual products during the use and the end of life (EOL) stages. The EOL stage (e.g. shredding, incineration, landfilling, recycling) of NOAA-containing articles has received almost no attention (Asmatulu et al., 2012), although the US NANORELEASE project identified it as the stage where significant release could occur, especially for products where the NOAA are bound in a matrix. Some few results for textiles, paints and nanocomposites (Gottschalk and Nowack, 2011) suggest that the released particles have undergone significant transformation and aging, and exhibit different environmental behaviour and effects compared to the pristine NOAA (Auffan et al., 2010; Labille et al., 2010). F7 NANOHOUSE has also shown that NOAA are released together with many other materials and thus comparison to a nano-free reference is crucial for assessing toxicity and ecotoxicity.

(Eco)toxicology and physico-chemical characterization

The state of the art in regard to the effects of NOAA in the environment is in an early stage. Knowledge is rapidly increasing through major projects such as MARINA and NANOVALID, but they almost entirely focus on short-term hazard testing of NOAA. Short-term experiments with single species are not tailored to assess long-term effects on ecosystems. Further, gene-studies using Next Generation Sequencing provide evidences of changes transferred along generations, causing epigenetic, mutational or reproductive effects (Vecchio et al., 2012). It is hypothesized that many nanomaterials are (designed to be) persistent, which might lead to long-term exposure. Long-term environmental effects are not comprehensively researched yet, especially for NOAA used in real products. Therefore, testing of long-term effects on multiple species should be developed, focusing on ecosystem services that are vital also to mankind. This will provide valuable data to use with the tiered strategy currently developed in MARINA to achieve long-term ecological RA of NOAA.

Toxicity data on NOAA are produced in many EU projects. For example, the central nervous response was investigated in NEURONANO, pulmonary, cardiovascular, hepatic, renal and developmental effects were studied in NANOSH, PARTICLE_RISK and more recently in NANOTEST and ENPRA. Immunotoxicity was investigated in NANOMMUNE. Effects of the protein corona were studied in NANOINTERACT, while NANOSUSTAIN investigated the functional genomics activation of several response pathways. In result, a coherent toxicity profile of NOAA begins to emerge. However, there are still shortcomings, including lack of longer-term inhalation studies essential for RA (as in most projects bolus instillation was used as substitute for inhalation), and adequate comparison of *in vitro* and *in vivo* results to reduce animal testing as part of a 3R (Replacement, Reduction, Refinement) strategy. The inhalation route has been investigated significantly more than ingestion, which is another important route of exposure, especially in consumer settings. In addition, much of the conducted toxicity studies involve pristine nanomaterials without inclusion in a complex matrix or aging.

There are lack of proper data on the characteristics of NOAA used in (eco)toxicity studies, which makes it difficult to understand their modes of action and select appropriate dose-exposure metrics (Hansen et al., 2007; Warheit, 2008). In addition to mass, relevant metrics in the literature include surface area (Oberdörster et al., 2007; Stoeger et al., 2005; Stoeger et al., 2007), particle number concentration (Wittmaack, 2006; 2007) and surface chemistry (Warheit et al., 2007a; Warheit et al., 2007b). Taking into consideration the physico-chemical diversity of nanomaterials and the complexity of nano-bio interactions, it is unlikely that a single metric would sufficiently describe their toxicity (Brouwer et al., 2009), e.g. size distribution is useful for understanding the extent of pulmonary deposition of NOAA, while particle size and surface area can associate with certain modes of action. Therefore, before an agreement is reached, both (eco)toxicity and exposure studies with NOAA should report doses in multiple metrics (Clark et al., 2010).

Current (eco)-toxicological approaches to assessing nano-material hazard are based either on classical toxicology approaches or on novel multiplexed assays. These approaches do not provide comprehensive assessments due to the many unique aspects of NOAA, such as their transport mechanisms (in the body and within cells) and, in particular, the relationship between their physico-chemical properties and (i) biological identity in various culture media; (ii) fate and behaviour (uptake, translocation, localization); and (iii) functional impacts at cellular and systems levels. In this context it has been largely recognized that it is essential to complement the toxicity testing of nanomaterials with comprehensive, yet optimized physico-chemical characterization in order to establish the relationships between their properties and the observed biological responses.

One reason behind the lack of convincing patterns could be that mainly primary characterization has been performed since NOAA are generally difficult to measure *in situ* (Tiede et al., 2008). Nevertheless, it has been shown that a variety of factors such as ionic strength, pH and other media-specific properties cause

changes (e.g. agglomeration, aggregation, surface modification) to the primary NOAA once they enter a media, which influence both their exposure and toxicity (Hassellov et al., 2008).

Persistence is a main driver for bioaccumulation. Similar to conventional hydrophobic persistent chemicals like DDT, organic NOAA like fullerenes, that are hydrophobic and resistant to environmental and biological degradation, have the potential to bioaccumulate. Without suitable information on the potential for NOAA to bioaccumulate it is infeasible to carry out higher-tier RA or derive Environmental Quality Standards. For this reason, it may be relevant to focus primary characterization on persistence and secondary characterization on nanomaterials surface/corona and in-situ state of agglomeration (Aschberger et al., 2012). For less persistent NOAA, there is still a potential for adverse effects, however there is less chance for irreversible systemic damage (e.g. fibrosis and mesothelioma associated with long persistent fibres). For such materials one might reasonably accept a lower standard of evidence for non-toxicity, such as *in vitro* screening assays, while for persistent NOAA the standard would need to address higher concerns, including *in vivo* testing of larger numbers of animals and a wider variety of species (Aschberger et al., 2012).

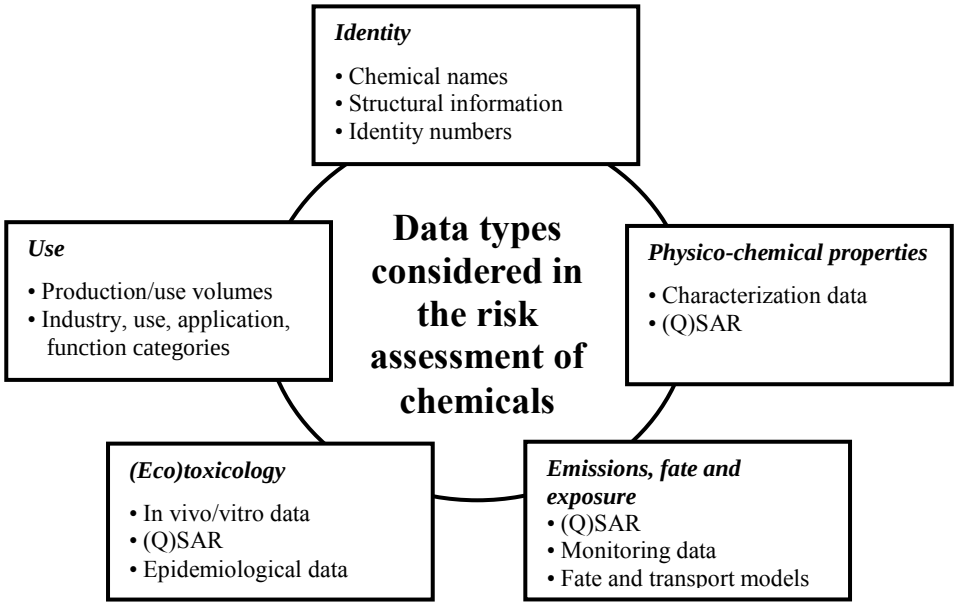
In the EU, the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and the European Food Safety Authority (EFSA) have recently reviewed the available data for RA of NOAA (EFSA, 2010; SCENIHR, 2007b; 2009b) and concluded that, despite the multiple gaps in the knowledge-base, the conventional RA framework is applicable to NOAA if properly adapted to address their novelties. In order to fill up the data gaps the European Commission funded about fifty projects under FP6 and FP7. These projects, together with a significant number of projects supported by government resources in the EU member states and the FP7 associated states, and other projects addressing safety as side objective, will gradually build the state-of-the-art in the nanosafety area

4.2 Data availability

The recognition that a core set of data is needed for the RA of chemicals dates back to the 1980s, when the OECD Council Act on the Minimum Premarketing Set of Data (MPD) for new chemicals (OECD, 1982) came into force. The OECD datasets appeared in result of a tradeoff among the interests of regulators, industry, scientists and the general public, as their selection was driven by the need to: reduce the cost of new data, define acceptable degrees of uncertainty and variability, and lower the use of experimental animals in toxicity testing (Van Leeuwen and Vermeire, 2007).

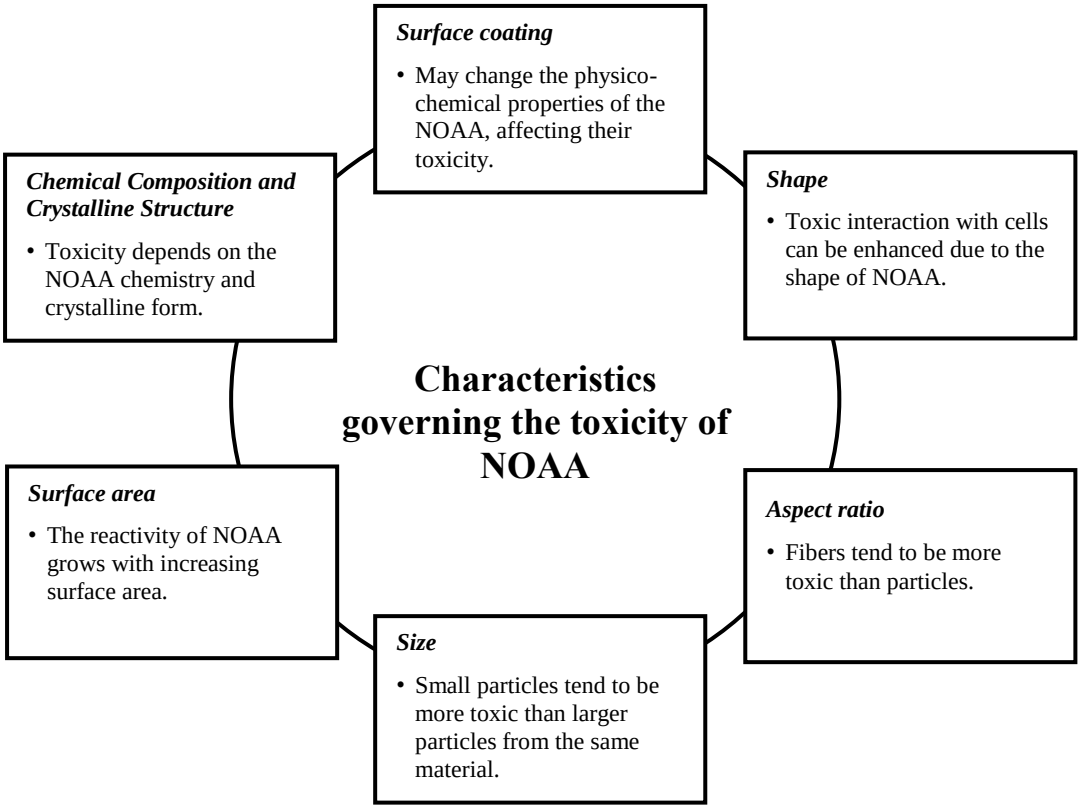
The data requirements for Chemical Safety Assessment (CSA) under REACH are based on the MPD. The CSA should include all available information on the identity, physico-chemical and (eco) toxicological properties, uses, emissions, exposures, environmental fate and behaviour of a chemical substance (Figure 4-1) (European Chemicals Agency, 2007a; b). Relevant information can be obtained from the literature and databases, as well as from *in silico* models, *in vivo* and *in vitro* experiments, and epidemiological studies.

Figure 4-9: Data for Chemical Safety Assessment (CSA) under the REACH Regulation. Modified after Van Leeuwen and Vermeire (2007).



This section looks at the subject of data needs and availability in the context of the CSA of NOAA. It has been largely recognized that in addition to the data required by REACH, further information needs to be considered. REACH data requirements do not yet cover relevant physico-chemical properties, expected to affect the toxicity of nanomaterials, including size, aspect ratio, surface area and reactivity, surface charge (Figure 4-2) (European Commission, 2008). Furthermore, it has been recognized that the capacity of most NOAA to disperse in aqueous media, instead to dissolve, would significantly influence their fate, which might require a shift from the conventional "solubility-hydrophilicity" paradigm, which drives our predictions of the environmental behaviour of most conventional chemicals, to a new "dispersivity" paradigm (Metcalfe et al., 2009). In this context, properties like the octanol-water partitioning coefficient are of minor relevance and alternative parameters may need to be introduced, based on characteristics such as size, surface charge, crystallinity and surface area (Metcalfe et al., 2009). It has become clear that CSA data requirements need to be adjusted to account for the novel properties of nanomaterials (European Commission 2008). For this reason the EC launched 3 REACH Implementation Projects on Nano (RIP-oN) and included their results as appendices to the existing REACH guidelines (Aitken et al., 2011; Hankin et al., 2011; JRC, 2011).

Figure 4-10: Important characteristics, affecting/determining the toxicity of NOAA.



Data relevant for CSA can be obtained from specialized libraries, documentation centres, and internet-based databases. Most data searches would now start by interrogating online sources, such as the US EPA's ECOTOX and IRIS, the TOXNET HSDB or the Danish (Q)SAR Database.

In order to find information relevant for CSA of NOAA, 3 types of online databases were surveyed: (i) chemical databases; (ii) bibliographic databases/digital libraries and (iii) project databases. The "chemical databases" store refined EHS data about substances (e.g. single values, excel sheets, text excerpts). For a risk assessor they are the most straightforward source of information. The "bibliographic databases" are organized as digital collections of abstracts and references to published literature, while the "digital libraries" go one step further, providing the full-text of the open contents. In case that the needed data are not available in the chemical databanks and they need to be obtained from the literature, online libraries provide an appropriate platform for search and download of publications. The "project databases" store information about on-going or completed projects (e.g. leader, objectives, duration, funding). The latter databanks were included in this survey since we consider that they are representative of the state of research in the nano-EHS area and provide a solid basis for assumptions about future data availability.

4.2.1 Data in online databases

A total of 42 chemical EHS databases were surveyed for useful data about hazard, exposure or risk from NOAA. The search entries were either the names or the Chemical Abstracts Service (CAS) registry numbers of the substances. Because most nanomaterials have no associated CAS numbers, nanoforms were

distinguished based on contextual information. Seven nanomaterial types were included in the survey, selected in terms of socioeconomic significance: carbon nanotubes (CNT); C_{60} fullerene; titanium dioxide (TiO_2), silver (Ag), zinc oxide (ZnO), iron (III) oxide (Fe_2O_3) and silica (SiO_2) nanoparticles. Due to the fact that in the different databases the materials appeared under alternative names (e.g. carbon nanotubes/buckytubes, fullerenes/buckyballs), the need to develop a comprehensive inventory of search entries was recognized, including both the standard names of the substances and their synonyms.

Out of the 42 databases, only 7 included results about nanomaterials:

(1) NANOhub (Open Science): http://www.napira.eu/;

(2) Hazardous Substances Data Bank (HSDB): http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB;

(3) Chemical Safety Database Searcher (CSDS): http://msds.chem.ox.ac.uk/msds-searcher.html;

(4) Stanford Chemical Safety Database (SCSD): https://chemtracker.stanford.edu/gdnchemsafety/;

(5) Chemical Carcinogenesis Research Information System (CCRIS): http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS;

(6) Woodrow Wilson International Centre for Scholars (WWICS) Inventory of Consumer Products: http://www.nanotechproject.org/inventories/consumer/; and

(7) WWICS Silver Nanotechnology Inventory: http://www.nanotechproject.org/inventories/silver/.

NANOhub (Open Science) is the only nano-specific database, but it is a “playground” where everyone can input data on voluntary basis and therefore includes many errors and data gaps. It contains data about TiO_2, ZnO, Ag and Fe_2O_3 nanoparticles, stored in records organised into several categories in accordance with the REACH requirements (e.g. physico-chemical properties, environmental fate and pathways, (eco)toxicological information, guidance to safe use). Unfortunately NANOhub (Open Science) does not have data extraction functionality because it is designed to report results to EC, instead of being used as an operational database where scientists can store and easily extract data.

Similarly to NANOhub, the HSDB contains relevant (eco)toxicological, environmental fate and exposure information about TiO_2, ZnO, Ag and Fe_2O_3 nanoparticles as well as CNT and C_{60} fullerene. The records are organized as refined text excerpts where the contained information is properly cited.

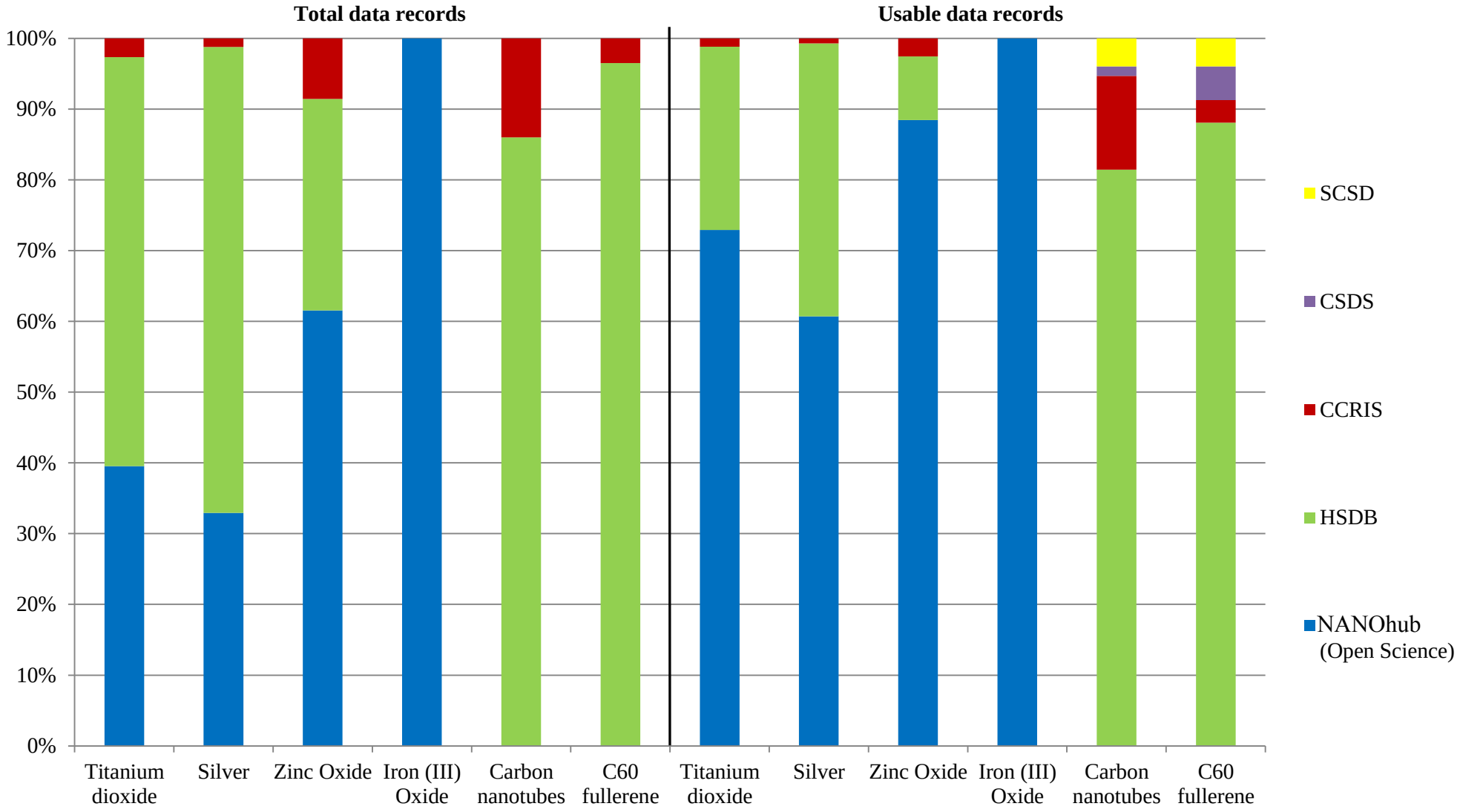
The CSDS and the SCSD store some data about the toxicity and the physico-chemical properties of CNT and C_{60} fullerene, respectively, but they are both very scarce and unreliable since they are not properly quoted. In the CCRIS some toxicological information was found about C_{60} fullerene and TiO_2 nanoparticles, including the type of toxicity study (e.g. mutagenicity), the test system (e.g. Chinese hamster lung cells), as well as the corresponding endpoints, doses and dose response curves. The data are stored in the form of quantitative values and qualitative statements.

The WWICS Inventory of Consumer Products (ICP) and the Silver Nanotechnology Inventory (SNI) deliver limited information from a risk assessor's point of view. However, since they report numbers of nano products on the market, these databanks are valuable sources of data for the formulation of consumer exposure scenarios. While still not comprehensive, the inventories include more than 1000 goods, containing nano-components. The SNI alone stores information about 244 nano-Ag products, while the ICP reports articles, containing C_{60} fullerene (7), CNT (24), TiO_2 (31), ZnO (24), Ag (256) and Fe_2O_3 (24) nanoparticles. In the SNI the data are organized in several classes (e.g. particle/substrate structure, synthesis method, use of nanotechnology, product testing, antimicrobial claims) and they are downloadable in PDF format, while in the ICP the information can be browsed by name, company or country and it is grouped into eight categories (e.g. Appliances, Automotive, Cross Cutting, Electronics and Computers, Food and Beverage, Home and Garden, Goods for Children and Health and Fitness).

A quantitative analysis has been carried out to estimate the relevant data and information in the above databases. They were found to contain data about 6 out of the 7 nanomaterials, included in the survey: CNT, C_{60} fullerene, TiO_2, ZnO, Ag and Fe_2O_3 nanoparticles (no information about silica nanoparticles). All records were counted for each nanomaterial and presented in 6 data categories: (i) manufacture, use and disposal; (ii) physical and chemical properties; (iii) environmental fate and pathways; (iv) ecotoxicological information; (v) toxicological information; and (vi) guidance on safe use. In the estimation it was distinguished between two types of entries, simply named "usable" and "unusable". A "usable" record incorporates both a result from a study and a properly cited reference, ensuring the reliability of the data, while an "unusable" one contains either no reference or no results. The latter case (i.e. reference but no results) is typical for NANOhub (Open Science), which is a "playground" where often only publication sources are reported, while the details and the results of the corresponding studies are left blank.

Figure 4-3 shows the distribution of records for each nanomaterial among the databases. It can be seen that the majority of both usable and total records are contained in the HSDB and in NANOhub (Open Science), while the SCSD and the CSDS store few, unreliable (i.e. unquoted) entries. Here it becomes evident that NANOhub loses a substantial number of usable records, especially for Ag and TiO_2.

Figure 4-11: Distribution of total and usable data records (in %) for each nanomaterial among the databases SCSD, CSDS, CCRIS, HSDB, NANOhub (Open Science).



Figures 4-4 and 4-5 show the distribution of usable and total (including unusable) records for each nanomaterial among the six data categories. Generally, the majority of available records concern nano-TiO_2, followed by ZnO and Fe_2O_3. Most of them are situated in the "toxicological information" domain, which is not surprising, considering the substantial number of toxicity studies carried out in the last several years to investigate the hazardous effects of these materials. However, for instance only 60% from all ZnO toxicity records are usable in practice, followed by 32% for TiO_2 and 20% for Fe_2O_3. In result, the relevant data in this field mainly refer to ZnO. The majority of usable TiO_2 toxicity records include results from acute, repeated dose, *in vivo* skin irritation/corrosion and *in vitro* genotoxicity studies, while the recorded Ag toxicological information is derived mainly from repeated dose toxicity and eye/skin *in vivo* irritation/corrosion experiments. For ZnO the databanks contain mainly acute toxicity, skin irritation/corrosion and carcinogenicity results, while for Fe_2O_3 mostly acute toxicity and *in vitro* genotoxicity data are available.

Figure 4-12: Distribution of usable data records for each nanomaterial (i.e., TiO_2, Ag, ZnO, Fe_2O_3, CNT, C_{60} fullerene) among six data categories (all databases).

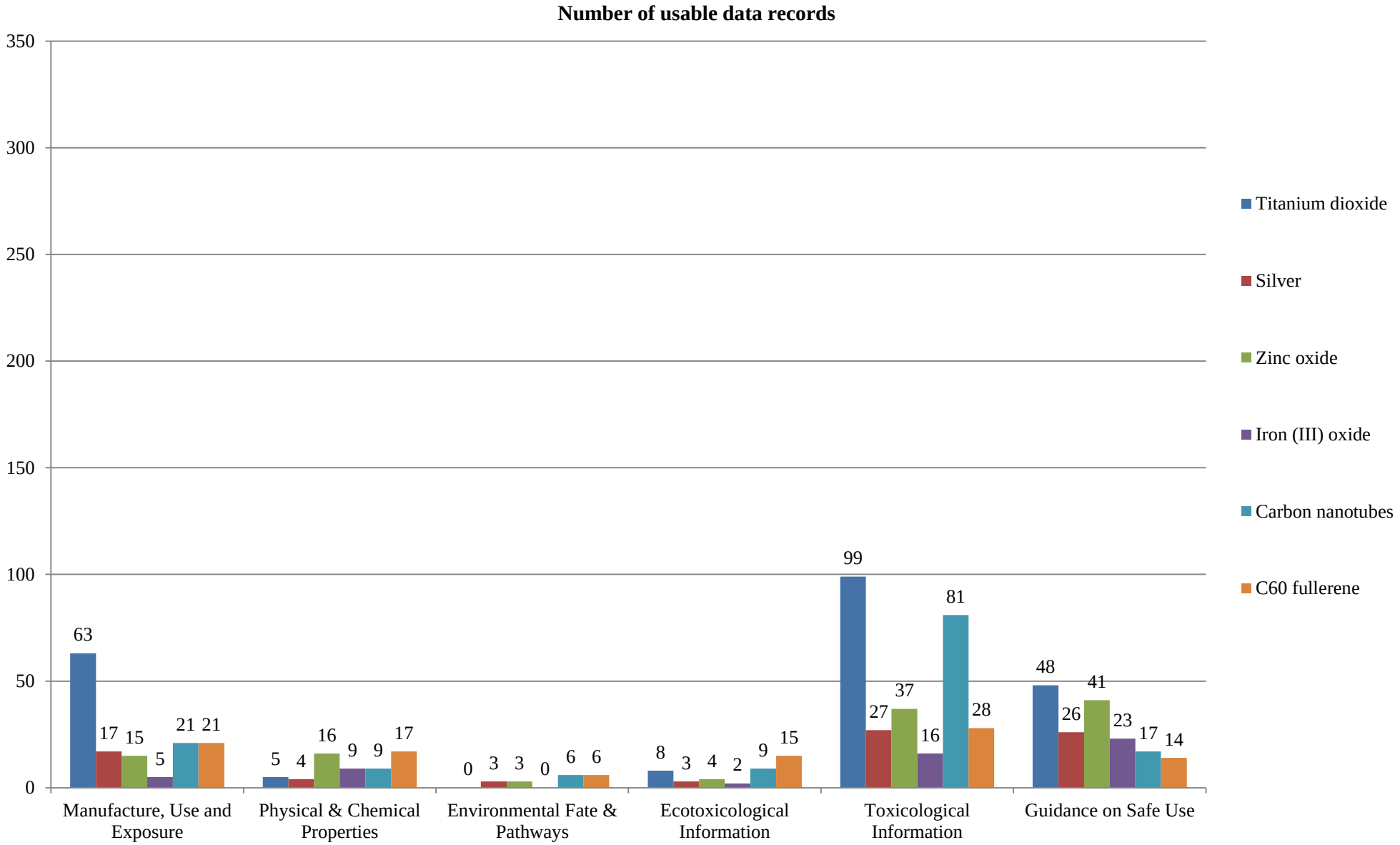
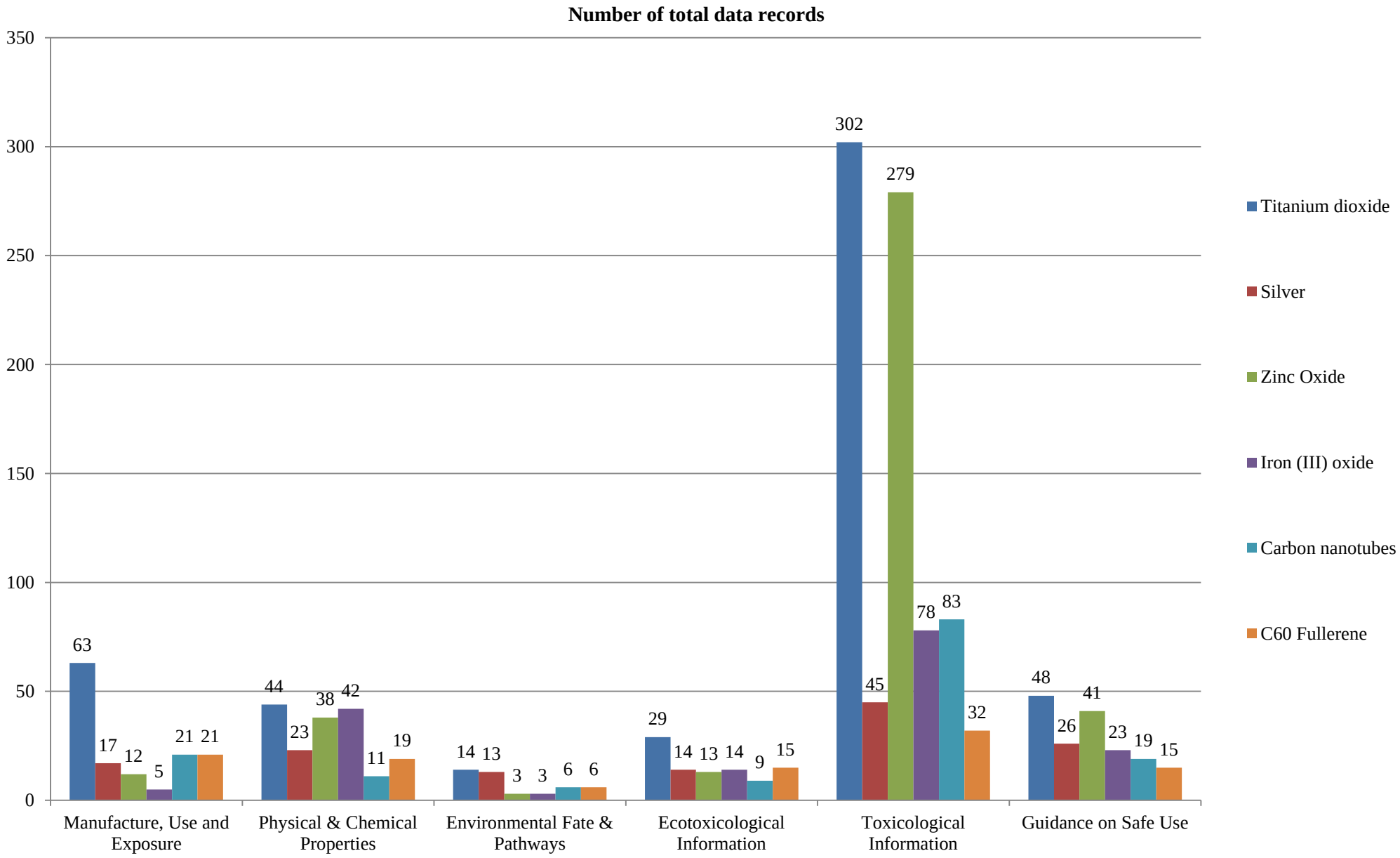


Figure 4-13: Distribution of total data records for each nanomaterial (i.e., TiO_2, Ag, ZnO, Fe_2O_3, CNT, C_{60} fullerene) among six data categories (all databases).



The useful records in the "physical & chemical properties", "manufacture, use and disposal" and "guidance on safe use" areas concern mainly the TiO_2 again, while most of the available "ecotoxicological information" involves the C_{60} fullerene. In all categories, there are only few usable entries for Ag, which is unexpected since the nano-Ag has received significant attention from the EHS research community over the last few years (Wijnhoven et al., 2009) and plenty of data have been generated. The generally low number of usable records in the online databases and particularly the scarcity of nano-Ag information suggest that the databanks are not representative of the overall nano-EHS data availability. Therefore most of the data, relevant for the RA of nanomaterials, still need to be obtained from the literature.

4.2.2 Data in the literature

The easiest way to gain access to scientific papers is to search for them in relevant bibliographic databanks/digital libraries. Some important sources of published literature on nano-EHS issues are:

(i) Toxicology Literature Online (TOXLINE): http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE;

(ii) Developmental and Reproductive Toxicology (DART): http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC;

(iii) International Council on Nanotechnology (ICON): http://icon.rice.edu/;

(iv) National Institute for Occupational Safety and Health (NIOSH) Nanoparticle Information Library (NIL): http://nanoparticlelibrary.net/index.asp;

(v) NIOSH NIOSHTIC-2: http://www2.cdc.gov/nioshtic-2/;

(vi) SAFENANO: http://www.safenano.org/Newsletter.aspx;

(vii) NCBI PubMed: http://www.ncbi.nlm.nih.gov/pubmed;

(viii) NCBI Bookshelf: http://www.ncbi.nlm.nih.gov/sites/entrez?db=books;

(ix) NCBI PubMed Central: http://www.ncbi.nlm.nih.gov/pmc/; and

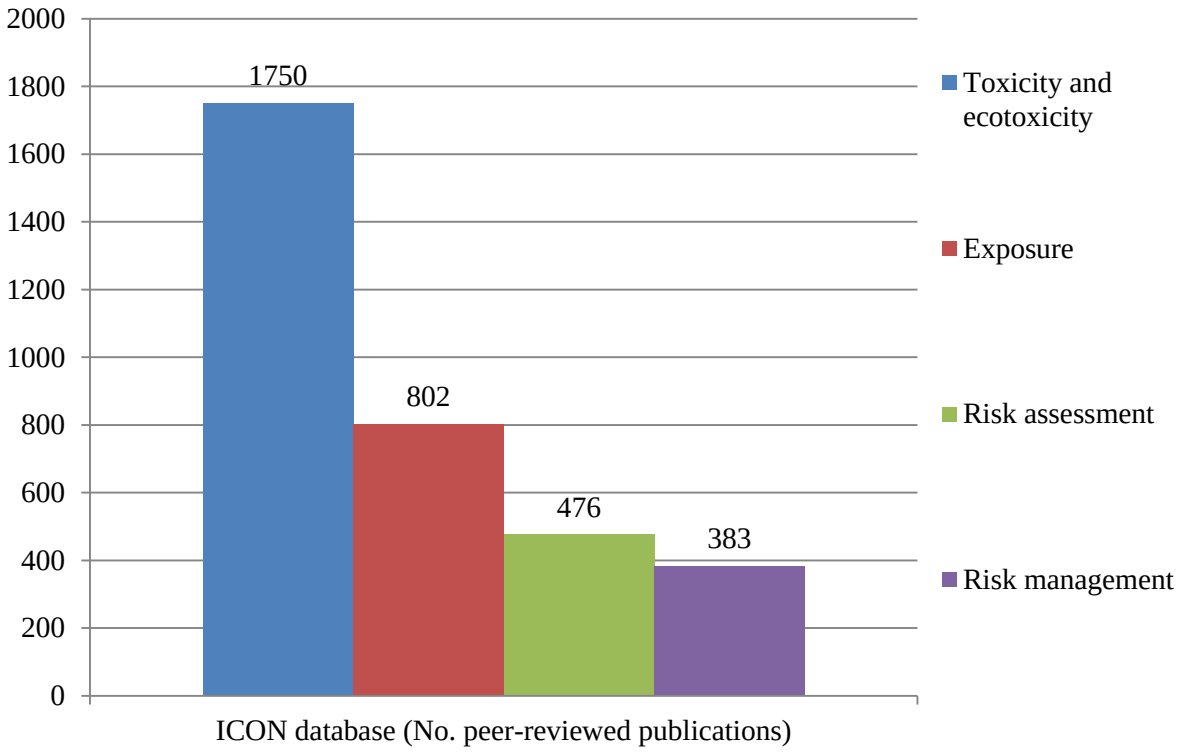
(x) ISI Web of Knowledge: http://apps.isiknowledge.com.

Using the above tools, it is possible to gain access to multiple documents, extract the relevant data from them and quantify their availability. Considering the large number of publications, however, such a major activity was impossible to undertake in the context of this review. That is why only conclusions about the current state of research, followed by informed assumptions about the probable distribution of the existing data and information in the literature are included below.

We searched the ISI Web of Knowledge and the ICON bibliographic databanks for peer-reviewed journal articles within several nano-EHS categories (e.g. toxicity, ecotoxicity, exposure, risk assessment) using search criteria defined by Grieger et al. (2010). As it is shown on Figure 4-6 the majority of the search

results fell in the “toxicity and ecotoxicity” category, while fewer publications were found on “Exposure” and “Risk assessment” topics.

Figure 4-14: Number of peer-reviewed publications in four categories: (Eco)toxicity, Exposure, Risk assessment and management. The results were obtained through a search in the International Council on Nanotechnology (ICON) database.



4.2.3 Future data availability

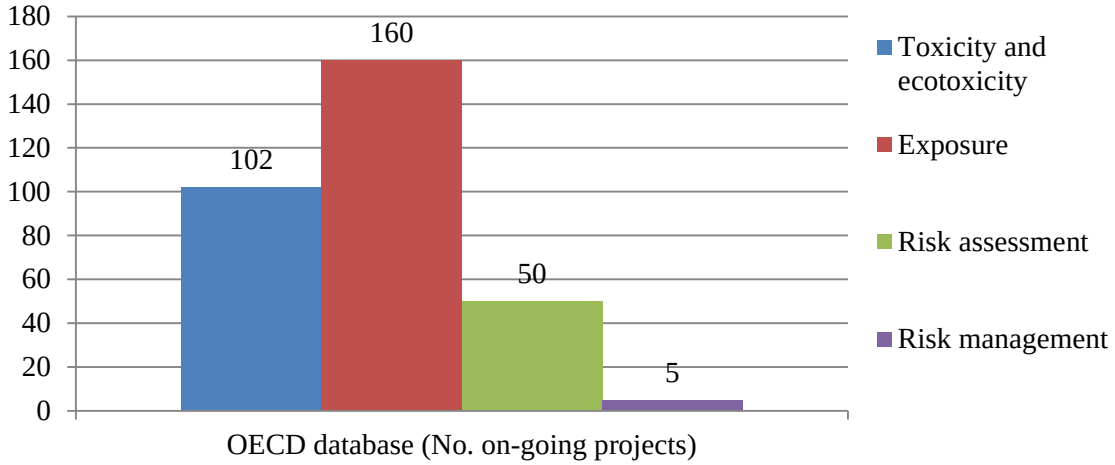
The majority of data found in the literature have been produced within national and/or European research projects. 2 major databases listing nanosafety projects are:

(i) The OECD Database on Research into Safety of Manufactured Nanomaterials (DRSMN): http://webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx;

(ii) The WWICS Inventory of Nanotechnology EHS Research: http://www.nanotechproject.org/inventories/ehs/.

They can provide information about on-going and planned projects to make informed assumptions about the future research trends and data availability. The OECD DRSMN was surveyed for on-going and planned nano-EHS projects using the search criteria defined by Grieger et al. (2010). The results are reported in Figure 4-7.

Figure 4-15: Number of on-going and planned nanosafety projects obtained through a search in the OECD Database on Research into Safety of Manufactured Nanomaterials.



Plotting the distribution of the on-going /planned nano-EHS projects in the above categories against the distribution of publications (i.e. results from past projects) (section 4.2.2), an apparent shift in the research efforts from the (eco)toxicity to the exposure domain is observed. Based on this, it can be assumed that in future more data and information relevant for the Exposure assessment of nanomaterials will become available.

4.3 Frameworks, methods, methodologies and tools

The recognised uncertainties and limitations outlined in section 4.1 often make the conventional RA unfeasible to apply to NOAA. EHS data are gradually generated to fill the gaps, but this process advances slowly and will take decades (Grieger et al., 2010), while RA results are urgently needed to inform adequate regulatory decisions (Hansen, 2009; Hristozov and Malsch, 2009). In this context several regulatory agencies, research institutes and companies (e.g. Environmental Defense and DuPont) proposed complementary/alternative approaches for near-term RA, taking into account the inherent novelties of NOAA.

It has been recognized that the literature does not explicitly differentiate among the terms “framework”, “method”, “methodology” and “tool”, when they are used in the context of the Risk assessment and governance of nanomaterials, which can sometimes cause perplexity and misunderstanding. Therefore, we consider it appropriate to introduce such a distinction in this review, thus avoiding any confusion, which can arise from using these terms. They are defined as follows:

- “Framework” is a set of practices, organized in a conceptual manner, that constitute a policy;
- “Method” is a series of steps taken to acquire knowledge;
- “Methodology” is a set of practices and tools organized in a well-structured system with interrelations and outputs clearly defined;
- “Tool” is a procedure or model used to generate data.

4.3.1 Frameworks

Risk assessment and management (RA/RM) are closely related, but different in their nature: RM represents the subjective, political part of the risk governance process, while the RA is its scientific backbone (Patton, 1993). Back in the 1980s, it was assumed that RM decisions should be made solely by reference to scientific evidence and expert opinion (JRC, 2004). This way of thinking describes the so called "technocratic" paradigm (JRC, 2004; Van Zwanenberg and Millstone, 2005), which neglected important political, socioeconomic, ethical and cultural factors, influencing the risk governance process. In the early 1990s a shift to the "decisionist" paradigm took place, which takes into account the above factors, but assigns minor importance to stakeholder communication. Today we observe a shift to the "transparent" paradigm (Van Zwanenberg and Millstone, 2005), focussing on risk communication embedded in an iterative dialogue, engaging all stakeholders at all phases of the decision making process.

Most adaptations of the RM framework for nanomaterials (Table 4-1) are "transparent" as the need for an iterative dialogue, engaging all stakeholders is emphasized and the socio-economic, cultural and political contexts are accounted for. They are all based on the conventional RA paradigm, which suggests that their authors deem it relevant if properly adapted to address the novelties of nanomaterials. However, they are not explicitly legislation-oriented, which means that they do not refer to regulatory provisions such as those required by REACH. Some frameworks tend to stress the importance of the Problem formulation step, which is essential for NOAA given the enormous complexity associated with their RA (Hristozov et al., 2012). Although some frameworks are more specific in their scope, most of them can fit in various decision contexts. In contrast, none of them incorporates specific mechanisms for timely decision making, but may be easily adaptable for this if necessary.

Some frameworks are iterative, which implies consideration of the Adaptive management (AM) concept in their design. Implementation of adaptive and more responsive RM is essential since the rapid nanoinnovation outpaces the nano-EHS research, which may result in lengthy, post-market safety investigations (Grieger et al., 2010) continuously generating new data to be considered in the risk governance process. AM can incorporate this flux of new information in a systematic way, acknowledging the uncertainties in the outset and developing strategies to reduce those (Linkov et al., 2007).

Since the current nano RA/RM frameworks are based on the traditional approach, they reflect the data requirements for conventional chemicals. The present deficit of quantitative nano-EHS information leads and will lead in the short term to uncertain and ambiguous, largely qualitative risk estimations, based on expert judgments, which may fail to support adequate regulatory decisions. Therefore, it is important to develop new complementary methods towards achieving quantitative RA of NOAA with the currently available data in order to enable near-term decision making and RM.

Table 4-1: Nano Risk assessment (RA) and management (RM) frameworks and their characteristics. HHRA=human health Risk assessment. ERA= ecological Risk assessment

Framework	Scope	RA/RM	Structure	Policy Model	Referred to the conventional RA?	REACH Oriented?	Data Requirements
Nano Risk Framework (Environmental Defense and DuPont, 2007)	HHRA[a]/ ERA[b]	RM	Iterative	Transparent	Yes	No	Physico-chemical properties; toxicity/ ecotoxicity effects; biological/environmental fate and behaviour; safety hazard data; exposure data.
Nanotechnology Risk Governance Framework (International Risk Governance Council, 2006)	HHRA/ ERA	RM	Iterative	Transparent	Yes	No	Physico-chemical properties; toxicity/ecotoxicity effects; biological/environmental fate and behaviour; exposure data.
SCENIHR Framework (SCENIHR, 2007)	HHRA/ ERA	RA	Non-iterative	N/A	Yes	No	Physico-chemical properties; toxicity/ ecotoxicity effects; exposure data.
(Oberdörster et al., 2005)	HHRA	RM	Non-iterative	Decisionist	Yes	No	Physico-chemical properties; toxicity/ ecotoxicity effects; exposure data.
Comprehensive Environmental Assessment (Davis et al., 2010)	ERA	RA	Iterative	Transparent	Yes	No	(Eco)toxicity effects; exposure, LCA inventory data.
Life Cycle Risk Assessment (LCRA) Framework (Shatkin, 2008)	ERA	RA	Iterative	Transparent	Yes	No	(Eco)toxicity effects; exposure, LCA inventory data.
Risk Assessment Framework for First Generation NOAA under REACH (Pronk et al., 2009)	HHRA/ ERA	RA	Iterative	Transparent	Yes	Yes	Physico-chemical properties; (eco)toxicity effects; exposure data.

[a] human health Risk assessment. [b] ecological Risk assessment.

4.3.2 Emerging methods

While the conventional RA is based on the notion that the chemical identity governs the biological effects of a substance, the toxicity of NOAA is determined by a set of characteristics (e.g. size, aspect ratio, surface area and reactivity, surface charge). Given the substantial complexity of nano-bio interactions their grouping in terms physico-chemical properties and biological effects has not yet been achieved. Therefore it is only possible to address the Hazard/Risk assessment of NOAA on case-by-case basis (Environmental Defense and DuPont, 2007; SCENIHR, 2007b). However, given the enormous diversity of NOAA, this is very resource-intensive and involves extensive testing. Therefore for nanomaterials we need a paradigm shift from animal testing towards hypothesis-driven approach to prioritise and reduce *in vivo* experiments on the basis of *in vitro* screening assays and *in silico* modelling. Such an Intelligent Testing Strategy (ITS) may significantly speed up the RA of NOAA, while at the same time reduce testing costs and animal use (EFSA, 2009; SCENIHR, 2007a; 2009a). In this context, Meng et al. (2009) proposed a hazard screening tool, which uses *in vivo* outputs to validate *in vitro* assays as being "predictive" and therefore valid for screening multiple batches of nanomaterials to obtain Quantitative Nano property-Activity Relationships (QNAR).

The increasing production of novel formulations of NOAA by the nanotechnology industry poses an immediate problem for RA, as many of them remain untested and thus QNAR, and *in silico* methods in general are very desirable. However, only few studies have attempted to develop QNAR (Burello and Worth, 2011; Petrova et al., 2011; Puzyn et al., 2011). Researchers agree that while QNAR is the ultimate goal, there is still a long way to go in terms of data production and model development before *in silico* methods can reliably predict the hazard of NOAA based on their physico-chemical properties.

In silico physiologically-based pharmacokinetic (PBPK) models can incorporate physico-chemical and biochemical characteristics along with species-specific physiological properties to study post-exposure absorption, distribution, metabolism and excretion (ADME) kinetics/dynamics of NOAA (Lee et al., 2009b; Riviere, 2009). A specific, blood-flow-limited PBPK model for quantum dots (QDs) was developed by Lee et al. (2009), while the first more generic model, applicable to different NOAA, was developed by the UK Institute of Occupational Medicine (IOM) in the context of the FP7 ENPRA project (Tran, 2011b). PBPK modelling can be used not only to characterize the ADME of NOAA, but also their biological interactions across a diverse range of species (Lee et al., 2009a). It can also help to develop NOAA-specific interspecies assessment factors for estimation of Derived No-effect level (DNEL) for RA (European Chemicals Agency, 2008).

In order to use *in vitro* data for RA purposes they need to be extrapolated to *in vivo* fist (Dekkers et al., 2011). Quantitative *in vitro-in vivo* extrapolations (IVIVE) typically start with dose-response modelling of raw continuous, quantal or ordinal toxicity data (Slob, 2002). Using an empirical approach one can look for correlations between *in vitro* and *in vivo* dose-response relationships. In this case, under the assumption that

the experimental results are standardised/comparable, the differences in the *in vitro* and *in vivo* data would be only due to variations in the physico-chemical properties of the dispersed NOAA (Bessems et al., 2011) or due to *in vivo* cellular interactions not considered by the *in vitro* models. If careful analysis of the data excludes the latter cause, a generic QSAR-like algorithm [i.e. a Quantitative Property (*in vitro*) Property (*in vivo*) Relationship (QPPR)] can be obtained. Using this QPPR, *in vivo* Benchmark Doses or Effective Concentrations can be derived out of *in vitro* data (Bessems et al., 2011). The disadvantage of this method is that it requires standardised data for a large number of NOAA, which are generally difficult to acquire. In this context an alternative mechanistic approach could be applied, which considers the complete toxicokinetic profiles of the NOAA by means of PBPK models.

A quantitative human health RA of NOAA would involve deterministic modelling of exposure-dose-response relationships and their extrapolation to humans. However, in the case of NOAA this would be affected by severe uncertainty and data variability. For this reason, Hristozov et al. (2012) recommended that the RA of NOAA is addressed in a probabilistic manner using stochastic approaches such as the Monte Carlo and the Latin Hypercube Simulations. In this case distributions of hazard estimates will be derived instead of single values, which can be plotted against distributions of exposure estimates in order to identify central tendencies of expected risk and associated high-end probability of exposure. Despite that the risk estimates will depend on the extent of uncertainty, by applying sensitivity analysis it is possible to identify the main factors contributing to the overall model uncertainty and develop a strategy to reduce it.

The above approaches promise to facilitate the regulatory RA of NOAA. However, they require significant data, which are currently unavailable for many materials. Their timely regulation requires flexible methodologies and tools, which can operate with limited information. This could be facilitated by emerging Weight of evidence (WoE) methods such as Multi-criteria decision analysis (MCDA) (Hansen, 2009; Hristozov and Malsch, 2009; Linkov et al., 2007).

WoE is an umbrella term for a number of approaches to systematically combine individual (qualitative and/or quantitative) Lines of Evidence (LoE) to make objective decisions in the face of uncertainty. Linkov et al (2009) proposed a conceptual framework for categorization of WoE methods into quantitative and qualitative (see section 3.2). MCDA includes a large number of quantitative WoE methods (Giove et al., 2009), designed to ensure that a synthesis of multiple sources of information is documented and directed towards a pre-defined goal (Linkov et al., 2011). A WoE-based risk analysis of NOAA would involve identification of measureable parameters derived from experimental or modelling results and their organization into LoE (Linkov et al., 2009; Linkov et al., 2006). Depending on the scope of the assessment various types of data can be used, including physico-chemical, (eco)toxicological, *in silico* and exposure information as well as results from pharmacokinetic modelling (Hristozov et al., 2012). Quantitative MCDA methods are generally preferred to qualitative approaches since their application yields multiple benefits such as the ability to incorporate conflicting information, to facilitate trade-offs among competing

alternatives, and to propagate uncertainty (Linkov et al., 2011). MCDA tools could help to choose relevant criteria to be considered in the RA process, determine the relative importance of each criterion, score it, and finally compare the scores to identify best alternatives (Linkov et al., 2006). In practice, the essential contribution of MCDA to the RA of NOAA is to establish links between the input data and the decision criteria/weighs, thus allowing the visualization and quantification of the trade-offs involved in the decision making process (Linkov et al., 2007). Until now WoE was applied in the context of NOAA only by (Zuin et al., 2010), while the use of MCDA for RA of NOAA was illustrated by Linkov at al., (Linkov et al., 2006) and Tervonen at al., (Tervonen et al., 2009) in hypothetical case studies involving no use of real data.

Another approach, often used in combination with the above methods, is the Expert Elicitation (EE) (Kandlikar et al., 2007; Morgan, 2005). Several methods to eliciting expert judgments have evolved, including Elicitation Protocol Design, Nominal Group Technique (NGT), Collective Judgement (CJ) and Delphi methods. For example US EPA used the NGT to prioritise research needs for nano-TiO_2 (US EPA, 2009) and the CJ for Comprehensive Environmental Assessment of nano-Silver (US EPA, 2012). Moreover, Kandlikar et al. (2007) introduced an approach to collect expert judgements for nanosafety evaluation, while Morgan (2005) proposed a tool to organize them into influence diagrams and thus support decisions while at the same time prioritise research gaps. The advantage of using influence diagrams to other pictorial representations (e.g. cognitive maps) is that they are computable in the sense that their variables and relationships are defined clearly enough to be used for both deterministic and probabilistic modelling, given sufficient data input (Morgan, 2005).

WoE and EE methods are currently largely applied in nano control banding approaches, where hazard and exposure bands are calculated. Overlapping these bands in a matrix allows predicting the level of occupational risk to identify suitable control measures. An action plan is then defined to guarantee the efficacy of the recommended preventive measures. If the measures indicated by the level of risk control are not achievable for technical or financial reasons, RA must be conducted in order to revise the action plan. A number of nano control banding tools have been proposed in the literature as discussed in the following section 4.3.3.

Table 4-2: Methods applied for risk analysis of nanomaterials.

Categories of tools	Risk-related field	Reviewed by
Integrated Testing Strategy (ITS) (Meng et al., 2009)	Hazard assessment	Hristozov et al., 2012
Physiologically-based pharmacokinetic (PBPK) modelling (Tran, 2011b)	Dose-response modelling	Hristozov et al., 2012
In vitro-in vivo extrapolation (IVIVE) (Bessems, 2011; Slob, 2002)	Dose-response modelling	Hristozov et al., 2012
Stochastic approaches (Tran, 2011)	Whole Risk assessment process	Hristozov et al., 2012
Weight of evidence (WoE) and Multi-criteria decision analysis (MCDA) (Linkov et al., 2007; Linkov et al., 2008; Tervonen et al., 2009)	Whole Risk assessment process	Grieger et al., 2012 Hristozov et al., 2012
Expert Elicitation (EE) (Kandlikar et al., 2007; Morgan, 2005)	Risk characterisation	Hristozov et al., 2012
Control banding (Fransman et al., 2010; Höck et al., 2010; Ostiguy et al., 2010; Paik et al., 2008)	Whole Risk assessment process	Grieger et al., 2012 Hristozov et al., 2012
Value of Information (VoI) (Linkov et al. 2011b)	Whole Risk assessment process	Hristozov et al., 2012

4.3.3 Methodologies and tools

A number of methodologies and tools facilitating human health RA of NOAA have been reported in the literature (Table 4-3). In spite of the substantial differences among them in terms of structure and scope, one common feature is that all use scoring procedures to estimate relative hazards/risks and group the materials on this basis.

Although none of the reviewed approaches precisely follows the steps and procedures of the conventional RA framework, all support certain characteristics of it and require similar datasets [e.g. physico-chemical characterization, environmental/human exposure, and (eco)toxicological data]. All of them are designed to use the currently available data, which makes them feasible to apply in the near term. There is no evidence that the methods by Tervonen et al. (2009) and Höck et al. (2010) were used in practice. However, the approaches by Robichaud et al. (2005) and Zuin et al. (2010) were tested on the pilot scale for hazard/risk screening of C_{60} fullerene, single-walled and multi-walled carbon nanotubes, cadmium selenide and zinc selenide quantum dots, carbon black, aluminum, TiO_2 and Ag nanoparticles. Despite this, however, their robustness cannot be confirmed before they are applied on the large scale and rigorously tested to fully evaluate their limitations (Grieger et al., 2010).

Most methodologies consider similar parameters in the relative assessment of nano risks. Both Höck et al. (2010) and Zuin et al. (2010) use the chemical stability of NOAA as a hazard-relevant indicator. In this context, stability takes into account the resistance of the NOAA to dissolution, chemical or physical change, particle agglomeration or degradation (Zuin et al., 2010). Since the size of the particles is a critical parameter in terms of biotic interaction (e.g. cellular internalization, localization in the body, excretion), it is important to verify if the materials remain stable during the transport from the source to the target,

maintaining their original size (Wick et al., 2007). The stability of NOAA is closely related to their bioavailability, another important determinant of the inherent hazards of NOAA, taken into account by Tervonen et al. (2009). Furthermore, both Tervonen et al. (2009) and Zuin et al. (2010) acknowledge the surface functionalization and the charge of the particles as hazard-relevant parameters, related to the reactivity of the materials, directly influencing their functionality in biological media and also their bioavailability. In contrast to the approaches of Tervonen et al. (2009) and Zuin et al. (2010), which consider mainly material-specific characteristics in the estimation of hazards/risks, the methodologies by Robichaud et al. (2005) and Höck et al. (2010) stay closer to the conventional RA, taking into account also Operational Conditions (OC) such as temperature and pressure, material input and output streams, releases, exposure frequency and duration. In all approaches toxicity is considered only implicitly and no clear distinction between ecotoxicity and human toxicity is made.

Although all reviewed methodologies employ scoring procedures to estimate relative hazards/risks, they use different methods to integrate data. The methodologies of Zuin et al. (2010) and Höck et al. (2010) are based on a semi-quantitative WoE approach. After identification of suitable LoE and corresponding indicators, they set ranges of rating classes to trigger a hazard ranking procedures. In contrast, Tervonen et al. (2009) uses stochastic multi-criteria acceptability analysis (SMAA-TRI), an outranking method based on ELECTRE TRI, to classify NOAA in terms of relative risk. The authors selected SMAA-TRI, since it extends the capabilities of ELECTRE-TRI by allowing the use of imprecise parameter values to allow decision making with limited information (Tervonen et al., 2009). The assessment procedure involves comparing the parameters, selected for a specific nanomaterial, against profiles that include ranges of criteria, corresponding to several risk classes. The final classification decision is based on assigned profile weights, which represent the subjective importance of the criteria. In contrast to the above approaches, the methodology proposed by Robichaud et al. (2005) is focused not on the nanomaterials themselves, but on the processes involved in their production. It maps the relevant parameters to relative risk classes on the basis of cut-off values, defined by the XL Insurance methodology (Robichaud et al., 2005).

Any RA carries uncertainties with it, which need to be characterized and clearly communicated in order to ensure robust decision making. The consideration of the level of ambiguity in the process is crucial, since it may directly impact the assessment results and their interpretation. Out of the reviewed methodologies, only Höck et al. (2010) explicitly incorporates uncertainty factors in the assessment, which are directly used in the calculation of "precautionary need". Despite that the use of a SMAA-TRI approach allows for the incorporation of uncertainty, it was not explicitly addressed by Tervonen et al. (2010). Similarly, both the methodologies by Zuin et al. (2010) and Robichaud et al. (2005) provide no characterization of uncertainty.

As mentioned before, both the Hazard identification and the Dose-response assessment of NOAA suffer from the substantial deficit of characterization data (Hansen et al., 2007; Stone et al., 2009), which makes it difficult to determine which properties account for their inherent toxicity and set appropriate dose metrics.

The enormous structural diversity within each group of NOAA (e.g. CNT) adds further complexity. To address these issues, Hansen et al. (2007) proposed a hazard classification approach, which categorizes the materials, based on the location of the nanoscale structures in their matrix, while SCHENIR (2005) suggested a very simple nano hazard screening algorithm, based on a decision tree. Both approaches emphasize the need for characterization of the test materials to enable correlation between NOAA properties and measured biological responses and to provide an adequate reference point for comparing testing and non-testing (e.g. QSAR) results.

In order to facilitate the occupational Exposure assessment of NOAA the Netherlands Organisation for Applied Scientific Research (TNO) released the Stoffenmanager Nano, which is an adaptation of Stoffenmanager 4.0 to nanomaterials. It is a qualitative risk banding approach that integrates hazard and Exposure assessment steps (Duuren-Stuurman et al., 2011; Fransman et al., 2010). Similarly, Maynard (2007) proposed a pragmatic approach, which uses two qualitative indices (i.e. impact and exposure index) to select appropriate exposure control measures (e.g. general ventilation) (Maynard, 2007). This tool is quite preliminary, using a limited set of variables with no defined mathematical relationships among them and uses plenty of expert judgment without an established elicitation protocol. The latter issues have been addressed by the Danish National Research Centre for Working Environment (NRCWE) and partners, who currently work on the NanoSafer: a first generation quantitative control banding tool, which operates with data on material properties and manufacturing processes (Jensen et al., 2012). Other methodologies recently published are the occupational French ANSES system (Ostiguy et al., 2010) and the consumer Danish NanoRiskCat (Hansen et al., 2011). Both tools are still on the pilot scale and need to be validated and tested.

The exposure to NOAA from consumer products largely depends on the location of the nanostructures in the articles (Hansen et al., 2008). In this context, Hansen et al. (2008) proposed an approach, which groups the articles into exposure categories on this basis. Although this is a solid starting point, the authors realize that for robust assessment quantification of exposure is needed, which is difficult achieve since the exact content of NOAA is known only for a small number of products (Hansen et al., 2008)

In 2009 SCENIHR reviewed the existing knowledge on the environmental exposure to NOAA and concluded that there was no adequate information available on this topic (SCENIHR, 2009). However a number of studies have been published reporting first attempts to model and predict the concentrations of certain NOAA in the environment.

Using assumptions about the market penetration and consumer use of nano-containing products Boxall et al. (2008) applied mathematical algorithms to predict the concentrations of NOAA in air, soil and water. Although the results of the authors are apparently consistent, this model is rather simplistic and considers a limited range of products, life cycle stages and environmental compartments (Boxall et al., 2008).

Mueller and Nowack (2008) advanced a step further and modelled for the first time the environmental releases of three NOAA (nano-Ag, nano-TiO_2 and CNT) from a complete life-cycle perspective in order to estimate their Predicted Environmental Concentrations (PEC) in air, water and soil. To obtain risk quotients (i.e. PEC/PNEC ratios) the authors plotted the calculated PEC against Predicted No-Effect Concentrations (PNEC). The analysis resulted in low risk from nano-Ag and CNT, while the modelled quantities of nano-TiO_2 raised some concern for the surface waters. Although it provided clear results, the study itself is not comprehensive because certain environmental compartments, such as sediment and groundwater, as well as some industrial processes with all their associated NOAA flows are excluded from the model (Gottschalk et al., 2010b). Moreover, the incorporation of uncertainties and variability of the model input parameters were restricted to a simplistic two-scenario analysis (Gottschalk et al., 2010b).

In order to solve the limitations of the above approaches, Gottschalk et al. (2010b) developed a Probabilistic Material Flow Analysis model (PMFA) based on a Monte Carlo and Markov Chain Monte Carlo approach, which is suitable to effectively calculate PEC in case of scarce or inconsistent data. In the recent study this PMFA was used to predict concentrations for different nanomaterials and regions: i.e. Europe, US and Switzerland.

A number of tools were reviewed in this section. Despite that most of them are fundamentally different one from another in terms of scope, aim and methodology, all are intended to facilitate near-term analysis of nano risks.

While most of the tools require input of purely experimental data [i.e. physico-chemical characteristics, (eco)toxicological results] or expert knowledge to operate, the approaches by Hansen et al. (2007) and Hansen et al. (2008) demand industry-derived information (i.e. the location of the nanostructures in the system/matrix of the nanomaterials). Such information is currently obscured from public knowledge and therefore it is difficult to obtain. The current corporate Confidential Business Information policy hinders the RA of nanomaterials, which is to the detriment of all stakeholders, including private companies (Hristozov and Malsch, 2009).

It is important to stress that most of the methodologies are not intended to facilitate regulatory decision making, but they serve as preliminary hazard/risk screening and/or research prioritization tools, aimed to help industry in identifying relevant sources of risk in the lifecycle of synthetic nanomaterials and pinpoint areas of knowledge deficits. While these approaches may be valuable in various aspects, suggesting innovative decision-making strategies and providing early nano-risk estimates, a main limitation is that many of them have not been yet thoroughly tested and applied in practice, and, therefore, their robustness is still unconfirmed (Grieger et al., 2012). Furthermore, although these methodologies can provide a useful baseline of nano-safety estimations, they do not satisfy the need to adequately inform the regulators about the potential risks from NOAA in the near term.

Table 4-3: Nano risk/hazard assessment methodologies and tools and their characteristics. RA=Risk assessment

Methodology	Scope	RA step supported	Methods used	Objective	Data requirements	Outputs	Potential users
Swiss Precautionary Matrix for Synthetic Nanomaterials (Höck et al., 2010)	HHRA[a]/ ERA[b]	All	Control banding, Expert judgement	Identify the need for precautionary measures in the lifecycle of the NOAA	Physico-chemical properties, biological/environmental fate (i.e. stability under physiological/ environmental conditions), exposure data (e.g. frequency, magnitude)	Precautionary need scores	SME[c] and Industry
Workplace Control Matrix (Maynard, 2007)	HHRA	Risk characterization	Control banding, Expert judgement	Set appropriate occupational controls for NOAA	Physico-chemical data/ material quantities data	Recommendation on the most suitable occupational exposure control (e.g. general ventilation, containment)	SME and Industry
Stoffenmanager Nano 1.0 (Duuren-Stuurman et al., 2011)	HHRA	All	Control banding	Estimate relative occupational risk and recommend risk reduction measures	Physico-chemical data/ exposure information	Estimated risk level and recommendation on the most suitable occupational exposure control	SME and Industry
Nanosafer (Jensen et al., in preparation)	HHRA	All	Control banding	Estimate adequate occupational risk control level and recommend risk reduction measures	Physico-chemical properties and/or manufacturing processes	Estimated occupational risk level and recommendation on the most suitable exposure control	SME and Industry
French ANSES system (Ostiguy et al., 2010)	HHRA	All	Control banding	Estimate adequate occupational risk control level and recommend risk reduction measures	Physico-chemical properties and/or manufacturing processes	Risk control action plan	SME and Industry

NanoRiskCat (Hansen et al., 2011)	HHRA/ ERA	All	Risk screening	Identify, categorize and rank exposure and hazards associated with nanomaterials embedded in products	Physico-chemical properties, manufacturing processes, Technical functions, releases from products into the Environment etc.	Hazard, exposure classification	SME and Industry
US XL Insurance Database Methodology (Robichaud et al., 2010)	HHRA/ ERA	All	Insurance Database Method.	Assess relative risk associated with the production of NOAA	Physico-chemical properties, toxicity/ecotoxicity effects, process conditions (e.g. material input, output streams), operational conditions (e.g. temperature), exposure data (i.e. emissions)	Relative risk scores	SME and Industry
Risk-based Clasificantion System for Nanomaterials (Tervonen et al., 2009)	HHRA/ ERA	All	SMA-TRI MCDA	Group NOAA in risk classes for screening level RA	Physico-chemical properties, toxicity/ecotoxicity effects, environmental fate (i.e. bioavailability, bioaccumulation)	Relative risk scores	SME, Industry, academia
Nano Hazard Assesment Approach (Zuin et al., 2010)	HHRA	Hazard identification	Indexing WoE, Expert judgement	Assess the relative hazard of NOAA	Physico-chemical properties, exposure- related characteristics (e.g. stability), toxicity effects	Relative hazard scores	SME, Industry, academia, regulators
Nano Hazard Categorization Framework (Hansen et al., 2007)	HHRA	Problem formulation	Qualitative WoE, Expert judgement	Categorize the NOAA on the basis of the location of the nanoscale structures in their system/matrix for the purpose of Hazard identification	Industry- derived data	Preliminary categorization of nanomaterials in 9 classes (e.g. bulk, multiphase, structured surface, film, structured film)	SME, Industry, academia

Nano Exposure Categorization Framework (Hansen et al., 2008)	HHRA/ ERA	Problem formulation	Qualitative WoE, Expert judgement	Categorize the NOAA on the basis of the location of the nanoscale structures in their system/matrix for the purpose of Exposure assessment	Industry- derived data	Preliminary categorization of nanomaterials in 3 classes (i.e. in the bulk, on the surface, as particles) and corresponding sub-classes	SME, Industry, academia
Environmental exposure model (Boxall et al., 2008)	ERA	Exposure assessment	Material flow transport and fate modelling	Predict environmental concentrations of NOAA in different compartments	Production/use volumes, product market penetrations, environmental releases of NOAA, characteristics of receiving media	Predicted environmental concentrations (PEC) in soil, water and air	Academia
Environmental exposure model (Mueller and Nowack, 2008)	ERA	Exposure assessment	Material flow transport and fate modelling	Predict environmental concentrations of NOAA in different compartments and calculate risk quotients	Data on production/use volumes, product market penetrations, environmental releases of NOAA, characteristics of receiving media	Predicted environmental concentrations (PEC) in soil, water and air and associated risk quotients	Academia
Probabilistic Material Flow Model (Gottschalk et al., 2010)	ERA	Exposure assessment	Material flow transport and fate modelling	Predict environmental concentrations of NOAA in different compartments	Data on production/use volumes, environmental releases of NOAA, characteristics of receiving media	Predicted environmental concentrations (PEC) in soil, water and air	Academia

[a] human health Risk assessment. [b] ecological Risk assessment. [c] Small and medium enterprises

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CHAPTER 5

Tiered human health Risk assessment of engineered nano-objects and their aggregates and agglomerates: framework and methodology

Contents included in:

Hristozov D, Gottardo S, Cinelli M, Isigonis P, Zabeo A, Critto A, Van Tongeren M, Tran L, Marcomini A. (2012). Application of a quantitative Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for Titanium dioxide and Carbon nanomaterials. Nanotoxicology (in press)

and

Hristozov D, Zabeo A, Linkov I, Critto A, Isigonis P, Marcomini A. (2012b). A Weight of evidence approach for hazard screening of engineered nanomaterials. Nanotoxicology (in press)

5.1 Objectives

This chapter describes a framework for quantitative human health Risk assessment (RA) of nano-objects and their aggregates and agglomerates (NOAA), which is intended to facilitate both industrial and regulatory decision making.

The objectives of this framework are:

1) to introduce a flexible methodology, which is compliant with the REACH regulation; and
2) to incorporate a sound strategy for uncertainty analysis.

5.2 Conceptual framework

The framework consists of three tiers (Figure 5-1):

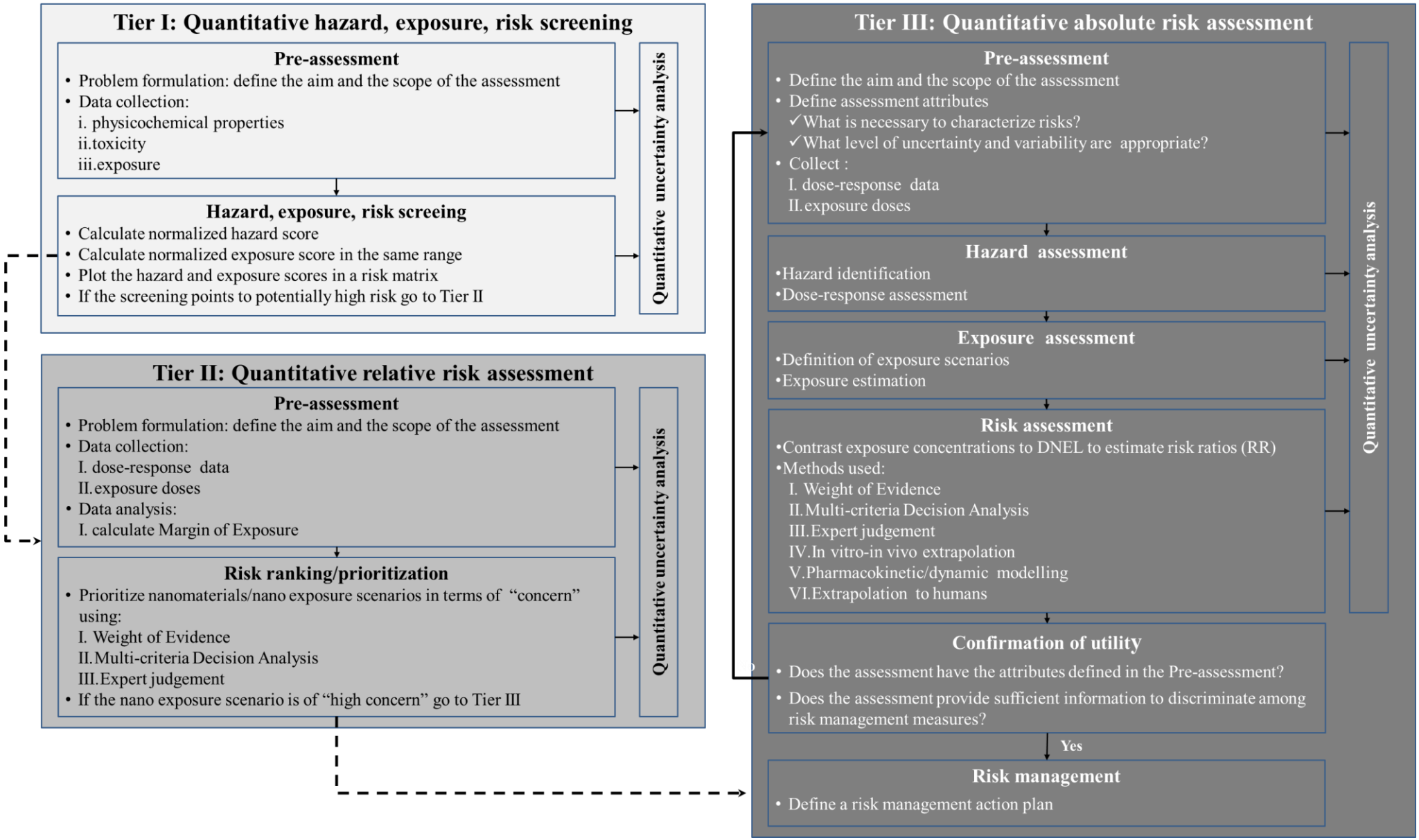
Tier I: Quantitative hazard, exposure, risk screening

Tier II: Quantitative relative Risk assessment

Tier III: Quantitative actual Risk assessment

The selection of the relevant tier by the assessor depends on two criteria: (i) aim of the assessment, and/or (ii) available data. The lower Tier I involved less data-intensive screening-level risk analyses intended for use by Small and Medium Enterprises (SME) or industries to support decisions about safe production and handling of NOAA. The intermediate Tier II is designed for use by industries, academia and/or regulators for prioritization of high-concern exposure scenarios (ES) and NOAA to be considered for actual RA in Tier III. It is important to note that higher tiers are relevant only if adjacent lower tiers set priorities for further testing and risk analysis or, in other words, if crude estimates indicate that margins between estimated exposure and hazard are large.

Figure 5-1: Tiered framework for health Risk assessment of engineered nano-objects and their aggregates and agglomerates.



5.2.1 Tier I: Quantitative hazard, exposure, risk screening

Tier I consists of the following steps:

1) Pre-assessment; and

2) Hazard, exposure, risk screening.

Pre-assessment

Pre-assessment is divided into (i) Problem Formulation; and (ii) Data Collection.

Problem Formulation is a systematic planning activity that identifies the major elements to consider in the study. In this step the goals and the scope of the assessment are defined, the target nanomaterials are introduced, the system boundaries (e.g. occupational settings) are identified.

In Data Collection, all available data on the elements to be considered in the study should be collected and stored in a database. Both qualitative and quantitative toxicological, physico-chemical and exposure data are relevant and should be considered. This compilation will form the foundation for further analysis.

Hazard, Exposure, Risk Screening

This analytical step involves Weight of evidence (WoE) models for nano hazard and exposure evaluation (described in section 5.3.1), which can be either used independently or integrated into an approach for risk screening of NOAA.

5.2.2 Tier II: Quantitative relative Risk assessment

Tier II involves the following steps to obtain relative risk ranking of NOAA (or other emerging stressors) and prioritise them for further testing and RA:

1) Pre-assessment;

2) Ranking and prioritization; and

3) Uncertainty characterization.

Pre-assessment

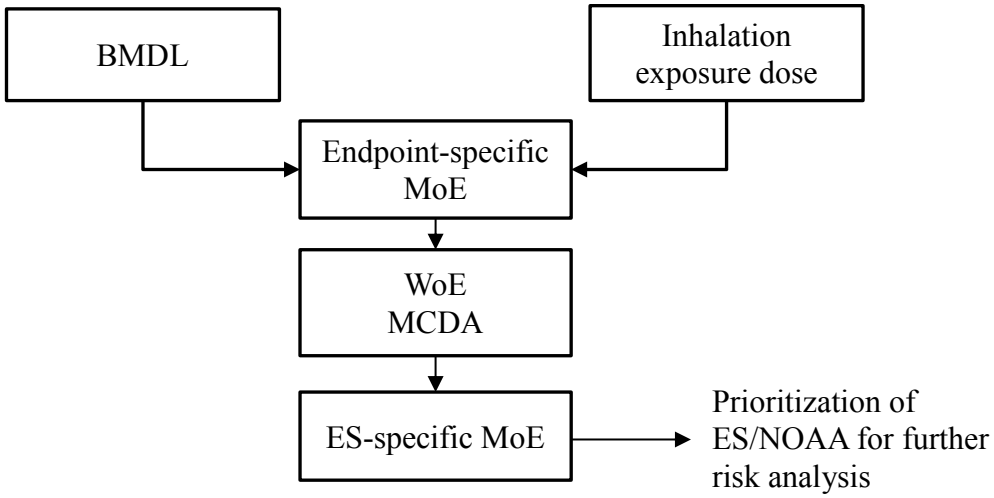
In Tier II, the Pre-assessment involves (i) Problem Formulation, and (ii) Data Collection and Analysis. Exposure doses (for various scenarios) and Points of Departure (PoD) (for different toxicological endpoints and organ systems) are collected. These data are then used to calculate Margins of exposure (MoE). MoE is the ratio of a PoD to an exposure dose (WHO/IPCS, 2009), representing the level of "concern" associated with handling or using a substance (Barlow et al., 2006; Benford et al., 2010; Omenn et al., 1997). This process is schematically represented in Figure 5-2.

Since the Benchmark Dose (BMD) method is considered statistically more powerful than the conventional No Observable Adverse Effect Level (EFSA, 2010; US EPA, 2000) (see chapter 3, section 3.1.3), we recommend using the BMD lower confidence limit (BMDL) as the PoD in calculating MoE.

Ranking and prioritization

The MoE estimated in the previous step are directly used in a quantitative WoE/Multi-criteria decision analysis (MCDA) approach (described in section 5.3.2) to prioritise high-concern ES/NOAA (Figure 5-2) for further risk analysis in Tier III.

Figure 5-2: Relative Risk assessment steps/components for calculation of ES-specific Margin of exposure. BMD=lower confidence limit of the Benchmark dose; NOAA= Nano-objects and their aggregates and agglomerates; ES=Exposure scenario; MCDA= Multi-criteria decision analysis; MoE=Margin of exposure; WoE=Weight of evidence.



Uncertainty characterization

Tier II employs a strategy for quantitative Uncertainty characterization, which is described in section 5.3.2.2.

5.2.3 Tier III: Quantitative actual Risk assessment

The high concern ES/NOAA identified in Tier II should be further analysed and their risk should be assessed in absolute terms. To this end, Tier III follows the steps of the conventional RA paradigm as described in the Chemical Safety Assessment (CSA) required by REACH, i.e.:

1) Pre-assessment;
2) Hazard assessment;
3) Exposure assessment;
4) Risk characterization; and
5) Uncertainty characterization.

Pre-assessment

Similarly to Tiers I and II the Pre-assessment is composed of Problem Formulation and Data Collection. Unlike the preceding tiers here the scope/goal is actual occupational or consumer RA of NOAA. OECD (2010) discussed the following problem formulation needs in regulatory risk analysis of nanomaterials, which are respected in the proposed framework:

- consider the "particle nature" of the material, such as the surface properties and interactions, the relation of metrics used, the characteristics of the material;
- assess and accommodate Risk assessment approaches with regard to the effects of test methods and exposure matrix (e.g. dispersion methods) on testing outcomes and on inter-comparability of the data used in the assessment; and
- include particular attention to the complex nature of the material (e.g. variation in size, surface properties, and composition that create a heterogeneous range of particle types) and its interaction with environmental components and transport mechanisms in exposure and toxicity contexts (OECD, 2010).

The dataset(s) collected for the lower-level assessments are complemented with new information and if the available evidences are still insufficient for regulatory RA, more data should be produced in the following steps.

Hazard assessment

The Hazard assessment involves two steps: (i) Hazard identification and (ii) Dose-response assessment. Detailed description of these processes is provided in chapter 3, sections 3.1.1 and 3.1.3. Hazard identification consists in gathering and evaluating relevant health and safety information (e.g. physico-chemical, toxicological data) in order to draw conclusions about the intrinsic hazard of a substance (European Chemicals Agency, 2007). Dose-response assessment is intended to quantitatively characterize the relationship between dose and the observed adverse response and to estimate an effect threshold above which humans should not be exposed, such as Derived No-effect Level (DNEL) (European Chemicals Agency, 2007).

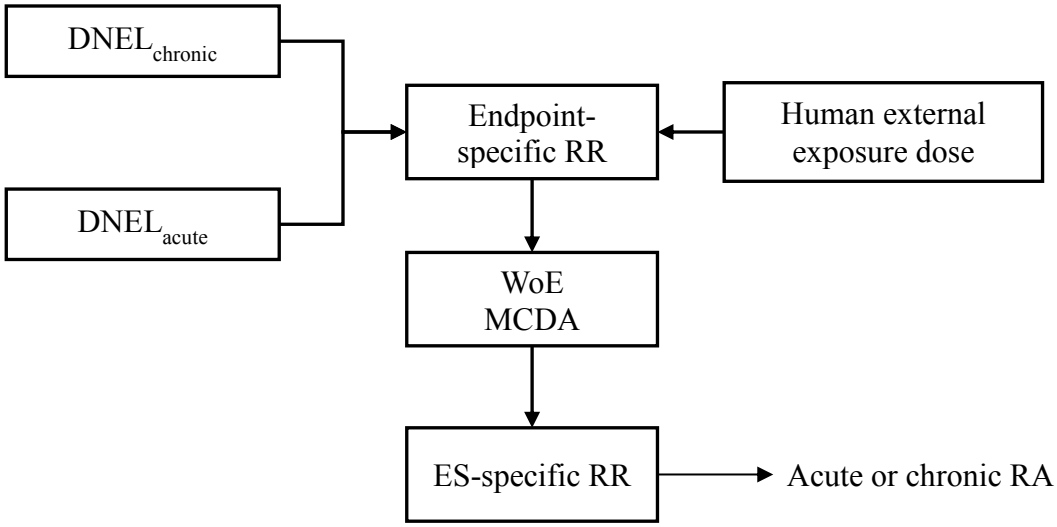
Exposure assessment

The Exposure assessment is introduced in detail in chapter 3, section 3.1.2. It generally starts with the formulation of one or more ES, addressing how a chemical is used by workers and/or consumers in different stages of its lifecycle. Then exposure is estimated for one or more routes (e.g. inhalation, dermal) under the conditions of use described in the ES. This includes, for instance, the estimation of indoor concentrations in consumer or occupational settings; the amount of the substance coming into contact with the skin, the intestine or the lungs; as well as exposure frequency and duration. Exposure estimates are derived typically either from measurements or modelling.

Risk characterization

In the Risk characterization the estimated exposure concentrations are compared to DNEL to obtain a risk ratio (RR) for each body system-endpoint combination. The RR is the ratio of a human exposure dose and acute or long-term DNEL. These RR values are then integrated into a final ES-specific risk score using a WoE model. The process is schematically presented in Figure 5-3 and described in detail in section 5.3.3

Figure 5-3: Actual Risk assessment steps/components for calculation of ES-specific Risk Ratio. RR=Risk Ratio; WoE=Weight of evidence; MCDA= Multi-criteria decision analysis; ES=Exposure scenario.



Uncertainty characterization

Tier III uses a methodology for quantitative Uncertainty characterization, described in section 5.3.2.2.

5.3 Methodological approach

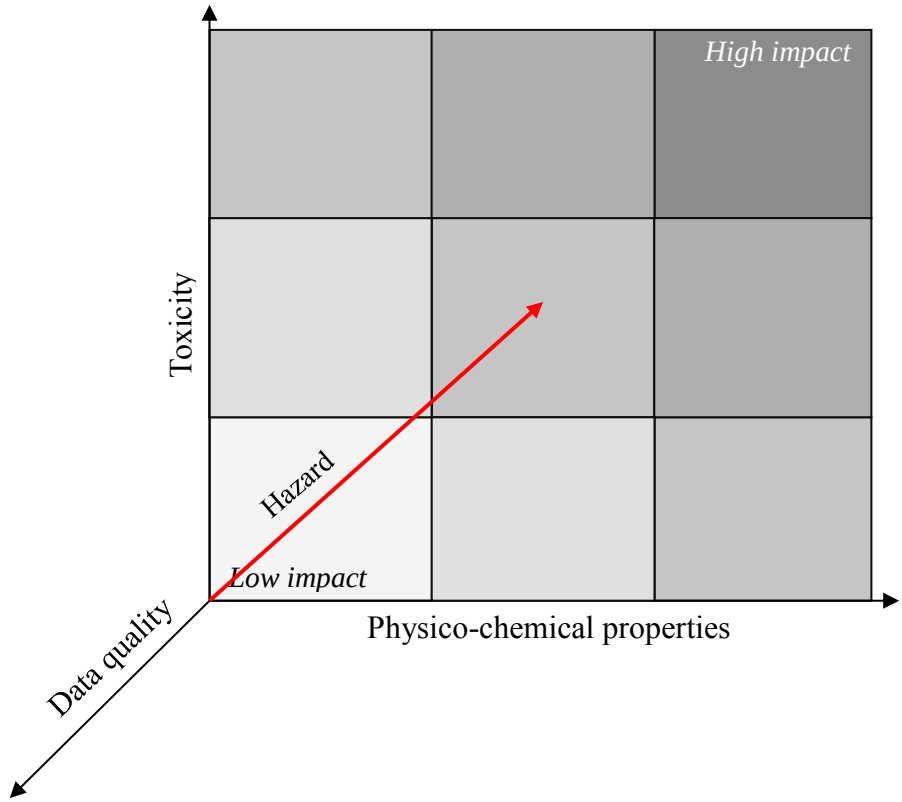
5.3.1 Tier I: Quantitative hazard, exposure and risk screening

Tier I involves WoE models for nano hazard and exposure screening, which can be either used independently or integrated into an approach for risk banding, as described below.

5.3.1.1 Hazard screening methodology

This section introduces a novel approach for hazard screening of emerging pollutants, also applicable to NOAA. In order to effectively account for the high uncertainty in the evidence base, the proposed model incorporates a system for data evaluation. In result, hazard is estimated based on three sets of criteria: physico-chemical properties, toxicity and data quality (Figure 5-4).

Figure 5-4: Hazard of nanomaterials is calculated on the basis of three sets of criteria, i.e. physico-chemical properties, toxicity and data quality.



5.3.1.1.1 LoE and aggregation algorithms

The approach incorporates both Index and Quantitative WoE (Linkov et al., 2009) (see chapter 3, section 3.2) and follows a Logic method, where one proceeds through the below uniform set of decision steps in which each study is treated as an individual Line of Evidence (LoE).

Step 1: Create an index corresponding to hazard classes based on physico-chemical data and assign a category distribution to each LoE.

A relevant set of criteria are identified, referring to physico-chemical properties affecting hazard of a particular kind of chemical stressor. A number of hazard classes $c_{i,1}^{p.chem}, \ldots, c_{i,n}^{p.chem}$ are mapped for each criterion onto a scoring system in the [0,100] range based on knowledge from the literature or expert judgment. The individual values are then aggregated by means of the arithmetic mean operator into a LoE-specific index $S_j^{p.chem.}$, which refers to the degree of hazard, intrinsic to the investigated nanomaterial. This aggregation step is represented by equation 1.

$$S_j^{p.chem.} = \frac{1}{n}\sum_{i=1}^{n} c_{i,(j)}^{p.chem}, \quad (1)$$

Step 2: Create an index corresponding to hazard classes based on evidence from toxicity studies and assign a class distribution to each LoE.

Hazard classes, based on toxicity data are mapped onto an index system such that a gradation exists between them. Each class (c_*^{tox}) is assigned some discrete value in the [0,100] range. For each LoE, the toxicological conclusion is determined to fall into one or more classes.

If a LOE corresponds strongly to a single category, it is described as fitting 100% in it. If its conclusion falls in multiple categories, a distribution value is assigned as the fraction of the conclusion in each of them. For example, if a study found positive genotoxicity, but it was flawed in some way, 50% (0.5) would be placed in the "likely to be toxic" class, while 50% (0.5) would fall into "inadequate information". For each LoE (j), the score (c_k^{tox}) assigned to class k is multiplied by its distribution value D_{ij} and the results are then summed to determine a LoE-specific index value, (S_j^{tox}), which refers to degree of intrinsic hazard. This is represented by equation 2.

$$S_j^{tox} = \sum_{k=1}^{5} c_k^{tox} D_{k,j} \tag{2}$$

Step 3: Aggregate the physico-chemical and the toxicity index values into a global index value for each LoE.

Both the physico-chemical and the toxicity data are indicative of intrinsic hazard. However they do not have equal weight in the assessment. In order to account for the difference in reliability we suggest using the Weighted Sum (WS) MCDA operator

WS is one of the best known MCDA methods. If we suppose that there are *m* alternatives and *n* decision criteria and that w_j denotes the relative weight of importance of the criterion C_j and a_{ij} is the performance value of alternative A_i, when it is evaluated in terms of the criterion C_j, then the total importance of alternative A_i, denoted as A_i^{WS}, is defined as follows:

$$A_i^{WS} = \sum_{j=0}^{n} w_j a_{ij}, \quad for\ i=1,2,3,\ldots,m. \tag{3}$$

In order to fit to our application framework equation 3 was transformed into equation 4, where S_j denotes the combined LoE index, $S_j^{p.chem.}$ and $S_j^{tox.}$ the physico-chemical and the toxicity index values, and $w_{p.chem}$ and w_{tox} are their respective weights, as $w_{p.chem} < w_{tox}$.

$$S_j = S_j^{p.chem.} \cdot w_{p.chem} + S_j^{tox.} \cdot w_{tox} \tag{4}$$

Step 4: Determine LoE weights within the information pool.

In order to determine the relative weight of each LoE a Logic model is applied, which uses regulatory data quality criteria.

Step 5: Obtain a collected weighted LoE index value.

In order to define the relative contribution of a particular study to the overall hazard assessment, its S_j should be multiplied by its weight (w_j). To do so our model uses the Multi-attribute value theory (MAVT) method (Keeney and Raiffa, 1993). MAVT allows integrating heterogeneous data into one-dimensional value function based on weighting of criteria and metrics associated with different sources of information. It can take nonlinear form, but this study uses linear approximation as represented by equation 5.

$$V(X^a) = \sum_i w_i \, v_i \, (x_i^a), \tag{5}$$

Here w_i is the weight for criterion i,($\sum_i w_i = 1$), v_i (x_i^a) is the single-attribute value function, and $V(X^a)$ is the overall value of information source a. This rule applies strictly under the condition of additive independence. In order to respect the $\sum_i w_i = 1$ condition of the proposed MAVT method, the weights should be normalized by dividing each of them by their sum as represented by equation 6.

$$WI_j = S_j w_j' \text{ , where } w_j' = \frac{w_j}{\sum_{\forall j} w_j} \tag{6}$$

The derived weighted LoE indices (WI_j) can be used to obtain relative hazard ranking of studies, or alternatively aggregated into a total weighted index value, V as it is represented by equation 7.

$$V = \sum_{j=1}^{n} WI_j \tag{7}$$

V can be compared with V' calculated for another stressor/nanomaterial for relative ranking of hazard.

5.3.1.1.2 Uncertainty analysis

The uncertainties in the proposed methodology are primarily associated with use of expert judgment to interpret evidence or establish weights for certain criteria and metrics. Monte Carlo was selected as the most relevant approach to analyse these types of uncertainty. This probabilistic method involves random sampling from the distribution of input values and weights in successive model simulations to derive a distribution of results that can be statistically analysed to obtain parameters such as mean and standard deviation (σ).

We suggest the following four uncertainty analysis steps:

1) Probability distributions for the input parameters are defined as the full range of the normalization scale (i.e. 0-100 for criteria/metrics and 0-1 for weights).
2) Input values are sampled from their corresponding probability distributions. The sampling is uniformly distributed within the interval.
3) The hazard assessment is run 1000-10000 times.

Changes in input parameters cause variations in the simulated outputs obtained as result of the Monte Carlo analysis, which gives information about the level of uncertainty in the obtained results as well as the sensitivity of the model with respect to certain input parameters.

5.3.1.2 Exposure screening methodology

This section describes a quantitative WoE approach for screening level Exposure assessment of NOAA, which can be applied for ranking/prioritization of occupational ES. The following paragraphs present the conceptual structure of the model, the rationale behind the selection of LoE and indicators and the mathematical algorithms used to aggregate them.

5.3.1.2.1 LoE and indicators

The proposed approach is composed of ten indicators organised in four LoE (Figure 5-5). Each of them is derived from recently developed models and tools, such as Stoffenmanager Nano (Duuren-Stuurman et al., 2011), Advanced Reach Tool (Fransman et al., 2009), US Control Banding Tool (Paik et al., 2008) and Precautionary Matrix for Synthetic Nanomaterials (Höck et al., 2010). One exemption is the "Process" indicator, defined based on expert judgment.

The Material characteristics (M) LoE is composed of two indicators, i.e. Physical environment (m_1), and Weight fraction (m_2) of the NOAA in the formulation. The Process characteristics (P) LoE involves only one indicator, i.e. Handling of solids and liquids (p_1). Some tasks refer to specific synthesis processes (e.g. laser ablation, flame pyrolysis, mechanical reduction) while others indicate more general activities (e.g. handling of small amounts) which are often performed in two alternative situations, i.e. with or without containment. If containment such as a fume hood is present the rating class is modified by an attenuation factor of 70%, as suggested by the developers of the Advanced Reach Tool (Fransman et al., 2009).

The Operational conditions (C) LoE involves indicators such as Duration (c_1) and Frequency (c_2), Process type (c_3), Amount of handled material (c_4), Use and Type of general ventilation (c_5).

The Risk management measures (R) LoE takes into account the factors that contribute to attenuation of exposure such as Use of local exhaust ventilation (LEV) (r_1) and respiratory protection equipment (RPE) (r_2).

A rating class in the [0,1] range is assigned to each indicator (Table 5-1). The available dataset is not always complete or explicit. For this reason, where real data are lacking the gaps are filled by deductions (e.g. from the information "handling of dry powder" we can deduce that the physical environment is air) or by assumptions based on conservative expert judgement (e.g. we assume no local exhaust ventilation if no information exists).

Figure 5-5: Conceptual structure of the Weight of evidence exposure model.

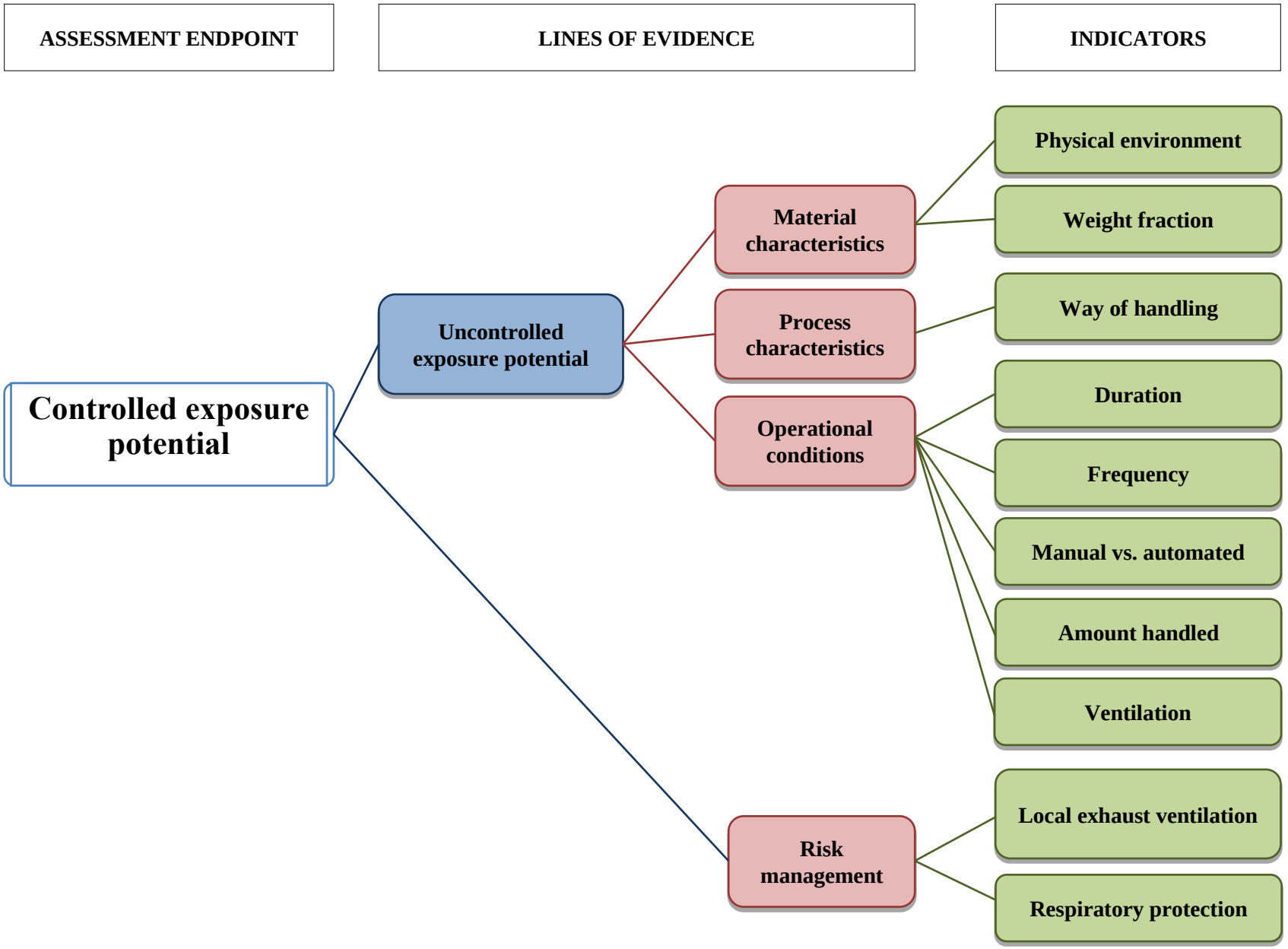


Table 5-1: Lines of Evidence (LoE), indicators and related rating classes used for ranking of occupational exposure scenarios. Proportions of data, deductions and assumptions used in regard to each indicator for the investigated exposure scenarios. NOAA = Nano-objects and their aggregates and agglomerates; S = solids; L = liquids; WC = with containment; NC = no containment.

LoE	Indicator	Rating class	Value	Symbol	Reference
Material characteristics (M)	Physical environment	solid matrix, stable under conditions of use, NOAA not mobile	0.0001	m_1	Precautionary Matrix for Synthetic Nanomaterials (Höck et al., 2010)
		solid matrix, stable under conditions of use, NOAA mobile	0.01		
		aerosol diameter > 10 µm, or liquid, or solid matrix, not stable under conditions of use	0.1		
		air, or aerosol diameter < 10 µm	1		
	Weight fraction	extremely small, i.e. < 0.01 %	0.00005	m_2	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		very small, i.e. 0.01 – 1 %	0.005		
		small, i.e. 1 – 10 %	0.05		
		substantial, i.e. 10 – 50 %	0.3		
		main component, i.e. 50 – 99 %	0.75		
		pure material, i.e. 100%	1		
Process characteristics (P)	Handling	S and L: in tightly closed containers	0	p_1	Stoffenmanager Nano (Duuren-Stuurman et al., 2011), modified after ART mechanistic model (Fransman et al., 2009)
		L: wet chemistry (synthesis - within solution)	0.00001		
		L: using low pressure, low speed with large or medium quantities (WC)	0.0003		
		S: mechanical reduction (preparing for imaging) L: using low pressure, low speed with large or medium quantities (NC)	0.001		
		S: in small amounts (up to 100 g) or in situations where only low quantities of product are likely to be released, (WC)	0.0009		
		S: in small amounts (up to 100 g) or in situations where only low quantities of product are likely to be released (NC); with low speed or with little force in medium quantities (several Kg) (WC); L: Sintering; wet chemistry (synthesis - into solution); wet chemistry (functionalization); laser ablation;	0.003		

		S: chemical vapour condensation; with low speed or with little force in medium quantities (several Kg) (NC)	0.01		
		S: with low speed or force, which leads to some dispersion of dust (WC) L: using low pressure, but high speed, resulting in generation of a mist or spray/haze,(WC)	0.009		
		S: Mechanical reduction (machining); with low speed or force, which leads to some dispersion of dust (NC); with medium speed or force, which leads to dispersion of dust (WC); L: using low pressure, but high speed, resulting in generation of a mist or spray/haze (NC)	0.03		
		S: with medium speed or force, which leads to dispersion of dust (NC); L: Flame pyrolysis	0.1		
		S: with a relatively high speed/force which may lead to some dispersion of dust (WC); L: at high pressure resulting in substantial generation of mist or spray/haze (WC)	0.09		
		S: with a relatively high speed/force which may lead to some dispersion of dust (NC); where due to high pressure, speed or high force, large quantities of dust are generated and dispersed (WC); L: at high pressure resulting in substantial generation of visible mist or spray/haze (NC)	0.3		
		S: where due to high pressure, speed or high force, large quantities of dust are generated and dispersed (NC)	1		
Operational conditions (C)	Duration (min/h)	1-30 min a day	0.06	c_1	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		0.5-2 h a day	0.25		
		2-4 h a day	0.5		
		Unknown	0.75		
		4-8 h a day	1		
	Frequency (1 d/year, month, week)	1 day a year	0.01	c_2	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		1 day a month	0.05		
		1 day per 2 weeks	0.1		
		1 day a week	0.2		

		2-3 days a week	0.6		
		Unknown	0.75		
		4-5 days a week	1		
	Process type	Automated	0.3	c_3	Expert judgement
		Manual	1		
	Amount handled (mg)	0-10 mg	0.25	c_4	Zalk et al. (2009)
		11-100 mg	0.5		
		Unknown	0.75		
		> 100 mg	1		
	Use of general ventilation	spraying booth, room volume < 100 m3	0.01	c_5	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		spraying booth, room volume 100- 1000 m3	0.03		
		natural/mechanical ventilation, room volume >100 m3; no general ventilation, room volume > 1000 m3; natural/mechanical ventilation, work is done outside; spraying booth, room volume > 1000 m3	0.1		
		natural/mechanical ventilation, room volume < 100m3; no general ventilation, room volume > 100 m3	0.3		
		no general ventilation, room volume < 100m3	1		
Risk management measures (R)	Use of local exhaust ventilation (LEV)	yes	0.3	r_1	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		no	1		
	Use of respiratory protective equipment (RPE)	yes	0.4	r_2	Stoffenmanager Nano (Duuren-Stuurman et al., 2011)
		no	1		

5.3.1.2.2 Aggregation algorithms

The indicator values are aggregated into scores for the M, P and C LoE, which are integrated into a single Uncontrolled Exposure Potential (Ep) index for each ES. Finally, Controlled Exposure Potential (Ec) is calculated by multiplying the Ep by an attenuation factor referring to the R LoE. The aggregation algorithms used are based on Weighted Average (WA) and Ordered Weighted Average (OWA) MCDA methods.

Equation 8 is used to obtain scores for the M LoE.

$$M = w_{m1}*m_1+w_{m2}*m_2 \quad (8)$$

Here m_1 and m_2 are indicators for M, and w_{m1} and w_{m2} are their respective weights.

The P LoE is composed of 1 indicator only, therefore aggregation is not needed.

The C LoE score is calculated by Equation 9.

$$C = w_{c1}*(c_1*c_2)+w_{c2}*c_3+ w_{c3}*c_4 + w_{c4}*c_5 \quad (9)$$

Here $c_{1\text{-}5}$ are indicators for *C*, and $w_{c1\text{-}4}$ are their respective weights. Since Duration (c_1) and Frequency (c_2) determine the total amount of exposure time, they are coupled and multiplied, while the other parameters are independent from each other and therefore treated separately.

Exposure attenuation factors are multiplied into the R LoE index by Equation 10:

$$R = r_1*r_2 \quad (10)$$

The next step involves calculating the Uncontrolled Exposure Potential (Ep) from the M, P and C LoE, which is represented by Equation 11.

$$Ep = w_1*OWA(M,P)+w_2*C \quad (11)$$

Here w_1 and w_2 refer to the weights used. Each of the M or P LoE is a significant determinant of exposure potential and as such is sufficient to determine low Ep even if the other LoE points to higher Ep. In order to account for this behaviour we aggregate them by the OWA operator.

The last aggregation step is represented by Equation 12.

$$Ec = Ep*R \quad (12)$$

Here the Ep is attenuated by the R in order to estimate the Ec, which represents controlled exposure potential after RMM are implemented.

The weights used in the calculations (Table 5-2) are based on expert judgment informed by the state of the art literature. The Physical environment indicator (m_1) is more important than Weight fraction (m_2) because an elevated fraction cannot cause high exposure if embedded in a (solid) material preventing the

NOAA from becoming airborne (Hansen et al., 2008; Höck et al., 2010; Paik et al., 2008). In the literature the Use of general ventilation (c_5) is considered a more important factor than other operational conditions used as indicators, and therefore it is given the highest weight (0.35), while the lowest (0.15) belongs to the Process type (c_3) (Duuren-Stuurman et al., 2011). Duration (c_1) and Frequency (c_2) are considered more important than the Amount of handled material (c_4) (Paik et al., 2008) and therefore their combined w_{c1} is higher than w_{c4} .Moreover, since both M and P are very strong potential exposure factors (Duuren-Stuurman et al., 2011; Höck et al., 2010), the w_1 assigned to the OWA operator is higher than the w_2 for C.

Table 5-2: Weights (w) used for calculation of exposure scores with their higher and lower confidence intervals. LoE=Line of evidence. OWA=Ordered weighted average. Na=Not applicable.

Score	w_1 low	w_1	w_1 high	w_2 low	w_2	w_2 high	w_3 low	w_3	w_3 high	w_4 low	w_4	w_4 high
Material characteristics LoE (M)	0.7	0.80	0.9	0.1	0.20	0.3	Na	Na	Na	Na	Na	Na
Operational conditions LoE (C)	0.2	0.30	0.4	0.05	0.15	0.25	0.1	0.20	0.3	0.25	0.35	0.45
Potential Exposure (Ep)	0.7	0.80	0.9	0.1	0.20	0.3	Na	Na	Na	Na	Na	Na
OWA operator	0.7	0.80	0.9	0.1	0.20	0.3	Na	Na	Na	Na	Na	Na

5.3.1.2.3 Reliability estimation

When real data are lacking deductions and assumptions are used to assign rating classes to the indicators. In order to account for the difference in reliability among the three types of evidence uncertainty scores α are defined, i.e. 1, 0.4, and 0.2 for “real data”, “deductions” and “assumptions” respectively. Such scores can be interpreted as errors due to the lack of (explicit) information and can be used to evaluate the reliability of the Ec for each scenario. Accordingly, an error U_i is calculated for each LoE, Ep and Ec by applying the error propagation rules in Table 5-3.

Table 5-3: Equations to derive reliability scores for different levels of aggregation.

Aggregation algorithm	Corresponding error (U_i)
$M = w_{m1}m_1 + w_{m2}m_2$	$U_M = \sqrt{(w_{m1}\sigma_{m1})^2 + (w_{m2}\sigma_{m2})^2}$
$P = p_1$	$U_P = \sigma_{p1}$
$C = w_{c1}(c_1 \cdot c_2) + w_{c2}c_3 + w_{c3}c_4 + w_{c4}c_5$	$U_C = \sqrt{\left(\left(\sqrt{\left(\frac{\sigma_{c1}}{c_1}\right)^2 + \left(\frac{\sigma_{c2}}{c_2}\right)^2} \cdot (c_1 c_2)\right) w_{c1}\right)^2 + (w_{c2}\sigma_{c3})^2 + (w_{c3}\sigma_{c4})^2 + (w_{c4}\sigma_{c5})^2}$
$R = r_1 \cdot r_2$	$U_R = \sqrt{\left(\frac{\sigma_{r1}}{r_1}\right)^2 + \left(\frac{\sigma_{r2}}{r_2}\right)^2} \cdot (r_1 \cdot r_2)$
$E_p = w_1 \cdot \min(M, P) + w_2 \cdot C$	$U_{E_p} = \sqrt{\left(w_1\sigma_{\min(M,P)}\right)^2 + (w_2\sigma_C)^2}$
$E_c = E_p \cdot R$	$U_{E_c} = \sqrt{\left(\frac{\sigma_{E_p}}{E_p}\right)^2 + \left(\frac{\sigma_R}{R}\right)^2} \cdot (E_p \cdot R)$

Legend:		
	M, P, C, R	Material (M) and process (P) characteristics, Operational conditions (O), and Risk management measures (R) LoE
	σ	Reliability score for a typology, i.e. real datum, deduction or assumption
	w	Weight
	U_i	Reliability score
	m_i, p_1, c_i, r_i	Indicator value
	Ep	Potential exposure
	Ec	Estimated exposure

The input error takes into account the distance between the value assigned to a certain indicator and both the adjacent higher and lower rating classes. If the indicator value is derived from assumptions, the associated error (σ_x) is calculated by summing up the 40% of the distance from the lower class and the 40% of the distance from the upper class. If the indicator value is derived from a deduction, the 20% of the distance from the lower and upper classes are considered. Error propagation rules are then applied to the σ_x values in order to calculate the Ec error (U_{Ec}).

5.3.1.2.4 Validation of results

In order to verify the obtained results, the WoE-based ranking of ES can be compared to a ranking of measured concentrations reported for the same ES. It has to be taken into account that monitoring data are typically obtained from different instruments operating in different size ranges and therefore they are often not directly comparable.

In order to allow comparison the values of the measured concentrations must be normalized to the [0,1] scale. This can be done in a sound way by using the [0, max] normalization scale where max is the maximum observed concentration in all scenarios of both groups. The two rankings can then be compared by using the procedure proposed by Nardo et al. (2005), where the average of the absolute differences in alternatives' ranks are calculated using Equation 13.

$$\Delta V = \frac{1}{|S'|} \sum_{\forall s \in S'} |V(R_0(s)) - V(R_1(s))| \quad (13)$$

Here ΔV is the average difference in values, $V(R_*(s))$ represents the value of scenario s in ranking R_* and |S´| is the cardinality of S´ (i.e. the number of scenarios in the group S´) (Nardo et al., 2005).

One additional parameter is calculated for each ES according to Equation 14.

$$\text{Diff\_value} = V(R_0(s)) - V(R_1(s)) \quad (14)$$

Here Diff_value is the non-absolute difference in value, obtained by comparing the two ranks for each ES. Specifically, Diff_value provides information on the magnitude of the discrepancy between a WoE estimate for a certain ES and the corresponding normalized concentration. In contrast to ΔV, Diff_value does not average values for all ES in a group, but treats individual ES.

5.3.1.2.5 Uncertainty analysis

Uncertainty is inherent in the model output and in order to evaluate it we should analyse the role of the (type of) input data and weights in establishing the final results. Monte Carlo was selected as the most relevant approach to do this. The adopted uncertainty analysis approach consists of the following three steps.

1) Probability distributions for the input parameters are defined, which consist in the distance between the rating classes. In the case that the indicator value is assigned by an assumption, 40% of the distance between the rating class above and below is considered. This interval is 20% when a deduction is performed and 0 in the case of real data. These percentage ranges were decided by expert judgment on the basis of the notion that the real data are more reliable than deductions and assumptions, as the latter are the least reliable among the three.
2) Input values are sampled from their correspondent probability distributions. The sampling is equally distributed within the interval.
3) The WoE-based model is run 1000 times and a ranking of scenarios is obtained for each simulation.

Changes in input parameters cause variations in the 1000 rankings obtained as result of the Monte Carlo analysis. This variability is assessed and presented by means of the ΔV parameter again.

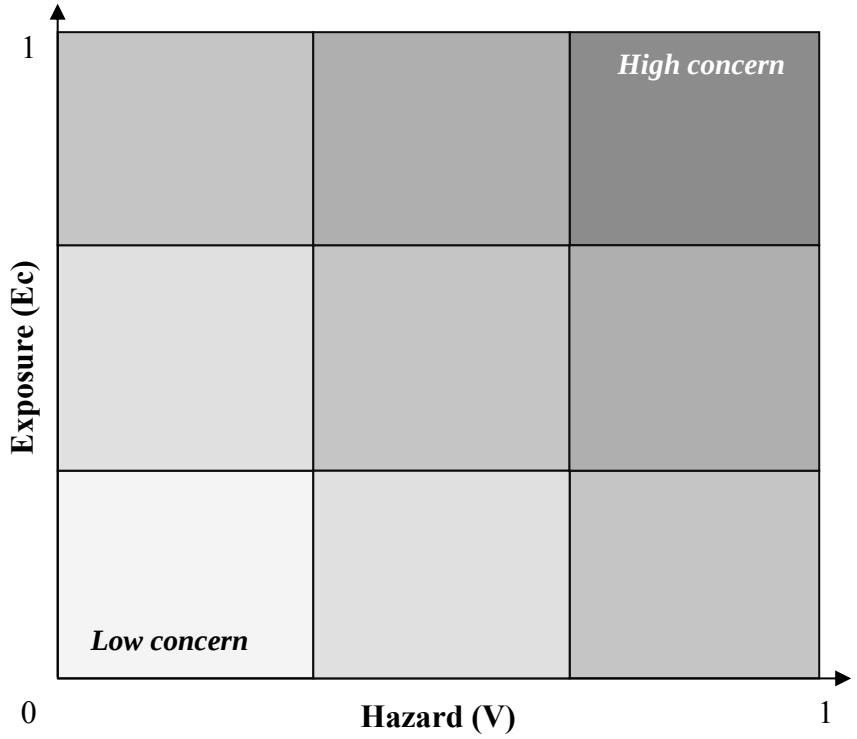
In order to further evaluate the stability of the model, we apply uncertainty analysis to the input weights as well. To define a probability distribution for each weight we used expert judgment to define upper and lower confidence intervals of +/- 0.1 (i.e. +/- 10%) of its original value (Table 5-2). Inside these intervals Monte Carlo picks up random values used to runs the WoE model to compute 1000 rankings. Here again the ΔV is used to illustrate variability.

5.3.1.3 Integrating Hazard and Exposure

The hazard screening approach introduced in section 5.3.1.1 calculates collected weighted LoE index values (V) normalized in the [0,100] interval. The exposure model presented in section 5.3.1.2 estimates Ec in the [0,1] range. Once they are normalized to the same scale, V is multiplied by the Ec to obtain a value Q,

which does not represent actual risk but relative concern. Using this value two or more combinations of NOAA and ES (NOAA/ES) can be compared. In order to visualize the integration of estimated hazard and exposure we can use a matrix similar to the one on Figure 5-6.

Figure 5-6: A risk matrix to integrate hazard and exposure estimates.



5.3.2 Tier II: Quantitative relative Risk assessment

5.3.2.1 LoE, indicators and aggregation algorithms

Tier II uses a quantitative Index WoE/MCDA methodology to prioritise NOAA (or other emerging stressors) for further testing and RA. It follows 3 steps:

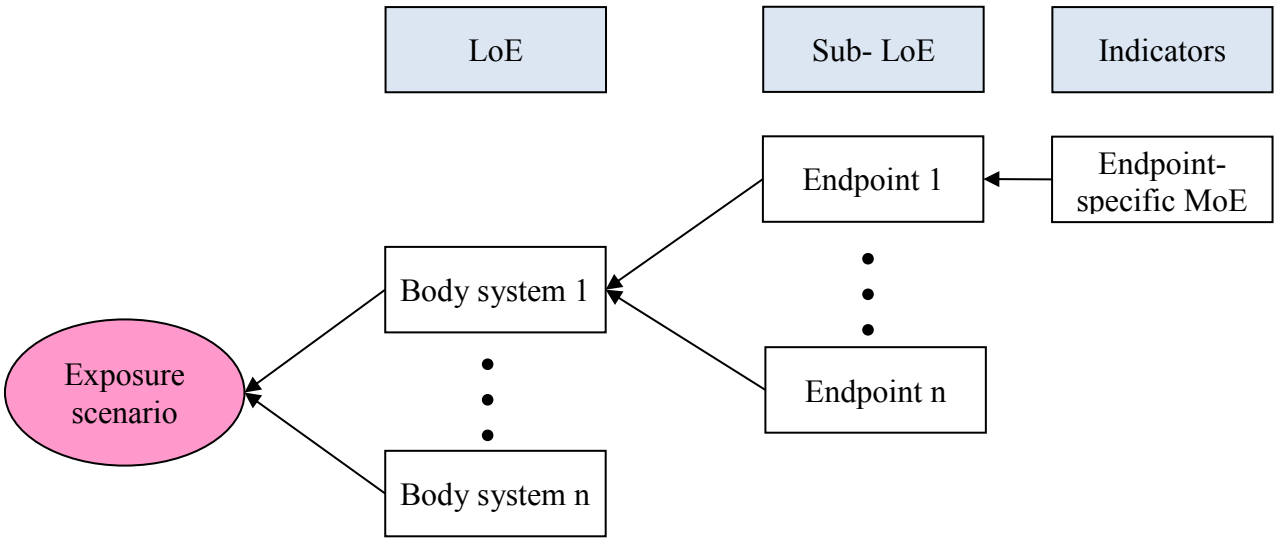
1) normalization of indicator values (i.e. endpoint -specific MoE corresponding to particular body systems);
2) aggregation of the normalized indicators into LoE scores (i.e. endpoint -specific MoE into body system-specific MoE and then into ES/NOAA-specific MoE); and
3) relative risk ranking/prioritization of ES and corresponding NOAA (based on the ES/NOAA-specific MoE).

The maximum (M_{max}) and the minimum (M_{min}) MoE among all available values are used to define the upper and the lower limits of the [0,1] normalization scale. Each MoE value ($M_{p,s,b,e,}$) is normalized and given a numerical value ($M'_{p,s,b,e}$) within this range. The normalization algorithm is represented by equation 15, where p = nanomaterial, s = exposure scenario, b = body system, and e = toxicological endpoint.

$$M'_{p,s,b,e} = 1 - \frac{M_{p,s,b,e} - M_{min}}{M_{max} - M_{min}} \quad (15)$$

Since higher MoE indicate lower concern (WHO/IPCS, 2009), an inverse scale is used so that the maximum MoE will correspond to a score of 0 (i.e. null/negligible concern) and the minimum MoE to 1 (i.e. high concern). After normalization, the endpoint-specific MoE values are directly used in a WoE approach to calculate final ES-specific MoE. Its conceptual structure is shown in Figure 5-7.

Figure 5-7: Hierarchical structure of the WoE approach developed for Tier II. LoE = Line of Evidence, MoE=Margin of exposure.



In order to aggregate the normalized endpoint-specific MoE values into a score for a single body system, we use an aggregation operator consisting in the WS of OWA (Dongrui and Mendel, 2010). This aggregator has been selected on the basis of the notion that under the same exposure conditions (i.e. concentration, duration, and frequency) some endpoints constitute higher risk than others. Based on this consideration, two groups of endpoints were identified: high risk (HR) (e.g. genotoxicity, fibrogenecity and functional toxicity, developmental toxicity) and low risk (LR) (e.g. oxidative stress or inflammation) endpoints.

The WS (equation 3) is one of the simplest MCDA methods, but is applicable only when all the data are expressed in the same unit. In our approach this condition is satisfied since all input MoE values are normalized into a scale between 0-1. For the purpose of this aggregation step, the above equation 3 was transformed into the following expression.

$$M'_{p,s,b} = w_{LR} LR_{p,s,b} + w_{HR} HR_{p,s,b} \quad (16)$$

Here $M'_{p,s,b}$ is the index for a body system b derived from summing $LR_{p,s,b}$ and $HR_{p,s,b}$: i.e. scores obtained from aggregating the sets of LR and HR endpoints by applying the OWA operator. The w_{LR} and w_{HR}weights are assigned by expert judgment and they should sum to 1.

The OWA is another simple and widely used MCDA operator. The formal definition of the OWA operator for n criteria $c_1, \dots, c_n$ with weights $w_1, \dots, w_n$ is given below.

$$\mathrm{OWA}(c_1, \dots, c_n) = \sum_{i=1}^{n} w_i \cdot c_{\sigma(i)} \tag{17}$$

Here σ is a permutation that orders the elements $c_{\sigma(1)} \leq \cdots \leq c_{\sigma(n)}$ and all the weights w_i are non-negative and summing up to 1.

In order to obtain $LR_{p,s,b}$ and $HR_{p,s,b}$ we apply the OWA operator with two different sets of weights $\{wr_1, .., wr_{nr}\}$ and $\{wi_1, \dots, wi_{ni}\}$ so that:

$$LR_{p,s,b} = \mathrm{OWA}\left(Mr'_{p,s,b,1}, \dots, Mr'_{p,s,b,nr}\right) = \sum_{j=1}^{nr} wr_j \cdot Mr'_{p,s,b,\sigma(j)} \tag{18}$$

$$HR_{p,s,b} = \mathrm{OWA}\left(Mi'_{p,s,b,1}, \dots, Mi'_{p,s,b,ni}\right) = \sum_{j=1}^{ni} wi_j \cdot Mi'_{p,s,b,\sigma(j)} \tag{19}$$

All weights are assigned on the basis of expert judgment.

By applying these equations a single score for each body system is derived. The obtained scores are aggregated into a final relative risk score for each investigated ES/NOAA. On the basis of the assumption that a single body system at high concern is enough to establish an overall high concern for the whole organism, the maximum operator was used for this aggregation step (equation 20).

$$M'_{p,s} = \max\left(M'_{p,s,b1}, \dots, M'_{p,s,bn}\right) \tag{20}$$

The scores estimated for each pair of NOAA and ES are used to build a complete order ranking. This enables the assessors to compare ES for the same or different NOAA in terms of potential risk in order to identify high-concern scenarios and nanomaterials for further testing and RA. All uncertainties related to the production of data, use of models and the application of the WoE-based aggregation procedure are characterized in the following Uncertainty characterization step.

5.3.2.2 Uncertainty characterization

Probabilistic Monte Carlo uncertainty analysis will be performed to test the performance of the above risk model with respect to deviations in the BMDL and the exposure doses. As these two quantities represent two different aspects of the methodology they should be analysed independently. The adopted Tier II uncertainty analysis approach consists of the following three steps.

1) Define a probability distribution for each input parameter (i.e. BMDL, exposure dose) accounting for the full range of uncertainty.

a. The BMDL is the lower limit of the BMD confidence interval. Uncertainties, associated with the design and performance of the toxicological tests used to obtain BMD are not reflected in it. In order to take them into account the interval can be widened by means of expert judgment. The result is a normally distributed probability density function with mean at the BMDL and variance accounting for the extended range of uncertainty.

b. Exposure doses are estimated based on measurements and/or modelling. Measurements are obtained by instruments, which present known measurement errors. However, many other aspects of the sampling process (e.g. position of the instrument, time of the measurement) add more uncertainties, often larger than the measurement error itself. Some exposure models estimate and report the errors in their results; however this is not always the case. In order to account for the full range of uncertainties we suggest applying normal probability distribution, centered at the measured exposure concentration or model estimate, with variance considering the instrument-specific measurement error or modelling error plus an expert judgment extension of the confidence interval to account for further uncertainties from experimental design and/or modelling.

2) Input values (i.e. BMDL and exposure doses) are sampled from their correspondent probability distributions. The sampling is normally distributed within the interval.

3) The WoE-based model is run 1000 times and a ranking of scenarios is obtained for each simulation.

Changes in input parameters cause variations in the 1000 rankings obtained as result of the Monte Carlo analysis. This variability is assessed and presented by means of the ΔV parameter (see section 5.3.1.2.4) representing the average difference in values between each simulated ranking and the reference one.

5.3.3 Tier III: Quantitative actual Risk assessment

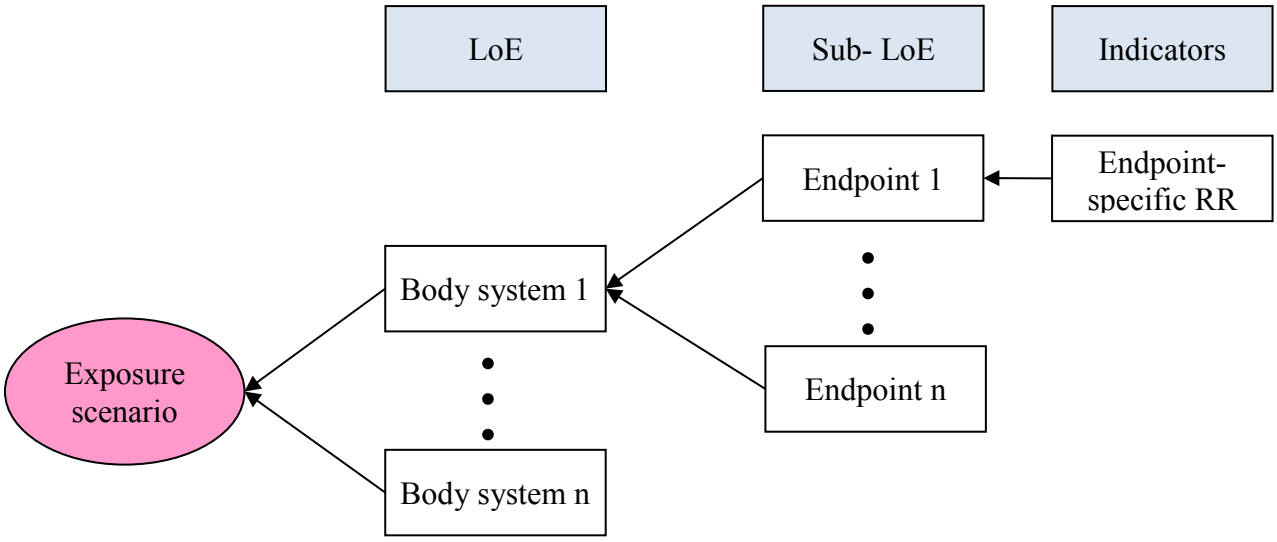
5.3.2.1 LoE, indicators and aggregation algorithms

The high-concern ES/NOAA identified in Tier II are further investigated in Tier III and data are generated to calculate endpoint-specific acute and/or chronic RR values, which are normalized to a single [0,1] scale by the following equation:

$$RR = \begin{cases} 1 & , Dose > \text{DNEL} \\ \dfrac{Dose}{\text{DNEL}} & , else \end{cases} \quad (21)$$

Once a $RR_{p,s,b,e}$ is calculated for each nanomaterial p, scenario s, body system b and endpoint e, they are aggregated towards ES-specific $RR_{p,s}$ following the hierarchy on Figure 5-8.

Figure 5-8: Hierarchical structure of the WoE approach for Tier III actual Risk assessment. LoE = Line of Evidence, RR=Risk ratio.



Similarly to Tier II, endpoints are subdivided into low risk (LR) and high risk (HR), and the OWA operator is used to aggregate the corresponding endpoint-specific RR into $LR_{p,s,b}$ and $HR_{p,s,b}$ indices. In Tier III these indices are rescaled using the sin function presented in the equations below:

$$LR'_{p,s,b} = \sin\left(\frac{(LR_{p,s,b} - 1)\pi}{2}\right) + 1 \quad (22)$$

$$HR'_{p,s,b} = \sin\left(\frac{HR_{p,s,b}\,\pi}{2}\right) \quad (23)$$

The final aggregation of endpoint scores into a body system index $RR_{p,s,b}$ is represented by equation 24.

$$RR_{p,s,b} = w_{LR} LR'_{p,s,b} + w_{HR} HR'_{p,s,b} \quad (24)$$

In order to emphasize the higher importance of HR factors the weight w_{LR} should be smaller than w_{HR}, as their values should be assigned by means of expert judgement.

In the final aggregation step a single RR for each ES is obtained from the aggregation of body system scores. Under the assumption that a single body system at high risk is enough to establish an overall high risk for the organism the maximum operator is used as presented by equation 25.

$$RR_{p,s} = \max\left(RR_{p,s,b_1}, \ldots, RR_{p,s,b_n}\right) \quad (25)$$

If the final RR equals 1, then the risks are NOT adequately controlled and appropriate Risk management measures should be implemented. If RR < 1 the risks are adequately controlled. The distance of RR from 1 provides information to the decision maker about the level of risk control. RR smaller, but close to 1 should be given special attention.

5.3.2.2 Uncertainty characterization

Tier III uses the Tier II Uncertainty characterization strategy, which is described in section 5.3.2.2.

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CHAPTER 6

Tiered human health Risk assessment of engineered nano-objects and their aggregates and agglomerates: results and discussion

Contents included in:

Hristozov D, Gottardo S, Cinelli M, Isigonis P, Zabeo A, Critto A, Van Tongeren M, Tran L, Marcomini A. (2012). Application of a quantitative Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for Titanium dioxide and Carbon nanomaterials. Nanotoxicology (in press)

and

Hristozov D, Zabeo A, Linkov I, Critto A, Isigonis P, Marcomini A. (2012b). A Weight of evidence approach for hazard screening of engineered nanomaterials. Nanotoxicology (in press)

This chapter reports and discusses the results of applying the tiered framework for Risk assessment (RA) to several commercially available nano-objects and their aggregates and agglomerates (NOAA), i.e. carbon nanotubes (CNT), titanium dioxide (TiO_2), zinc oxide (ZnO) and silver (Ag). Tier I was applied with heterogeneous data from the peer-reviewed literature. Tiers II and III used standard toxicity data from the ENPRA project and exposure estimates derived using the NanoSafer model (Jensen et al., in preparation) with data from the NANEX, NANOSH and NANO-INNOV projects well as from the United States NIOSH agency.

6.1 Tier I: Quantitative risk screening

6.1.1 Pre-assessment

Problem formulation

TiO_2 (molecular weight 79.9) was selected as a representative nanomaterial to illustrate the application of the proposed Tier I approach. TiO_2 appears as white powder at room temperature and has two representative crystal structures: rutile and anatase. The TiO_2 nanomaterials are largely defined as particles with primary size ranging from approximately 1 to 100 nm and aggregates of the primary particles. The physico-chemical properties of the nano-TiO_2 used in toxicity tests (e.g. size distribution, crystallinity, surface coating) vary significantly among studies.

Compared to coarser TiO_2, the nanoparticles have the following specific characteristics:

1) large specific surface area per unit weight;
2) increases the absorption of ultraviolet radiation and decreases the scattering of the visible light.

The enhanced photocatalitic activity of nano-TiO_2 is a result of the first characteristic and its increased application in sunscreen formulations is due to the second.

Nano-TiO_2 is primarily used in paints, inks, pigments and plastics. Its production in the US is expected to grow from about 30 000 million tons in 2012 to the estimated 2 400 000 million tons in 2026 and it will account for about 99% of the total US TiO_2 production (Robichaud et al., 2010). Although TiO_2 is generally considered a biologically inert material, nano-TiO_2 may pose adverse health effects due to its enhanced reactivity (Stone et al., 2009). Workers are likely to be exposed to highest nano-TiO_2 concentrations via inhalation. Therefore the aim of this screening level assessment is to predict the hazard/risk from nano-TiO_2 inhaled in occupational settings.

Data collection

We collected 29 peer reviewed articles (Annex 3) and reports from major projects funded by EU that provide information on nano-TiO_2 physico-chemical properties and toxicity, as well as sufficient information to assess data quality. Priority has been given to sources of information that are most current, peer reviewed, transparent and publicly available. These 29 papers report nano-TiO_2 toxicity data for 62 endpoints.

We have developed a database that includes information for these 62 endpoints. A number of physico-chemical characteristics related to absorption and bioaccumulation potential, ability to cross biological barriers (e.g. blood-brain, blood-placenta), to deposit in the lungs or interact with other chemicals were selected to represent health hazards of nano-TiO_2 (Borm and Kreyling, 2011; Maynard and Kuempel, 2005). They were classified in two groups based on primary influence on (i) effects, and (ii) internal exposure. The physico-chemical criteria selected to represent NOAA adverse biological effects are reactivity, composition, purity, and morphology/shape. Properties influencing NOAA uptake, translocation and bioaccumulation are size, colloidal stability and surface chemistry.

Moreover we collated data from the catalogue of the NANEX project, which contains 25 exposure scenarios (ES) for nano-TiO_2. 81% of them report particles number concentrations, while 6.5% report mass concentrations, and12.5% no measurements at all. Almost 40% of the particle number concentrations were derived by scanning mobility particle sizers (SMPS), while 22% and 10% are obtained by condensation particle counters (CPC) and optical particle counters (OPC), respectively.

In order to compare the exposure potential of nano-TiO_2 with other nanomaterials, we collected data for CNT and fullerenes, also from the NANEX database. Since these data were derived by different instruments operating in different size ranges they could not be directly compared. Therefore two homogeneous groups of comparable ES (A and B) were identified. Group A contains 12 ES with background-adjusted by subtraction number concentrations for particles in the 10-1000 nm range measured by CPC. Group B

includes 8 ES with activity-specific number concentrations for particle sizes smaller than 100 nm measured by SMPS. They are reported in Table 6-1.

Table 6-1: Exposure scenarios (ES) in groups A and B. Group A scenarios report background-adjusted by subtraction number concentrations for particle size range 10-1000 nm [Condensation particle counter (CPC)]. Group B scenarios report activity-specific number concentrations for particles < 100 nm [Scanning Mobility Particle Sizer (SMPS)]. NOAA= Nano-objects and their aggregates and agglomerates; ES ID = Scenario identification number in the NANEX database. MWCNT=Multi-walled carbon nanotube.

Group	NOAA type	ES ID	ES description	Measured concentration (Particle number)
A	TiO_2	3.4	Lab: Creating stock solutions in fume hood	0
	MWCNT	57.1	Opening growth chamber (with exhaust)	300
	MWCNT	53.2	Weighing functionalised MWCNT	680
	MWCNT	53.4	Sonication of functionalised MWCNT	730
	TiO_2	3.5	Lab: Transferring material during weighing or into vials for solution prep	1350
	Fullerenes	54.1	Weighing C_{60} fullerenes	1476
	MWCNT	53.1	Weighing raw MWCNT	1530
	Fullerenes	54.2	Sonication of C_{60} fullerenes	2176
	MWCNT	53.3	Sonication of raw MWCNT	2500
	TiO_2	3.1	Manufacturer: Dumping into mixing tank using focused LEV	3500
	TiO_2	3.3	Manufacturer: Manual (un)loading trays inside booth	15500
	MWCNT	57.2	Opening growth chamber (no exhaust)	42200
B	CNT	15.2	Weighing of CNT powder	0
	TiO_2	8.1	Weighing of TiO_2 powder	0
	TiO_2	16.1	Laser ablation	0
	CNT	13.1	Dry mounting of CNT on to EM (electron microscopy) grids	95
	CNT	15.1	Transfer of liquid containing CNT	462
	CNT	14.1	Production of filaments of CNT	1373
	TiO_2	21.1	Dumping large amount of powder into vessel	4032
	TiO_2	18.2	Bag/bin filling	8645

6.1.2 Hazard screening

The European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) sets forth a process for Hazard identification of chemical substances (European Chemicals Agency, 2007). According to REACH, the process starts with collection of physico-chemical and toxicological information. REACH

guidelines also require an assessment of the quality of the available dataset by evaluating the “relevance”, “reliability” and “adequacy” of each datum in order to establish its relative weight in the information pool.

6.1.2.1 Criteria and metrics

Physico-chemical properties

The toxicity of NOAA may depend on a number of physico-chemical characteristics, including size, specific surface area, shape, and presence of toxic impurities in the bulk of the material (Borm and Kreyling, 2011; Nel et al., 2006; Oberdörster et al., 1994; Wittmaack, 2007; Zuin et al., 2007). In addition, the capacity of NOAA to deposit in the upper lungs, enter the systemic circulation, cross biological barriers, reach secondary organs or penetrate into cells is largely determined by material properties such as size (distribution), agglomeration/aggregation rate, and surface charge (Borm and Kreyling, 2011; Maynard and Kuempel, 2005; Zuin et al., 2007). Therefore the physico-chemical characteristics of NOAA in general and nano-TiO_2 in particular are a relevant category of criteria to judge their intrinsic hazard, which can be further decomposed into two sub-categories: physico-chemical properties influencing (i) effects; and (ii) uptake, distribution and bioaccumulation. Each sub-category has been further divided into a number of indicators corresponding to relevant material properties (Figure 6-1; Table 6-2).

NOAA tend to be more reactive than corresponding bulk forms due to their larger surface area per unit mass (Nakagawa et al., 1997). Many authors who observed that smaller nano-TiO_2 particles are more toxic than their larger counterparts attribute this to their larger surface area (Duffin et al., 2002; Grassian et al., 2007; Grassian et al., 2006; Sager and Castranova, 2009; Sager et al., 2008; Warheit et al., 2009; Warheit et al., 2007a; Warheit et al., 2007b; Warheit et al., 2006). Therefore it is a relevant criterion to judge the reactivity of nano-TiO_2 as a proxy for toxicity.

Trace impurities of transition-metal oxide catalysts (e.g. iron and nickel), solvents in the carbon NOAA (e.g. tetrahydrofuran), or toxic elements in the core of the quantum dots (e.g. cadmium, selenium, lead) can contribute to the NOAA toxicity (Derfus et al., 2004; Pulskamp et al., 2007; Zuin et al., 2010). In this context, two composition-based indicators were selected: the presence of (i) toxic substances in the bulk of the materials; and (ii) toxic impurities.

Recent studies reported that long, persistent carbon nanotubes (CNT), when injected into the abdominal cavity of mice, produce marked inflammatory reaction and fibrogenic effect similar to that of asbestos (Donaldson et al., 2010; Poland et al., 2008). In this context, an aspect ratio of at least 3:1 was selected to discriminate between nanoparticles and nanofibres, which is in line with the World Health Organization (WHO) definition of man-made mineral fibres (World Health Organization, 1985).

It is common knowledge that the interaction of NOAA with biological systems, e.g. inhalation, localization, translocation, and excretion, is influenced by their size distribution (Kreyling et al., 2006; Nel et al., 2006; Oberdörster et al., 2005; Oberdörster et al., 2007; Semmler-Behnke et al., 2008). Two particle size-based parameters are used in this assessment framework. The first takes into account the entrance and deposition of NOAA into the respiratory system. Zuin et al. (2010) defined the index categories for the first criterion on the basis of the International Commission on Radiological Protection (ICRP) deposition rate model (ICRP, 1994). The second particle sized based parameter represents the potency of NOAA to translocate into blood circulation from the lungs and be distributed among secondary organs. In order to define the categories for this parameter, the authors used results from the EU-funded Particle Risk project (Semmler-Behnke et al., 2008).

The size distribution of nanomaterials depends largely on their colloidal stability. Nano-systems are intrinsically unstable since nanoobjects have the tendency to agglomerate/aggregate in various media (e.g. water, body fluids or media cell culture). Their stability may depend on their coating and the presence of stabilizing agents, and it can be predicted by measuring the ζ-potential, an indicator of surface charge. Because none of the studies in our database reported ζ-potential measurements, we were forced to select the qualitative criterion "Presence of anti-agglomeration coating/steric stabilization".

Functional groups (e.g. carboxyl, thiols) on the particle surface can make NOAA hydrophilic, which may also affect biological uptake and localization (Ryman-Rasmussen et al., 2006; Sayes et al., 2006; Zuin et al., 2010). On the basis of this consideration, the presence of a hydrophilic surface group is used as an indicator for NOAA bioaccumulation potential.

Figure 6-1: Conceptual structure of the Multi-criteria decision analysis (MCDA) methodology for hazard screening of engineered nano-objects and their aggregates and agglomerates.

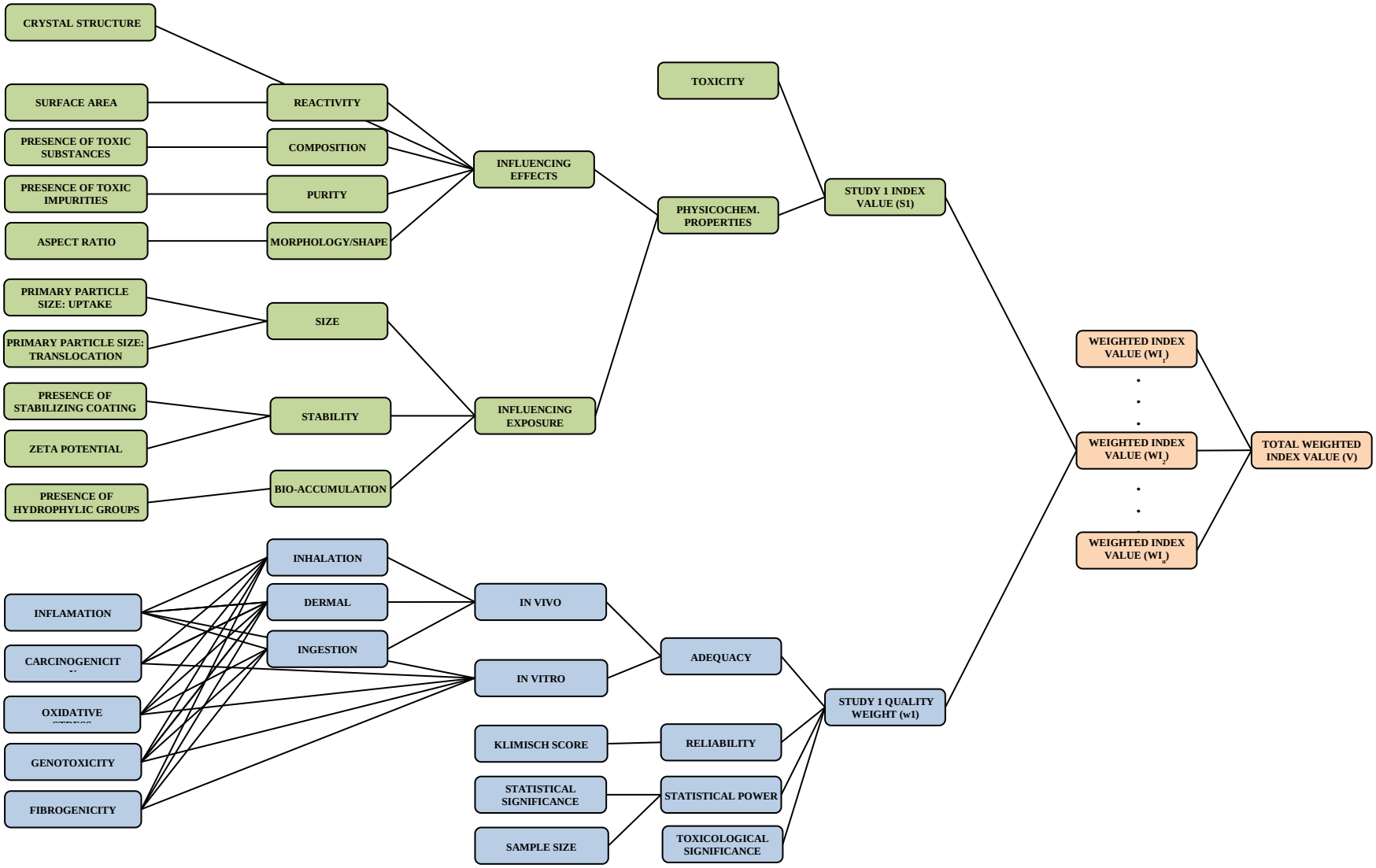


Table 6-2: (Sub-) categories, classes and scores of the criterion "physico-chemical properties".

Criterion	Sub- Criteria		Category index	Citation
Physico-chemical properties influencing toxicity	Reactivity	BET specific surface area	Low surface area (0 - 5 max m^2/g)=25; medium surface area (5 - 15 m^2/g)=50; high surface area (>15 m^2/g)=100; unknown=75	Expert judgement
	Composition	Presence of toxic substances in the bulk of the materials (incl. coating)	Boolean rating values: Yes/No; Yes= high hazard potential= 100; No= low hazard potential= 25	(Donaldson et al., 2004; Pulskamp et al., 2007)
	Purity	Presence of toxic impurities (e.g. PAH, heavy metals) in the material composition	< 96% (high impurity)=high hazard potential=100; 96 ÷ 99% (moderate impurity)=medium hazard potential=75; > 99% (low impurity)=low hazard potential= 25	(Derfus et al., 2004; Pulskamp et al., 2007; Zuin et al., 2010)
	Morphology/shape	Diameter-to-length (aspect) ratio	≥1:3 (fibres)= high hazard potential= 100; <1:3(particles)= low hazard potential= 25	(Donaldson et al., 2010; Poland et al., 2008; Seaton and Donaldson, 2005; World Health Organization, 1985)
Physico-chemical properties influencing uptake, distribution and bioaccumulation	Size	Primary particle size: inhalation uptake	> 30 nm (low lung deposition)=low uptake potential= 25; 10- 30 nm (medium lung deposition)= medium uptake potential= 75; < 10 nm (high lung deposition)=high uptake potential= 100	(James et al., 1991; Zuin et al., 2010)
		Primary particle size: translocation from lungs to secondary organs	> 5 nm= low translocation= 25; 2.5- 5 nm= medium translocation= 75; < 2.5 nm= high translocation= 100	(Geiser and Kreyling, 2010; Kreyling et al., 2006; Semmler-Behnke et al., 2008)

	Stability	Presence of anti- agglomeration coating/ steric stabilization	Boolean rating values: Yes/No; Yes (high stability)= high uptake/ distribution potential= 100; No (low stability)= low uptake/ distribution potential= 25	(Hassellov et al., 2008; Tiede et al., 2008)
		ζ - potential (mV) in aqueous suspension at pH 7	> +30 mV/< -30 mV (high stability)= high uptake/ distribution potential= 100; -30 mV- +30 mV (low stability)= low uptake/ distribution potential= 25	(Auffan et al., 2009; Zuin et al., 2010; Zuin et al., 2007)
	Bioaccumulation	Presence of hydrophilic surface groups	Boolean rating values: Yes/No; Yes (high stability)= high uptake/ distribution potential= 100; No (low stability)= low uptake/ distribution potential= 25	(Ryman-Rasmussen et al., 2006; Sayes et al., 2006; Zuin et al., 2010)

Toxicity

For the "toxicity" criterion five hazard classes were mapped onto an index system and assigned some discrete value in the [0,100] range. For each study to be used as a LoE the toxicological conclusion of the authors falls in one or more selected classes. On the basis of an approach adopted by US EPA for chemical carcinogenicity evaluation (US EPA, 1995), we suggest the following classification.

Class 5: Toxic to humans=100

The authors indicate strong evidence of human toxicity. The descriptor applies when there is convincing evidence of a causal relationship between a nanomaterial and an effect.

Class 4: Likely to be toxic to humans=75

Evidence for human toxicity is described as strong but does not merit the more stringent classification of Toxic to humans.

Class 3: Suggestive evidence of toxic potential=50

Indicative of toxicity, but the conclusion of the authors does not support a stronger conclusion. Small increases in toxicity compared to the controls would warrant inclusion of the LoE in this class.

Class 2: Inadequate information to assess toxic potential=25

Inclusion in this class indicates that the authors did not have enough information to draw a conclusion about toxicity. These studies either contain little pertinent information, conflicting evidence, or inconclusive results.

Class 1: Not likely to be toxic to humans=0

The authors of this paper conclude that there is no basis for human concern. Robust evidence is presented that the animal mode of action does not apply to humans or that there is a lack of animal toxicity. In addition, data disproving particular exposure routes, and evidence that effects are not likely below a particular dose may fall into this category.

Data Quality

Figure 6-1 presents the criteria hierarchy developed to determine the quality of each study or, in other words, the weight of each LoE. Regulatory data quality criteria are defined and discussed in section 3.1.1. Since the criterion "relevance" covers the extent to which data are appropriate for a particular assessment (e.g. evaluation of lung cancer risks from small, negatively charged nano-TiO_2), it is irrelevant for our holistic hazard screening covering the full spectrum of physico-chemical characteristics and effects of nano-TiO_2. Therefore we substituted this criterion for "statistical power", which refers to the probability that the test will reject the null hypothesis when it is actually false. The statistical power is influenced by the

"statistical significance". For a specific effect level, statistical significance is a function of "sample size". Therefore, statistical significance considered without also considering sample size may bias the evaluation towards larger projects. These two parameters were evaluated by means of expert judgment; a value in the 0-1 range was assigned to each of them and then averaged into a study-specific "statistical power" index.

In addition, we added a new criterion, i.e. "toxicological significance" referring to the relevance of the dose used in the test system. The "toxicological significance" represents the relevance of selected dose in the toxicological experiment and was again evaluated by means of expert judgment, assigning a value in the 0-1 range.

"Adequacy" is defined by Klimisch et al. (2007) and understood by regulatory agencies as the "usefulness of data for Hazard/Risk assessment purposes". If, for example, the state of the knowledge is that nano-TiO_2 particles do not penetrate the skin and do not cause skin irritation or sensitization, there is no reason to perform Hazard/Risk assessment considering the dermal route. Instead, we should focus on the inhalation route, where most effects are observed and highest exposure is likely. Therefore dermal toxicity data is less "adequate" for Hazard/Risk assessment than the inhalation data. The same is true for some endpoints, which are more relevant than other endpoints. The state of knowledge, therefore, is an indirect reflection of the literature as assimilated by experts. In the application of this method for regulatory or policy purposes, a suite of experts should be consulted in determining "adequacy."

The "adequacy" criteria are used to judge the usefulness of the data for the purpose of hazard assessment. The two main types of toxicity studies, i.e. *in vivo* and *in vitro*, were selected as its sub-categories. *In vivo* can be further divided into sub-categories and criteria referring to exposure routes (i.e. inhalation, ingestion and dermal) and to different toxicological endpoints (e.g. oxidative stress, inflammation, carcinogenicity). It is impossible to link *in vitro* studies to specific exposure routes because they investigate effects after delivery of doses directly to organ cells, neglecting their absorption, distribution, metabolism, and excretion (ADME) kinetics of the potential stressor. For the latter reason *in vivo* data are generally considered more adequate in human health Hazard and Risk assessment than *in vitro* results.

Inhalation is considered as the primary route by which particles suspended in air can enter the human organism (Stone et al., 2009). Highest airborne concentrations of nano-TiO_2 are expected in occupational settings (Brouwer et al., 2011; Mueller and Nowack, 2008; Stone et al., 2009). Once inhaled, particles may deposit in all regions of the respiratory tract and can cause local toxic effects (Lee et al., 1985). There is already sufficient evidence that from the respiratory tract particles can translocate through the blood to secondary organs and cause systemic effects (Borm and Kreyling, 2011). In this context inhalation, Intratracheal and nasal instillation toxicity studies are considered of higher adequacy for the hazard assessment of nanomaterials than dermal or ingestion tests.

The US National Institute for Occupational Safety and Health (NIOSH) concluded that nano-TiO_2 is carcinogenic to rats and it cannot be ruled out as a human carcinogen (NIOSH, 2005). The suspected mode of action is chronic inflammation following pulmonary overload, caused by the particle nature of nano-TiO_2 (NIOSH, 2005). Thus we consider carcinogenicity studies as adequate evidences of nano-TiO_2 toxicity.

The current dominant mechanistic paradigm for nanomaterials toxicity is oxidative stress (Nel et al., 2006), caused by free radical formation, leading to inflammation (Dick et al., 2003; Hussain et al., 2005; Stone et al., 2009) and cell injury (Stone and Donaldson, 2006). Particle- induced genotoxicity, involved in cancer development, is strongly linked to oxidative stress and inflammation (Poland et al., 2008). Therefore we consider the latter two endpoints "adequate".

Although there is a presumed correlation between inflammation-induced genotoxicity and nano-TiO_2 carcinogenicity, the existing evidence is insufficient to support this hypothesis (Stone et al., 2009). However, in order to be precautionary in the face of uncertainty, we give the "genotoxicity" endpoint a relatively high weight.

Fibrosis is a debilitating disease because there is a net loss to normal organ tissue and it may also lead to neoplasia, which is most commonly associated with inflammation. Strong fibrotic effects were observed for carbon nanotubes (Donaldson et al., 2010; Poland et al., 2008) and quartz particles. There is also evidence that nano-TiO_2 can cause overload-associated pathology through fibro-proliferative changes in rats (Bermudez et al, 2004).Therefore we deem this endpoint a relevant determinant of nano-TiO_2 toxicity.

On the basis of the above considerations and expert judgment a relative adequacy score in the 0-1 range was assigned to each "test type", "exposure route", and "endpoint" sub-category. These weights are material-specific and they pertain solely to our case study.

Characterizing the adequacy of each study normally involves identification of the test type, one exposure route, one or several endpoints. An arithmetic mean operator has been used in order to aggregate all endpoint-specific weights into a collective study-specific "adequacy" index.

Following the REACH guidelines we used the Klimisch scoring system to evaluate "reliability" on the basis of the applied test methods and the plausibility of the results. A reliability category was assigned to each datum: i.e. 1= reliable without restriction, 2= reliable with restrictions, 3=not reliable and 4=not assignable (Klimisch et al., 1997). The four classes were normalized in the 0-1 range.

The scores obtained for the study "adequacy", "reliability", "statistical power", and "toxicological significance" were aggregated by means of weighted average to calculate LoE-specific weights. The relative weights given by the experts to each of the three criteria are reported in Table 6-3. Because the reliability of the data is a key consideration, which is usually used for initial screening prior to further adequacy evaluation (OECD, 2004) this criterion is considered more important.

Table 6-3: Weights of the data quality (sub-) criteria.

Criterion	Sub-criteria	weight
Adequacy (w=0.35)	**Test type**	
	In vivo	1.00
	In vitro	0.30
	Exposure route	
	Inhalation	1.00
	Ingestion	0.60
	Dermal	0.30
	Endpoint	
	Carcinogenicity	1.00
	Inflammation	1.00
	Oxidative stress	1.00
	Genotoxicity	0.80
	Fibrogenecity	0.80
Reliability (w=0.5)	**Klimisch score**	
	Reliable without restriction	1.00
	Reliable with restrictions	0.75
	Not reliable	0.50
	Not assignable	0.25
Statistical power (w=0.15)	Statistical significance	0.60
	Sample size	0.40

6.1.2.2 Application results

The MCDA/WOE method described in section 5.3.1.1 was systematically applied for hazard screening of the nano-TiO_2 case study. In Step 1 of the MCDA approach, the collated physico-chemical data were evaluated. An index value has been assigned to each study and parameter in the [0,100] range. The individual index values were then aggregated by means of the arithmetic mean operator into a LoE-specific score, which refers to the degree of intrinsic hazard of the material (Table 6-4). The lowest LoE score is 25.00, while the largest is 57.14 with average of 39.10. In Step 2, the collected toxicity data was analysed for each result. The distribution of each LoE among the five pre-defined toxicity categories was determined. The lowest derived LoE score is 0, while the largest is 100 with average of 56.85. One publication resulted in several LoE if several toxicity findings were presented.

The above physico-chemical and toxicity LoE indices were aggregated by means of weighted average to obtain a holistic LoE-specific value in the [0,100] range (Step 3). Because different studies differ in terms of "adequacy", "reliability" and "statistical power" it was important to develop weights for the LoE using a Logic model, incorporated in the MCDA approach (Step 4). The contribution of each study *j* to the overall hazard evaluation was then defined by the product of its LoE-specific index value (S_j) and normalized weight (w_j'). This resulted into a set of LoE-specific weighed hazard scores (WI_j), which were aggregated into a total weighted index value (*V)* (Stage 5), representative of the hazard of nano-TiO_2, estimated on the basis of the available dataset. The results of Stages 3-5 are presented in Table 6-4. The calculated *V* is 52.19. This value should be used for relative hazard ranking of nano-TiO_2 among other NOAA evaluated using the same approach.

Table 6-4: Summary of results: (weighted) LoE-specific hazard scores based on physico-chemical and/or toxicity data, individual study quality weights, and the total weighted value (V), corresponding to the overall hazard of nano-TiO_2 evaluated on the basis of the collated evidence base.

ID	Citation	LoE index values based on physico-chemical properties ($S_j^{p.chem}$)	LoE index values based on toxicity (S_j^{tox})	Total LoE index values (S_j)	Study quality weight (w_j)	Normalized study quality weight (w_j')	Weighted LoE index values (WI_j)
1	Oberdörster et al., 1994	46.43	87.5	75.18	0.49	0.02	1.18
2	Heinrich et al., 1995	39.29	0	11.79	0.48	0.02	0.18
3	Heinrich et al., 1995	39.29	87.5	73.04	0.63	0.02	1.47
4	Bermudez et al., 2004	39.29	75	64.29	0.52	0.02	1.08
5	Bermudez et al., 2004	39.29	75	64.29	0.57	0.02	1.18
6	Bermudez et al., 2004	39.29	75	64.29	0.52	0.02	1.08
7	Bermudez et al., 2004	39.29	0	11.79	0.50	0.02	0.19
8	Bermudez et al., 2004	39.29	0	11.79	0.57	0.02	0.22
9	Bermudez et al., 2004	39.29	0	11.79	0.47	0.02	0.18
10	Grassian et al., 2007	39.29	50	46.79	0.50	0.02	0.75
11	Ferin et al., 1992	35.71	62.5	54.46	0.47	0.02	0.83
12	Ferin et al., 1992	35.71	62.5	54.46	0.50	0.02	0.88
13	Oberdoerster et al., 1992	42.86	62.5	56.61	0.55	0.02	1.00
14	Rehn et al., 2003	39.29	100	81.79	0.57	0.02	1.50

15	Rehn et al., 2003	39.29	0	11.79	0.51	0.02	0.19
16	Renwick et al., 2004	46.43	100	83.93	0.54	0.02	1.46
17	Warheit et al., 2006	50.00	75	67.50	0.68	0.02	1.48
18	Warheit et al., 2006	46.43	0	13.93	0.54	0.02	0.24
19	Warheit et al., 2007b	39.29	87.5	73.04	0.52	0.02	1.21
20	Sager et al., 2008	57.14	100	87.14	0.57	0.02	1.60
21	Chen et al., 2006	25.00	75	60.00	0.54	0.02	1.05
22	Kobayashi et al., 2009	35.71	100	80.71	0.52	0.02	1.34
23	Kobayashi et al., 2009	57.14	87.5	78.39	0.54	0.02	1.35
24	Kobayashi et al., 2009	46.43	87.5	75.18	0.64	0.02	1.53
25	Lee et al., 1985*	57.14	100	87.14	0.57	0.02	1.58
26	Muhle et al., 1991*	35,71	0	10.71	0.50	0.02	0.17
27	Heinrich et al., 1995	35.71	100	80.71	0.54	0.02	1.39
28	Heinrich et al., 1995	32.14	0	9.64	0.55	0.02	0.17
29	Grassian et al., 2007	32.14	75	62.14	0.48	0.02	0.95
30	Chen et al., 2006	42.86	0	12.86	0.51	0.02	0.21
31	Wang et al., 2008	39.29	75	64.29	0.48	0.02	0.99
32	Wang et al., 2008	32.14	75	62.14	0.54	0.02	1.08
33	Park et al., 2008	32.14	100	79.64	0.42	0.01	1.06

34	Barlow et al., 2005	30.00	100	79.00	0.55	0.02	1.39
35	Zhang and Sun, 2004	40.00	100	82.00	0.49	0.02	1.28
36	Peters et al., 2004	40.00	0	12.00	0.46	0.01	0.18
37	Linnainmaa et al., 1997	40.00	0	12.00	0.45	0.01	0.17
38	Long et al., 2007	40.00	87.5	73.25	0.49	0.02	1.14
39	Long et al., 2007	30.00	87.5	70.25	0.45	0.01	1.00
40	Hussain et al., 2005	30.00	87.5	70.25	0.45	0.01	1.02
41	L'Azou et al., 2008	40.00	87.5	73.25	0.49	0.02	1.14
42	L'Azou et al., 2008	35.00	87.5	71.75	0.49	0.02	1.12
43	L'Azou et al., 2008	35.00	0	10.50	0.43	0.01	0.14
44	L'Azou et al., 2008	35.00	0	10.50	0.61	0.02	0.21
45	Park et al., 2008	35.00	100	80.50	0.46	0.01	1.18
46	Gurr et al., 2005	30.00	87.5	70.25	0.42	0.01	0.94
47	Hussain et al., 2005	40.00	0	12.00	0.49	0.02	0.19
48	Helfenstein et al., 2008	40.00	0	12.00	0.40	0.01	0.15
49	Helfenstein et al., 2008	55.00	81.25	73.38	0.42	0.01	0.98
50	Long et al., 2006	55,00	100	86.50	0.49	0.02	1.35
51	Long et al., 2007	35.00	100	80.50	0.58	0.02	1.49
52	Long et al., 2007	35.00	0	10.50	0.43	0.01	0.14

53	L'Azou et al., 2008	35.00	25	28.00	0.53	0.02	0.48
54	L'Azou et al., 2008	35.00	25	28.00	0.55	0.02	0.49
55	L'Azou et al., 2008	35.00	25	28.00	0.47	0.02	0.42
56	L'Azou et al., 2008	35.00	25	28.00	0.46	0.01	0.41
57	Gurr et al., 2005	35.00	81.25	67.38	0.52	0.02	1.11
58	Linnainmaa et al., 1997	33.33	12.5	18.75	0.40	0.01	0.24
59	Linnainmaa et al., 1997	40.00	12.5	20.75	0.39	0.01	0.26
60	Churg et al., 1999	40.00	62.5	55.75	0.54	0.02	0.96
61	Peters et al., 2004	45.00	87.5	74.75	0.39	0.01	0.92
62	Long et al., 2007	35.00	87.5	71.75	0.40	0.01	0.92

Legend: * Note this publication contained 2 results, or LoE, indicating different classes of toxicity. **V = 52.19**

** pigment grade TiO_2 used

6.1.2.3 Uncertainty characterization

Probabilistic Monte-Carlo analysis was performed for identifying the influence of the input variables in the output of the model, resulting from uncertainties in the data, as well as for an estimation of the sensitivity of the hazard screening model.

The input parameters and the bounds that were varied in the analysis were:

- The LoE-specific index of physico-chemical properties, $S_j^{p.chem.}$ in the range [0,100]
- The LoE-specific index of toxicity data, S_j^{tox} in the range [0,100]
- The normalized LoE weights, w_j' in the range [0,1]

Four analyses were performed on the input parameters:

1) Variations of the LoE-specific index of physico-chemical properties, $S_j^{p.chem.}$, while all other parameters were kept stable.
2) Variations of the LoE-specific index of toxicity data, S_j^{tox}, while all other parameters were kept stable.
3) Variations of the normalized LoE weights, w_j', while all other parameters were kept stable.
4) Variations at the same time of all the three above mentioned parameters ($S_j^{p.chem.}$, S_j^{tox}, w_j'), while all other parameters were kept stable.

The metric used is the absolute deviation ΔV_i, as shown in equation 6.1.

$$\Delta V_i = |V_i' - V| \; in \; [0,100] \tag{6.1}$$

Here V_i' (for $i = 1{:}\,10{,}000$) is the calculated total weighted index values of each Monte Carlo iteration and V is the calculated total weighted index value of the reference application of the model, as shown in Table 6-4. The results are presented below in four different figures.

Figure 6-2a reports the ΔV_i for the 10,000 iterations when random values in the range [0,100] are uniformly selected for the LoE-specific index of physico-chemical properties, $S_j^{p.chem.}$, and the other parameters are stable. The average ΔV_i is 7.98 units or 7.98% if expressed in percentage.

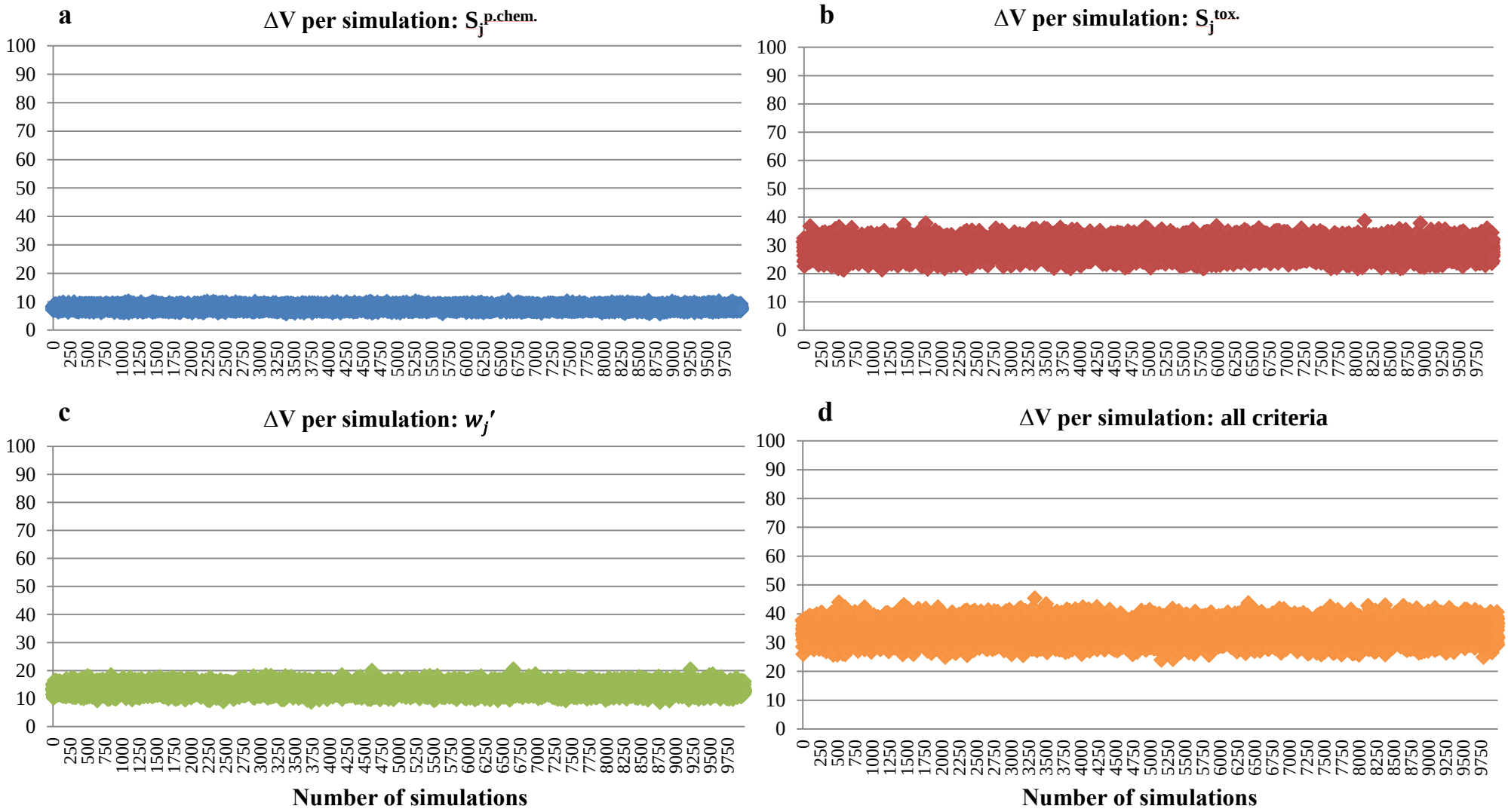
Figure 6-2b reports the ΔV_i for the 10,000 iterations when random values in the range [0,100] are uniformly selected for the LoE-specific index of toxicity data, S_j^{tox}, and the other parameters are stable. The average ΔV_i is 28.88 units or 28.88%.

Figure 6-2c reports the ΔV_i for the 10,000 iterations when random values in the range $[0,1]$ are uniformly selected for the normalized LoE weights, w_j', and the other parameters are stable. The average ΔV_i is 13.47 units or 13.47%.

Figure 6-2d reports the ΔV_i for the 10,000 iterations when random values in the above mentioned ranges are uniformly selected for the LoE-specific index of physico-chemical properties, $S_j^{p.chem.}$, the LoE-specific index of toxicity data, S_j^{tox}, and the normalized LoE weights, w_j', while the other parameters are stable. The average ΔV_i is 33.6 units or 33.6% if expressed in percentage.

The uncertainty analysis quantified the relative stability of the model; the average variation was just above 33 units when all the input parameters are considered uncertain and unstable across the entire range of possible inputs. The variation reflects the responsiveness of the model (i.e. 33%) to changing inputs. The relative sensitivity of the model is described by the first 3 scenarios, in which the index of physico-chemical properties, the index of toxicity and the relative weights are all varied sequentially. The model is most sensitive to changes in the index of toxicity and least sensitive to changes in the physiochemical properties index. Overall, the units/percentages are lower in all cases where only one input parameter is considered uncertain. The analysis shows that the model is most sensitive with respect to variation in the input derived from the evidence of toxicity.

Figure 6-2: Absolute difference (ΔV_i) between the total weighted index value (V_i') in each Monte Carlo iteration and the calculated total weighted index value (V) of the reference application of the model as shown in Table 6-4, after probabilistic uncertainty analysis applied to the LoE-specific index (a) for physico-chemical properties ($S_j^{p.chem.}$); (b) for toxicity ($S_j^{tox.}$); (c) to the weights (w_j') and (d) to all the three criteria simultaneously.

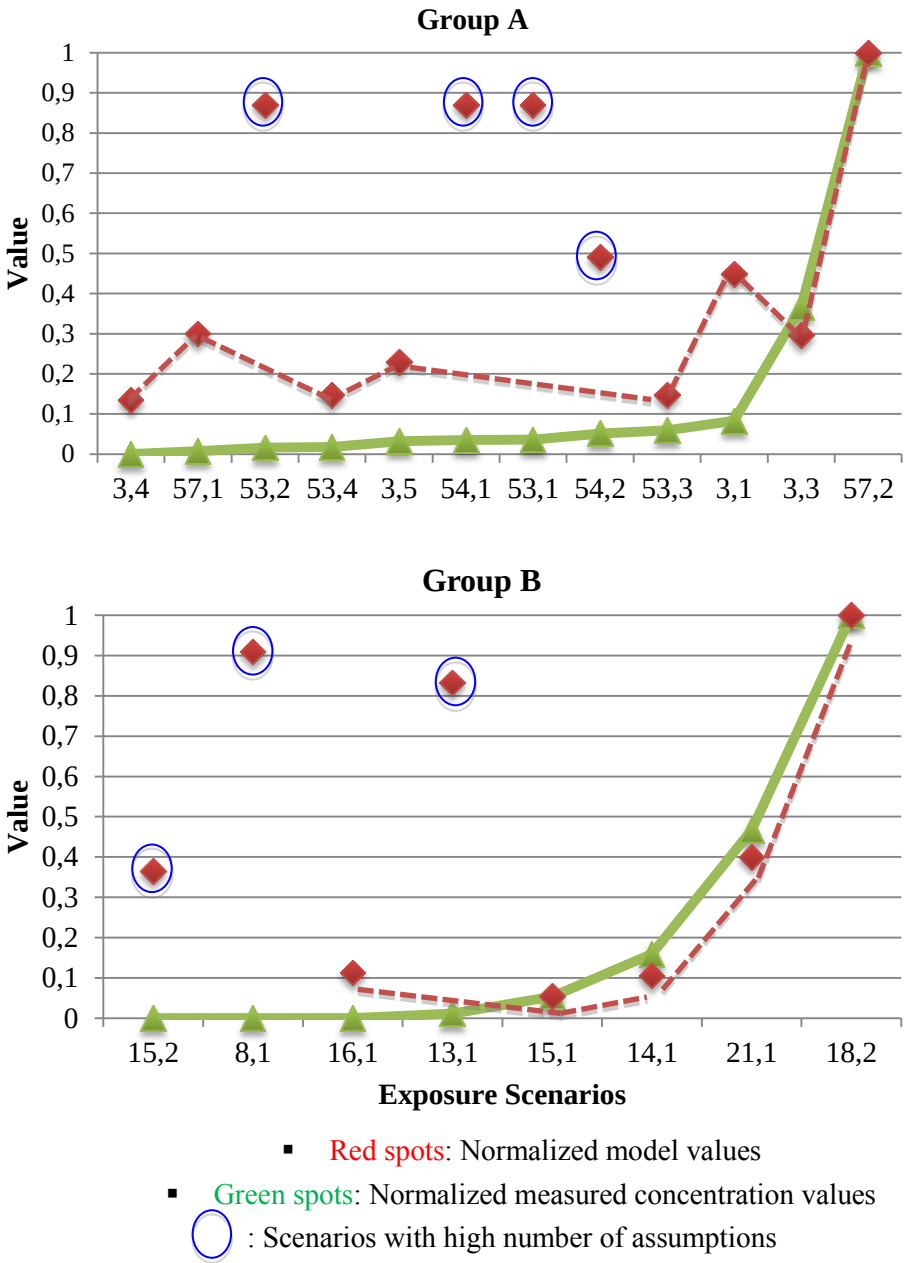


6.1.3 Exposure screening

6.1.3.1 Application results

The WoE exposure model described in Section 5.3.1.2 was systematically applied to estimate exposure potential for 20 occupational ES classified in 2 groups: i.e. A and B. In order to validate them, these estimates were ranked and compared to a parallel ranking of measured exposure concentrations (Conc) for the same ES in terms of difference in normalized value (ΔV) (Figure 6-3).

Figure 6-3: Exposure values estimated with the Weight of evidence model (in red) plotted versus normalized exposure concentrations (in green) for both groups of exposure scenarios A and B.

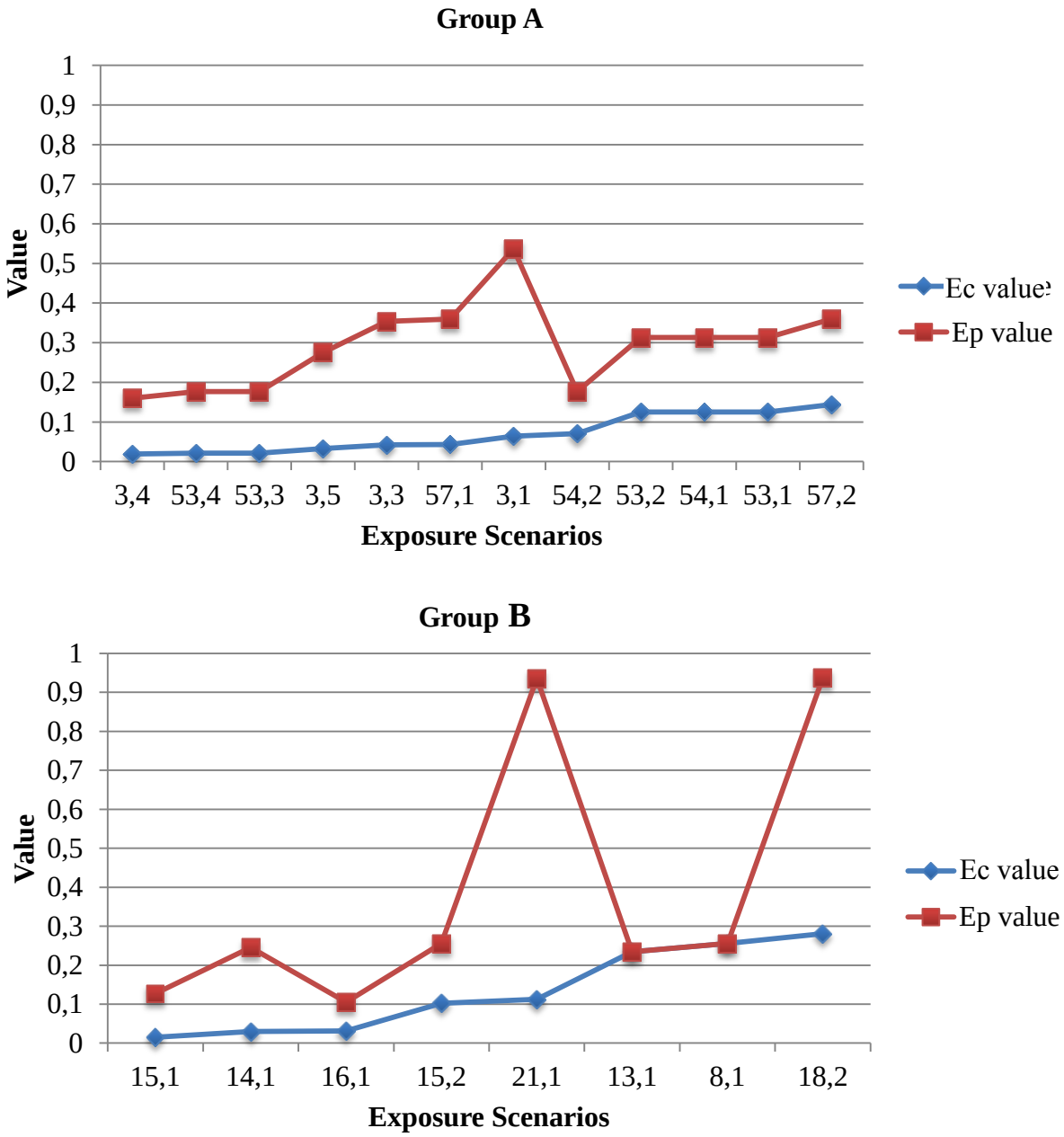


In Group A the worst-case scenario is 57.2 where the normalized WoE and Conc values are both equal to 1. Highest model overestimations with respect to the corresponding normalized measured exposure concentrations (i.e. between 38% and 90%) are observed for scenarios 53.2, 54.1, 53.1 and 3.1, where due to lack of data high numbers of assumptions were used. For ES 3.4, 57.1, 53.4, 54.2, 53.3, and 3.5 exposure is overestimated between 8% and 30% as this is seemingly again due to overuse of expert judgement. The model underestimates exposure by 7% only for ES 3.3, which refers to "(Un) loading trays filled with nano-TiO_2 inside a booth", which can be attributed to the influence of an exposure reduction measure on the results. In group A all scenarios except for ES 3.3 and 57.2 have Conc values below 0.1. The lowest reported concentration is for ES 3.4 and it differs from the corresponding model estimate by 20%.

The situation in group B is similar. WoE and Conc coincide for the worst-case ES 18.2, while for ES 21.1, 14.1 and 15.1 the model overestimates exposure by only 6%, 5% and 1%, respectively. In contrast, overestimations of over 80% are observed for ES 8.1 and 13.1, corresponding to activities where very small amounts of material were handled and the assessment was based on large numbers of expert deductions and assumptions. For ES 15.2 and 16.1 the model overestimates exposure by 36% and 11%, respectively, obviously due to inconsistencies in the measurements, which show no difference from background concentrations.

In order to highlight the importance of the measures adopted to reduce exposure and mitigate risks, the results for the Ep, which is derived by aggregating the M, P and C LoE were compared with the Ec, which also involves the R LoE encompassing all implemented RMM, and the results are reported in Figure 6-4. The role of the RMM is clearly visible for ES 3.1 in A and for ES 21.1 and 18.2 in B, whose Ep are reduced by almost 90%. For all other ES Ep values are significantly lowered by RMM as well.

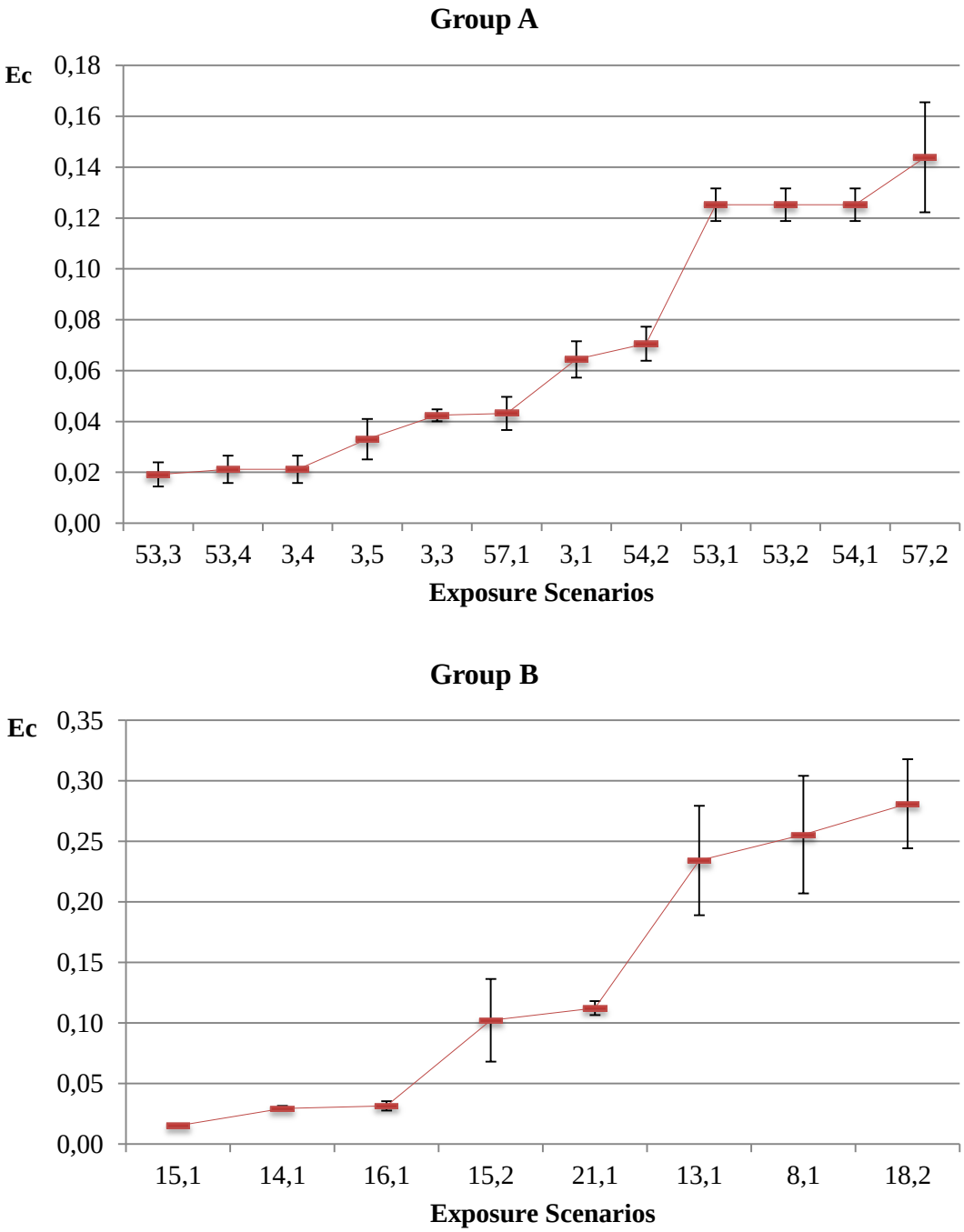
Figure 6-4: Controlled Exposure Potential (Ec) and uncontrolled Exposure Potential (Ep) values for the exposure scenarios in both groups A and B.



6.1.3.2 Error estimation

The error associated to the Controlled Exposure Potential (U_{Ec}) indicates how much the Ec score can vary as a consequence of the use of deductions/assumptions and specific aggregation formulas. Therefore the confidence intervals reported in Figure 6-5 give an idea of how stable the WoE model performs given variations in the input data.

Figure 6-5: Controlled Exposure Potential (Ec) and correspondent error (UEc) for each exposure scenario in groups A and B.

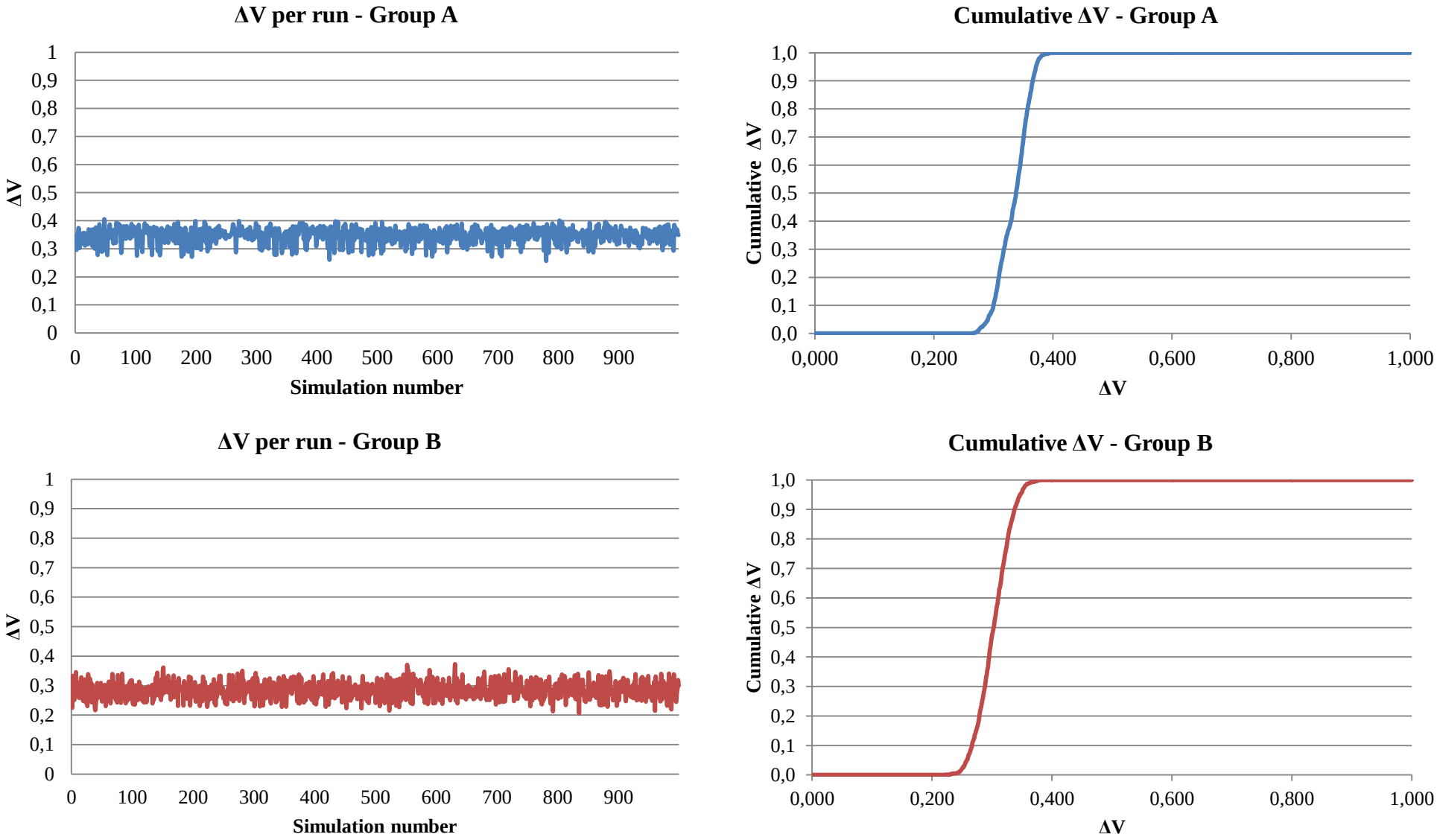


The U_{Ec} values vary and the more assumptions are used in assessing exposure relevant to an ES the wider its confidence interval is. Broadest ranges are observed for ES 57.2, 15.2, 13.1, 8.1 and 18.2, where in most cases 30-40% of the indicators were defined by assumption and 20-30% by deduction. However, it can be seen that for all the scenarios the changes caused by the estimated errors do not considerably influence the results. In fact, the scenarios in the right side of each graph maintain their positions, while the others may alter slightly their Ec, but without causing in this way consistent changes in the rankings, which demonstrates that the model is stable and the approach reliable.

6.1.3.3 Uncertainty analysis

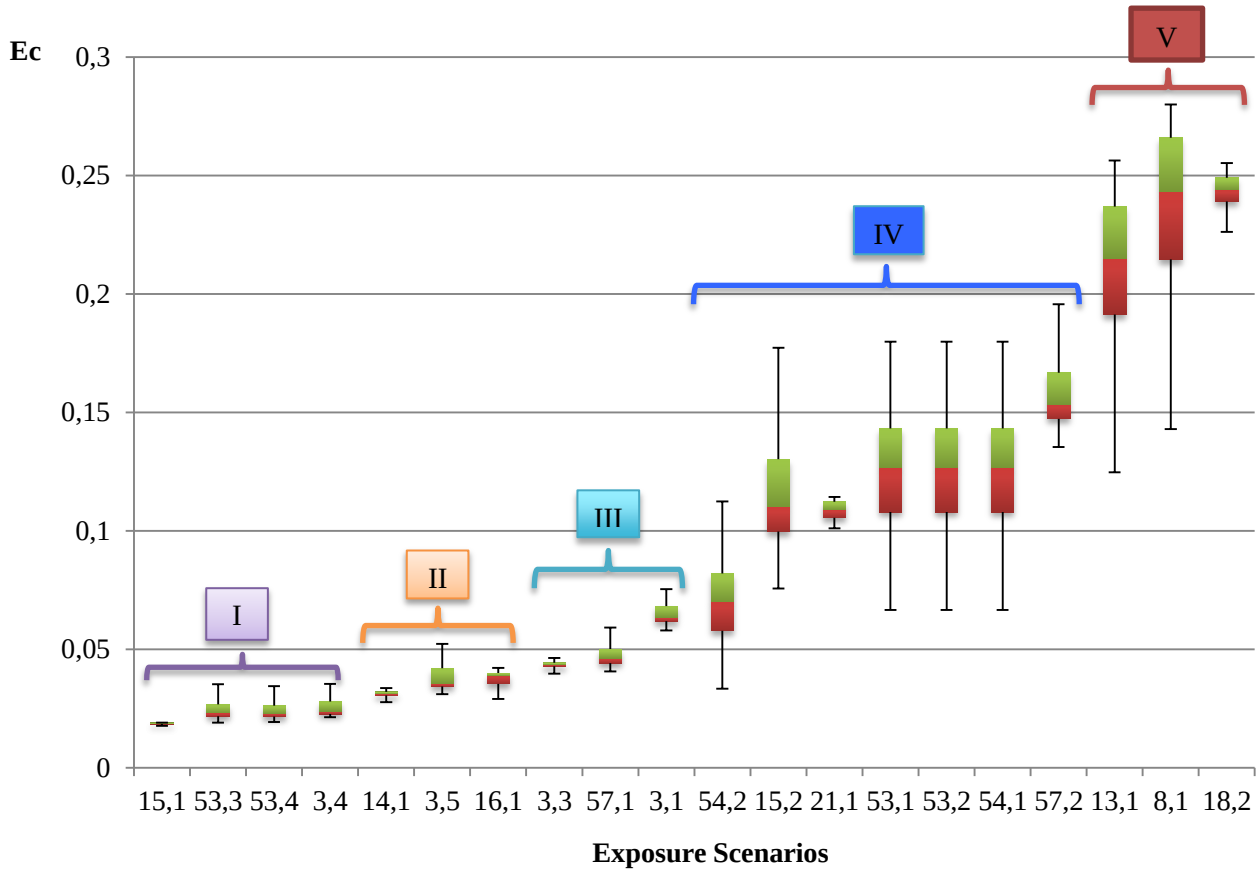
In order to understand how changes in the input parameters influence the performance of the WoE model and the obtained results a Monte Carlo simulation was performed. Figure 6-6 reports the (cumulative) distribution of ΔV for groups A and B and it clearly shows that in both cases the ΔV varies with less than 0.1 units in the 1000 simulated rankings. In group A ΔV equals 0.35 (35%) with a σ of 0.02. In group B ΔV is 0.28 (28%) with σ of 0.02. The low variance of the ΔV expressed by the low σ suggests that the performance of the model is stable.

Figure 6-6: Left side: distributions of mean differences in value (ΔV) for groups A and B derived from 1000 Monte Carlo simulations changing input data; Right side: cumulative distribution of ΔV for groups A and B.



The results of the uncertainty analysis can also be used to analyse each scenario individually. Figure 6-7 is a blot box illustrating the distribution of Ec for each ES. The red and the green parts of each box represent the intervals between the 25th to the 50th percentile, and the 50th to the 75th percentile, respectively. The edges of each box are the lower and the upper confidence limits.

Figure 6-7: Box plot values of Controlled Exposure Potential (Ec) distributions obtained with the uncertainty analysis on the input parameters for the exposure scenarios in groups A and B. Latin numbers I-V indicate clusters of scenarios with similar Ea.



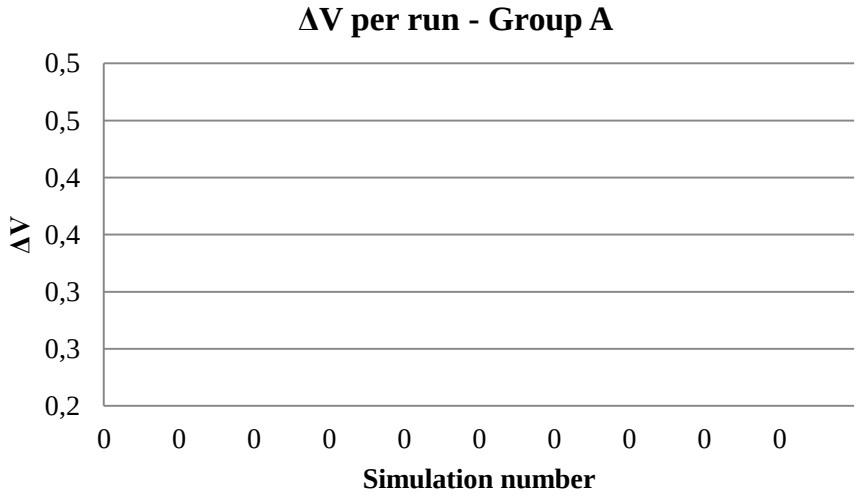
Scenarios demonstrating highest Ec variability are 15.2, 57.2, 13.1, 8.1 and 18.2, where the highest number of deductions and assumptions were used.

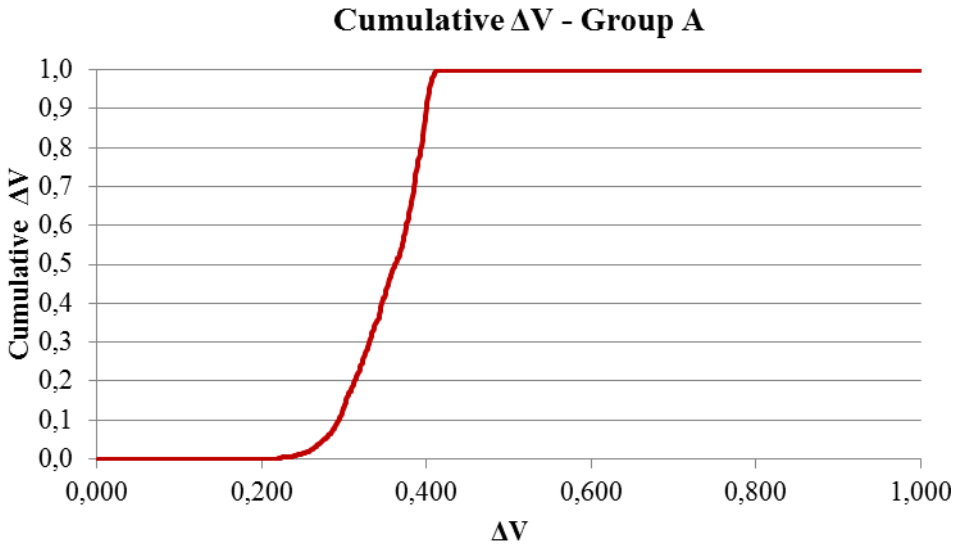
In Figure 6-7 clusters of ES can be distinguished. Cluster I (lowest Ec) consists of ES 15.1, 53.3, 53.4, 3.4, involving handling of liquids containing CNT and nano-TiO_2. It is followed by another set of low-emission activities where RMM were implemented (i.e. ES 14.1, 3.5 and 16.1 in cluster II). Cluster III involves high emission tasks, performed with LEV and/or RPE like for example opening growth chambers (ES 57.1) or dumping (ES 3.1 and 21.1). Cluster IV consists of weighing activities with relatively high Ec (i.e. ES 15.2, 53.1, 53.2, 54.1), especially in the absence of general and local ventilation. The worst-case cluster V scenarios 13.1, 8.1 and ES 18.2 involve highly emissive processes performed without any

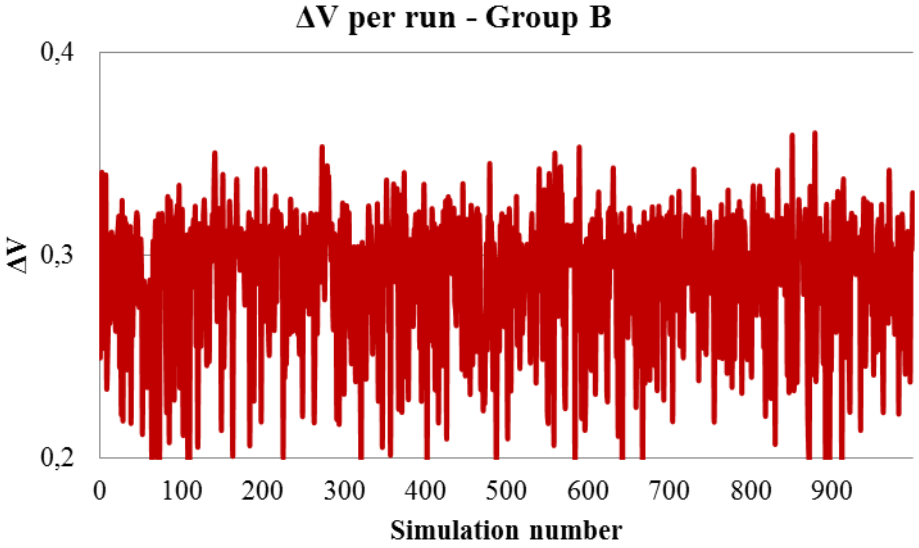
exposure controls. These results once again demonstrate that the model accounts for the significant attenuation effect of LEV, also demonstrated by Fransman et al. (2009).

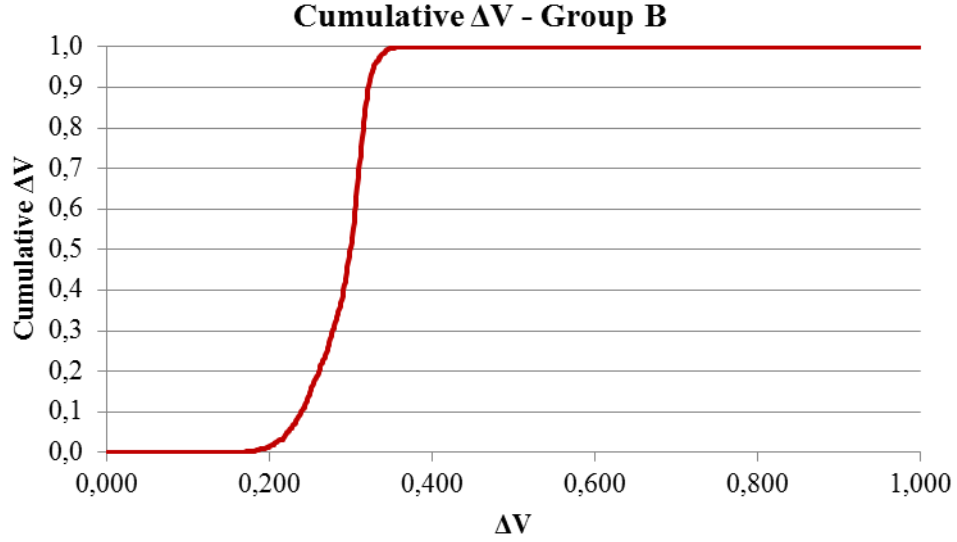
The results of applying the Monte Carlo uncertainty analysis to the input weights are illustrated by Figure 6-8.

Figure 6-8: Left side: distributions of mean differences in value (ΔV) for groups A and B derived from 1000 Monte Carlo simulations changing input weights; Right side: cumulative distribution of ΔV for groups A and B.



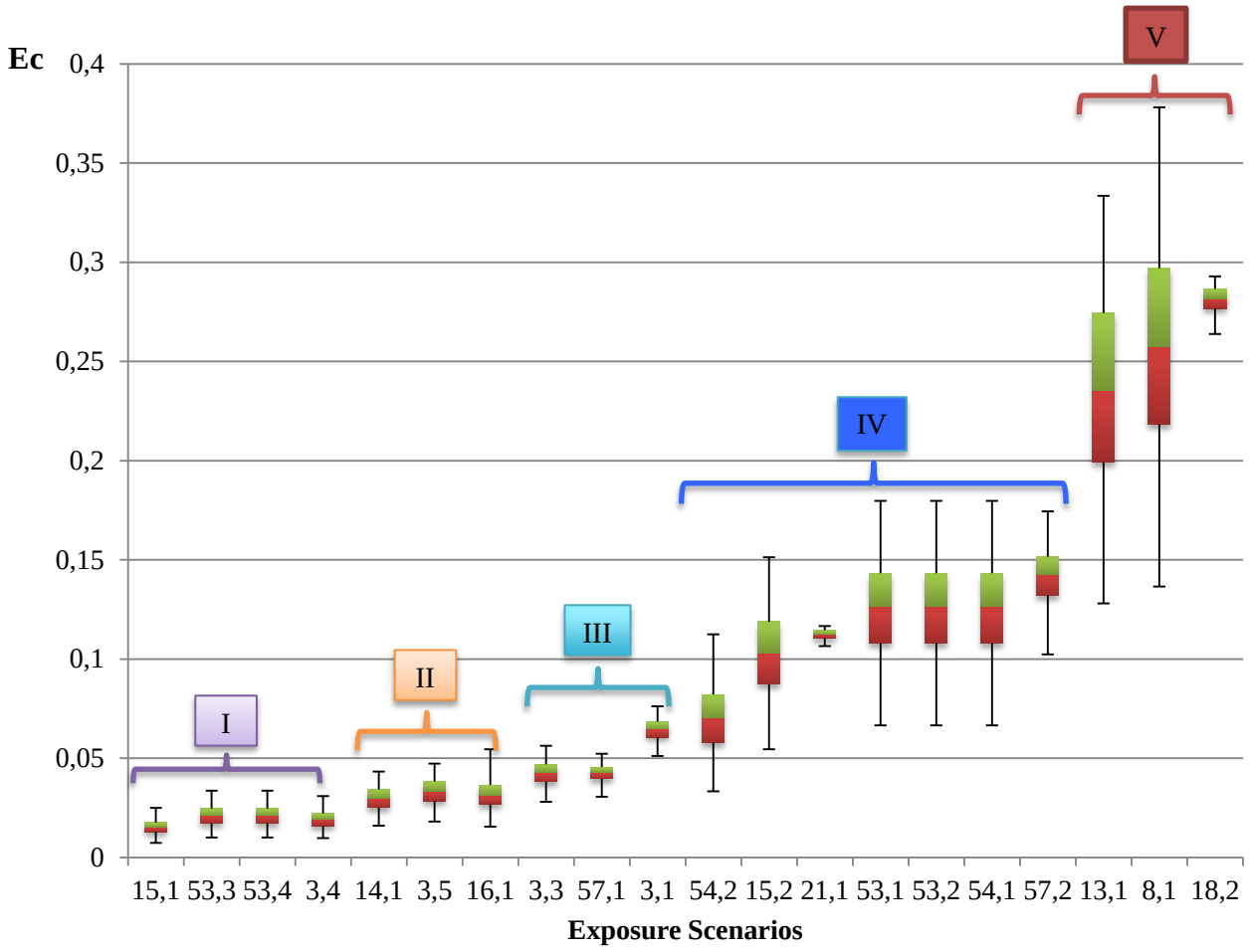






In each ES group ΔV varies with about 0.1 units in the 1000 simulated rankings, which confirms the stability of the model. Similarly to the results of the uncertainty analysis applied to the indicators, the model-based ranks are on average 32% different from the concentration-based ones. The low σ (0.04 for A and 0.03 for B) suggests that the model performance is not significantly affected by changes in the input weights. In the Figure 6-9 it is still possible to identify the clusters of scenarios with similar Ec identified in Figure 6-7, which confirms the stable behaviour of the model.

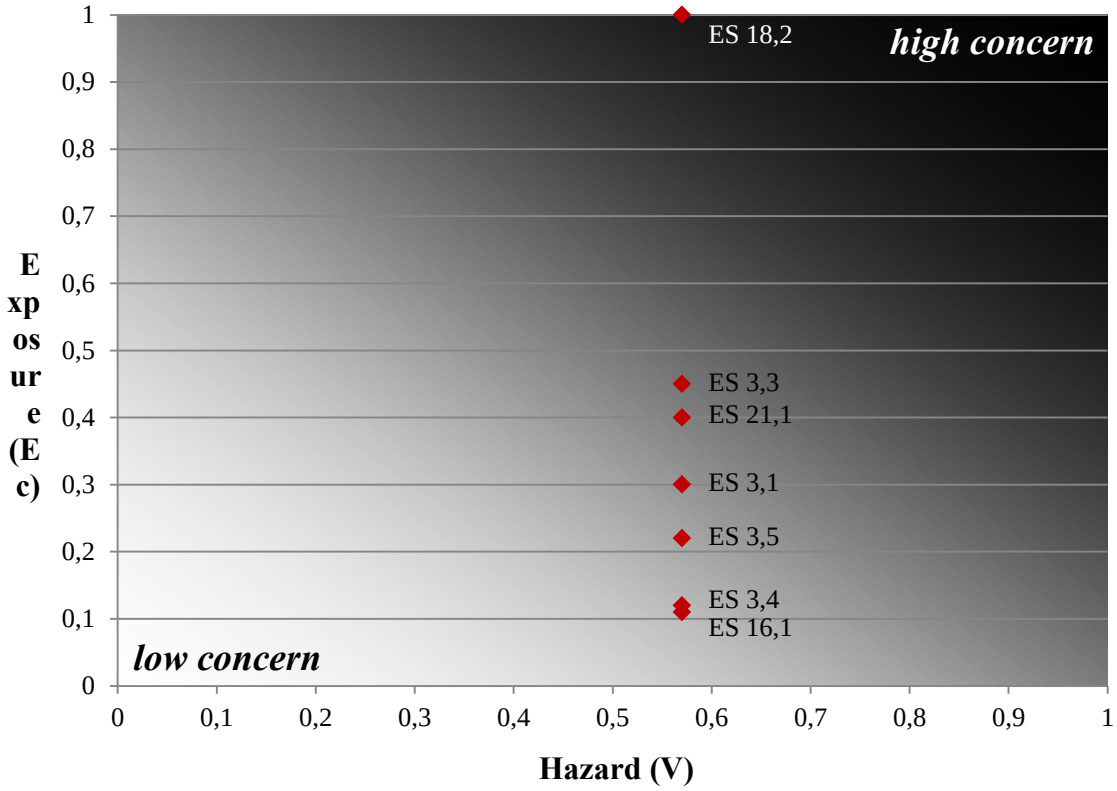
Figure 6-9: Box plot values of Controlled Exposure Potential (Ec) distributions obtained with the uncertainty analysis on the input weights for the exposure scenarios in groups A and B.



6.1.4 Integrating hazard and exposure

The hazard screening approach introduced in section 5.3.1.1 was applied in Section 6.1.2 to calculate a collected weighted LoE index value (V) for nano-TiO_2 (V = 56.94). In Section 6.1.3 the exposure model proposed in Section 5.3.1.2 was applied to obtain exposure estimates for a number of occupational nano-TiO_2 scenarios. To normalize the hazard and exposure values to the same scale, V was rescaled to [0,1]. In order to integrate hazard and exposure we used the matrix reported in Figure 6-10. Red points denote ES as the darker the background is the higher the ES-specific concern for the human health is.

Figure 6-10: Matrix visualizing human health concern associated with a number of nano-TiO_2 occupational exposure scenarios (ES). X-axis shows relative hazard of nano-TiO_2. Y-axis shows relative exposure potential for nano-TiO_2 ES (red points). Darker background indicates higher concern.



Data related to both nano and pigment-grade TiO_2 indicated that workers may be exposed to high concentrations of the materials at virtually all steps that involve handling of dry powder (e.g., bagging, filtering/drying, milling, shovelling, cleaning, maintenance) (Aitken et al., 2004; Brouwer, 2010; Hanai et al., 2009). Based on the current literature, however, it is considered that highest exposure may occur during the bagging of the particles (Aitken et al., 2004; Brouwer, 2010; Hanai et al., 2009). This notion is confirmed by our assessment, which concluded that ES 18.2 "Bag/bin filling" is most concerning as well as by on- site investigations performed in the context of the Japanese NEDO project (Hanai et al., 2009). Based these evidences we conclude that the bagging of nano- TiO_2 is the worst case ES for nano- TiO_2. It is followed by ES 3.3 "Manual (un)loading trays inside booth" and ES 21.1 "Dumping large amount of powder into vessel", which also involve manual handling of dry powder. ES of lower concern are ES 3.1: "Dumping into mixing tank using focused LEV" because LEV is present and ES 3.5 "Transferring material during weighing or into vials for solution prep" because very small amounts of material are handled. Scenarios of lowest concern are ES 3.4 "Creating stock solutions in fume hood" and ES 16.1 "Laser ablation", which are contained activities performed under controlled conditions.

6.2 Tier II: Quantitative relative Risk assessment

6.2.1 Pre-assessment

Problem formulation

The aim of Tier II is relative RA (including quantitative uncertainty analysis) of TiO_2, ZnO, Ag nanomaterials and multi-walled carbon nanotubes (MWCNT). The target of the assessment is the general workers population. The analysis focusses on 9 nanomaterials with contrasting characteristics (Table 6-5). Most batches were obtained from the Joint Research Centre (JRC) Repository and are similar to those used in the OECD WPMN programme.

The NRCWE rutile series (NRCWE 001-004) were used, which include nano-TiO_2 comparable in terms of phase, purity, and dispersibility, but different in size. Small-size nano-TiO_2 particles were functionalized to obtain positive (NRCWE-002) and negative (NRCWE-003) surface charge. In addition coated (NM-111) and uncoated (NM-110) nano-ZnO as well as bent (NM-400) and (partially) entangled (NM-402) MWCNT were used. The physico-chemical properties of the materials, summarized in Table 6-5, were characterized by the laboratories of the Department of Environmental Sciences, Informatics and Statistics of the Ca' Foscari University Venice and the Danish National Research Centre for the Working Environment (NRCWE).

Table 6-5: Physico-chemical characterization of ENPRA nanomaterials considered for risk evaluation. XRD=X-ray Diffraction. MWCNT= Multi-walled carbon nanotubes. TEM=Transmission Electron Microscopy.

	Uncoated TiO_2	**Coated TiO_2**	**Coated TiO_2**	**Uncoated TiO_2**	**Uncoated ZnO**	**Coated ZnO**	**Coated Ag**	**MWCNT**	**MWCNT**
Code	NRCWE-001	NRCWE-002	NRCWE-003	NRCWE-004	NM-110	NM-111	NM-300K	NM-400	NM-402
Primary size [TEM]	80-400 nm (80%)	80-400 nm	80-400 nm	1-2000 nm	20-350 nm	10-450 nm	~ 20 nm	Ø: 5-35 nm L: 0.7-3 µm	Ø: 6-20 nm (~80%) L: 0.7-4 µm (~80%)
XRD-size	10.4 ± 0.5	9.7 ± 0.4	10.1 ± 0.5	94	71	58	4	-	-
XRD-Phases	An:Br:Ru:Am: 6:0:75:19	An:Br:Ru:Am: 0:0:62:38	An:Br:Ru:Am: 0:0:70:30	An:Br:Ru:Am: 0:0:44:56	Zincite:Am 52:48	Zincite:Am 34:66	Ag_m	-	-
Specific surface area	99 (±0.5)	84.3 (±0.5)	84.2 (±0.5)	5.1 (±0.5)	14 (±0.1)	18 (±0.1)	-	298 (±1)	225 (±1)
Pore Volume	-	-	-	-	0.1	0.1	-	1.4	1.2
Impurities of concern	Fe, Co	Fe, Co	Fe	Fe, Co	-	-	Fe, Zn, As	Fe, Co, Zn	Fe
Shape [TEM]	Irregular polyhedral particles	Irregular polyhedral particles	Irregular polyhedral particles	Different morphologies identified	Polyhedral particles, some irregular, varied morphology	As NM110	Individual (dispersed) idiomorphic crystallites	Bent multiwalled	Bent and partially entangled multiwalled

6.2.1.2 Data collection and analysis

For each nanomaterial *in vivo* toxicity tests were performed in ENPRA addressing five body systems (i.e. pulmonary, cardio-vascular, hepatic, renal and lymphatic) and five toxicological endpoints (i.e. oxidative stress, cytotoxicity, inflammation and immune response, genotoxicity and organ pathology). Their results were processed with the Dutch PROAST model (http://www.rivm.nl/en/Library/Scientific/Models/PROAST) to obtain a dose-response relationship for each test. PROAST uses the Bench Mark Dose (BMD) approach (EFSA, 2010), described in section 3.1.3. Statistical uncertainties in the data are taken into account in the confidence interval around the BMD, as the lower limit of this interval (denoted as BMDL) is the PoD, or in other words: the highest safe dose (EFSA, 2010; US EPA, 2000). The complete dose–response analysis of the raw toxicity data resulted in derivation of test-specific BMDs and their lower 90-95% confidence limits. Multiple test-specific BMDL values have been obtained for each NOAA. Following a conservative approach, the lowest out of them was selected for each endpoint-body system combination to be used in conjunction with external exposure doses to calculate MoE. These endpoint/body system-specific BMDL are reported in Table A4-1 of Annex 4.

All *in vivo* data are from intratracheal instillation studies, mimicking the inhalation route. In order to use them for calculation of inhalation MoE we extrapolated them following two "extreme" approaches (Box 4-1, Annex 4). One extreme is to assume that the instillation bolus at the BMDL spreads out over one volume of animal breath (tidal volume). We define this value as "animal single inhalation BMDL". The other extreme is to divide the "single inhalation BMDL" by the total number of inhalations, which equals the breathing frequency of a test animal multiplied by the duration of a worker shift. This results in a "daily averaged animal inhalation BMDL". The true inhalation BMDL is a value between the 2 extremes. However, any of them can be used for calculation of MoE and for relative RA. We chose to use the "assumed single inhalation BMDL".

Workplace monitoring data form the NANOSH and NANO-INNOV projects are available in the NANEX database, however they do not correspond to the ENPRA nanomaterials. In order to enable fusion of the exposure and hazard datasets for risk analysis we constructed hypothetical ES based on operational conditions (e.g. exposure duration and frequency) data from NANEX and material characteristics (e.g. dustiness) data from ENPRA. Then we performed first order modelling of the nanomaterials dust concentrations in the near- and far-field using the background model in NanoSafer control banding tool (http://nanosafer.i-bar.dk/) (see Annex 5) to estimate both acute and long-term inhalation exposure doses (D_{inh}). This process is described in Annex 5, where the calculated exposure doses are reported in Table A5-2. The near-field acute D_{inh} were contrasted to the single inhalation BMDL to calculate endpoint/body system-specific MoE using the following equation 6.11.

$$\mathrm{MoE} = \frac{\mathrm{BMDL}\ (\frac{\mathrm{mg}}{\mathrm{m}^3})}{\mathrm{D_{inh}}\ (\frac{\mathrm{mg}}{\mathrm{m}^3})} \quad (6.11)$$

These MoE are reported in Table A6-1 (Annex 6).

6.2.2 Ranking and prioritization

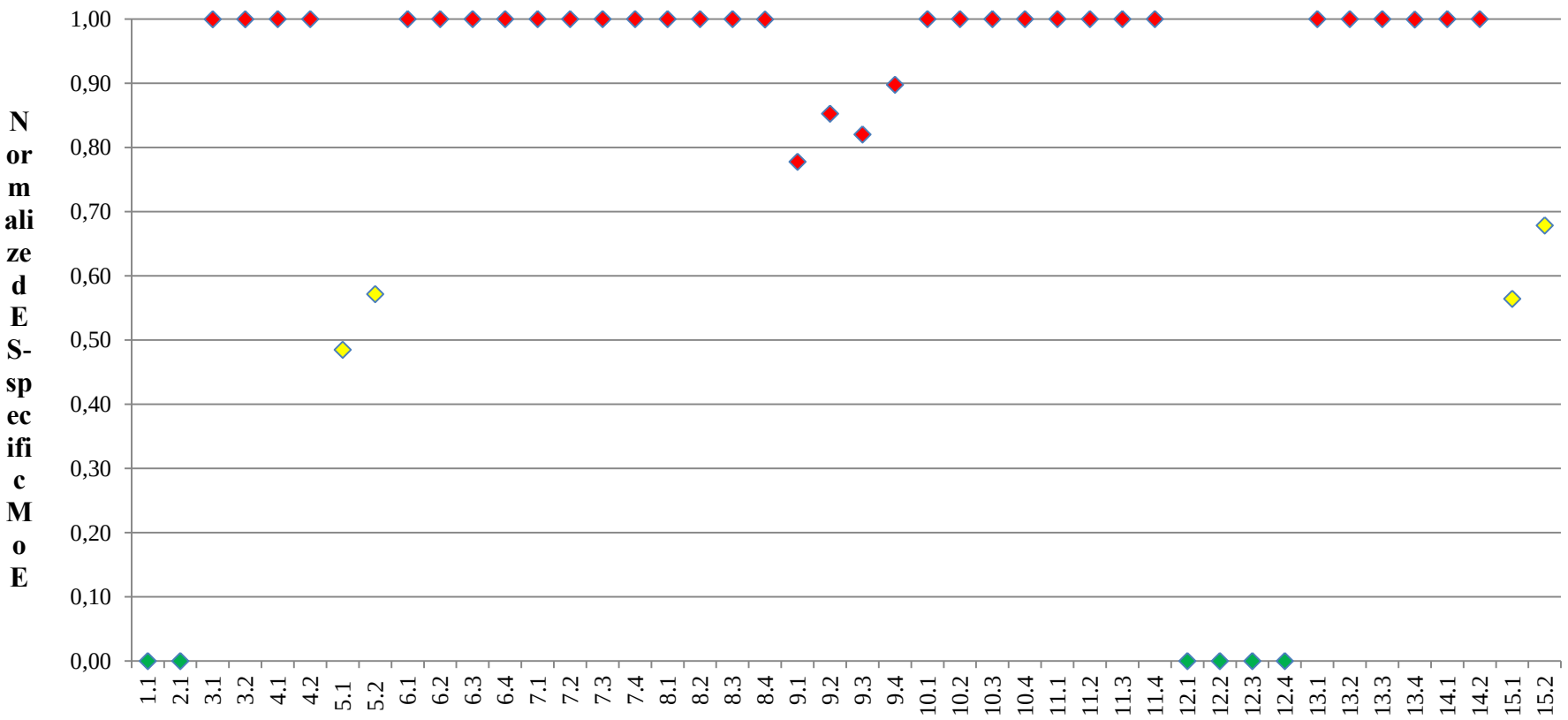
The endpoint-specific MoE are normalized to the [0,1] scale and used as input (i.e. indicators) for the WoE model, described in section 5.3.2. The outputs of the model are ES-specific relative risk indices (Table 6-6), which were used for ranking and prioritization of high-concern ES/NOAA as shown on Figure 6-11.

Table 6-6: Normalized Margin of exposure (MoE) values calculated for a number of occupational exposure scenarios using the Tier II risk model.

Material	Scenario	Normalized MoE
NM300	1.1: Production of nano-Ag during wet-chemistry process: opening the dryer door and transfer for grinding	0
NM300	2.1: Production of Nano-Ag during wet-chemistry process: opening the grinder hatch and transfer for packaging	0
NM400	3.1: Sonication of raw MWCNT	9,99741212987E-01
NM402	3.2: Sonication of raw MWCNT	9,99784764882E-01
NM400	4.1: Weighing raw MWCNT	9,99999933569E-01
NM402	4.2: Weighing raw MWCNT	9,99999953167E-01
NM400	5.1: Opening growth chamber with no exhaust and transfer of MWCNT	4,84869787132E-01
NM402	5.2: Opening growth chamber with no exhaust and transfer of MWCNT	5,71545547464E-01
NCRWE1	6.1: Manufacturer: Manual (un)loading trays inside booth	9,99999999998E-01
NCRWE2	6.2: Manufacturer: Manual (un)loading trays inside booth	9,99999999998E-01
NCRWE3	6.3: Manufacturer: Manual (un)loading trays inside booth	9,99999999996E-01
NCRWE4	6.4: Manufacturer: Manual (un)loading trays inside booth	9,99999998356E-01
NCRWE1	7.1: Manufacturer: Dumping into mixing tank using focused LEV	9,99999999998E-01
NCRWE2	7.2: Manufacturer: Dumping into mixing tank using focused LEV	9,99999999998E-01
NCRWE3	7.3: Manufacturer: Dumping into mixing tank using focused LEV	9,99999999997E-01
NCRWE4	7.4: Manufacturer: Dumping into mixing tank using focused LEV	9,99999998841E-01
NCRWE1	8.1: Lab: Transferring material during weighing or into vials for solution prep	9,99999483981E-01
NCRWE2	8.2: Lab: Transferring material during weighing or into vials for solution prep	9,99999289475E-01

NCRWE3	8.3: Lab: Transferring material during weighing or into vials for solution prep	9,99998744547E-01
NCRWE4	8.4: Lab: Transferring material during weighing or into vials for solution prep	9,99484941668E-01
NCRWE1	9.1: Lab: Creating stock solutions in fume hood	7,77750055466E-01
NCRWE2	9.2: Lab: Creating stock solutions in fume hood	8,52633350654E-01
NCRWE3	9.3: Lab: Creating stock solutions in fume hood	8,20243156231E-01
NCRWE4	9.4: Lab: Creating stock solutions in fume hood	8,97692736964E-01
NCRWE1	10.1: Dumping large amount of powder into vessel	1,0000000000E+00
NCRWE2	10.2: Dumping large amount of powder into vessel	1,0000000000E+00
NCRWE3	10.3: Dumping large amount of powder into vessel	1,0000000000E+00
NCRWE4	10.4: Dumping large amount of powder into vessel	9,99999999984E-01
NCRWE1	11.1: Bag/bin filling	9,99999999998E-01
NCRWE2	11.2: Bag/bin filling	9,99999999997E-01
NCRWE3	11.3: Bag/bin filling	9,99999999995E-01
NCRWE4	11.4: Bag/bin filling	9,99999997877E-01
NCRWE1	12.1: Laser ablation	0
NCRWE2	12.2: Laser ablation	0
NCRWE3	12.3: Laser ablation	0
NCRWE4	12.4: Laser ablation	0
NCRWE1	13.1: Weighing of TiO_2 powder	9,99999329470E-01
NCRWE2	13.2: Weighing of TiO_2 powder	9,99999076723E-01
NCRWE3	13.3Weighing of TiO_2 powder	9,99998368627E-01
NCRWE4	13.4: Weighing of TiO_2 powder	9,99330717890E-01
NM111	14.1: Preparation of inks / Preparation of nano-ZnO solution	9,99991263013E-01
NM110	14.2: Preparation of inks / Preparation of nano-ZnO solution	9,99992390849E-01
NM111	15.1: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	5,64265404117E-01
NM110	15.2: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	6,78519718819E-01

Figure 6-11: Ranking of normalized Margin of exposure (MoE) values calculated for a number of occupational exposure scenarios using the Tier II risk model.



Red spots: Relatively high-concern scenarios

Yellow spots: Intermediate-concern scenarios

Green spots: Low-concern scenarios

The Tier II analysis resulted in highest relative risk from scenarios concerned with “Manual (un)loading trays inside booth”, “Dumping into mixing tank using focused LEV”, “Dumping large amount of powder into vessel”; and “Bag/bin filling” since they involve manual handling of dry powders, which leads to high emissions (Aitken et al., 2004; Brouwer, 2010; Hanai et al., 2009). Less, but still concerning scenarios are “Weighing raw MWCNT”, “Weighing of TiO_2 powder”, “Sonication of raw MWCNT”, “Transferring material during weighing or into vials for solution prep”, and “Preparation of inks/Preparation of nano-ZnO solution” due to lower, but significant exposure potential. Scenarios of intermediate concern involve: “Creating stock solutions in fume hood”, “Opening growth chamber with no exhaust and transfer of MWCNT”, “Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate”, which are contained activities performed either under controlled conditions or involving handling of smaller amounts of material. Scenarios concerned with laser ablation and wet-chemistry synthesis processes are not concerning due to negligible emissions. Among the above scenarios more risky are the ones involving NCRWE1 (due to relatively high hepatic toxicity), NCRWE2 (cardiovascular toxicity), and NCRWE4 (pulmonary and cardiovascular toxicity), NM110 (pulmonary genotoxicity), and NM111 (pulmonary cytotoxicity). It is evident from the above application that the Tier II application is consistent with Tier I application for nano-TiO_2.

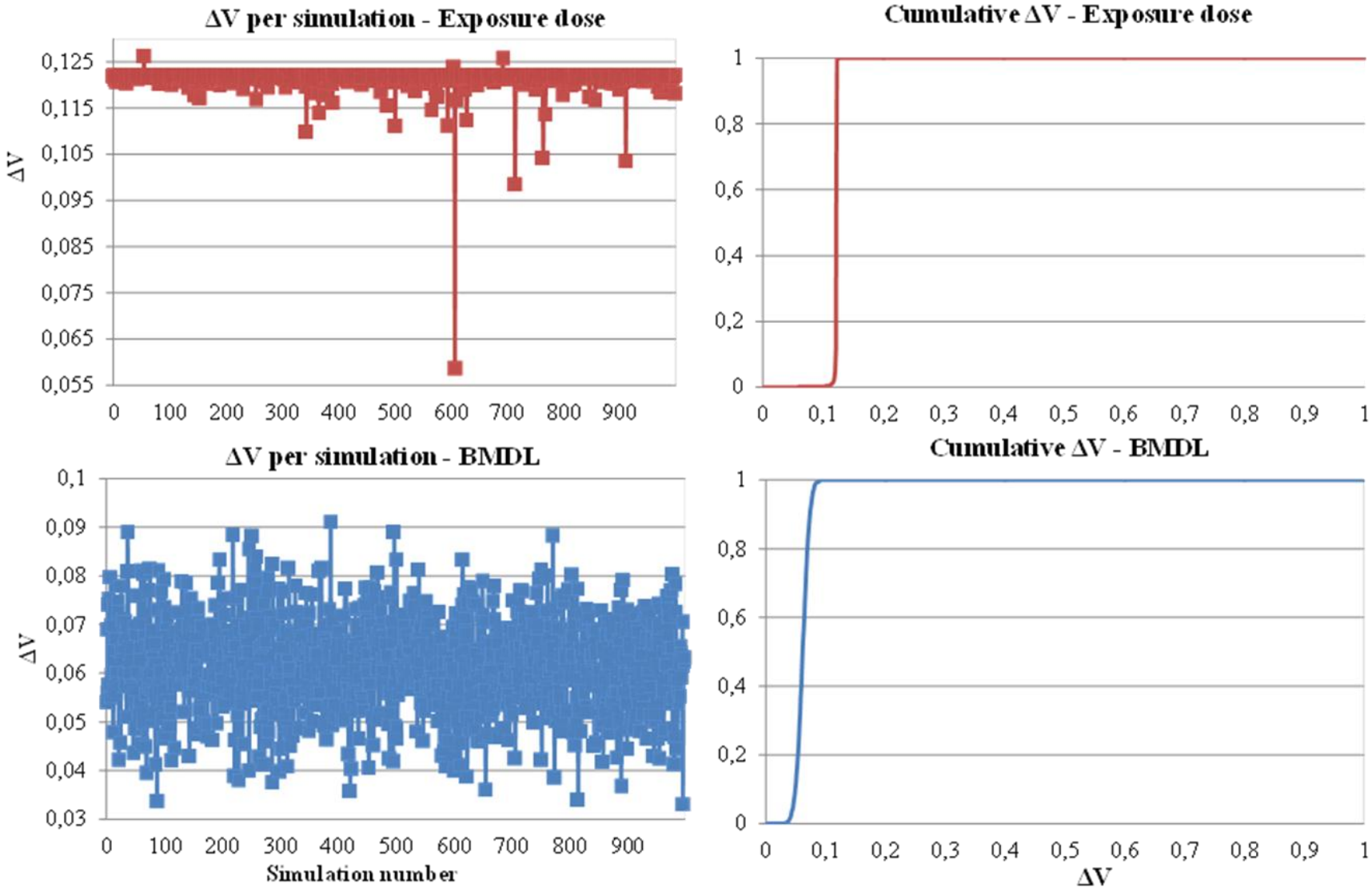
6.2.3 Uncertainty characterization

In order to understand how changes of the input parameters influence the performance of the WoE model and the obtained results, an uncertainty analysis based on a Monte Carlo simulation was performed. The effects of variations in both the BMDL and the exposure doses were studied independently.

For each exposure dose a uniform probability distribution was defined in the [a,b] interval with mean at the modelled estimate and interval endpoints calculated as values 1000% below [a] and above [b] of the estimate value, respectively. This extended range of uncertainty is based on expert judgment and includes uncertainties in the data used to construct ES as well as the Nanosafer model performance. Similarly, a probability distribution was defined for each endpoint/body system-specific dose-response relationship in the [c,d] interval centered at the BMDL with interval endpoints [c] and [d] calculated as values 100% above and below the BMDL to account for uncertainties in the design of the underlying toxicological experiments.

Figure 6-12 reports the (cumulative) distribution of ΔV (see sections 5.3.1.2.4 and 5.3.2.2) for variations in both inputs and it clearly shows that in both cases the ΔV varies with less than 0.002 units (for exposure doses) and 0.06 units (for BMDL) in the 1000 simulated rankings. In the simulations based on variation of exposure doses the average ΔV equals 0.122 (12.2%) with a standard deviation $\sigma = 0.0025$. In the BMDL-based analysis the average ΔV is 0.061 (6.1%) with a standard deviation $\sigma = 0.0096$. The low variance of the ΔV suggested by the low σ suggests that the performance of the model is stable.

Figure 6-12: Left side: distributions of mean differences in value (ΔV) for (i) exposure doses and (ii) Benchmark dose lower confidence intervals (BMDL) derived from 1000 Monte Carlo simulations changing input data; Right side: cumulative distribution of ΔV.



6.3 Tier III: Quantitative actual Risk assessment

6.3.1 Pre-assessment

Problem formulation

Although the aim of Tier III is actual occupational RA of the high-priority NOAA/ES identified in Tier II, we applied it to all nanomaterials and scenarios addressed in the lower tier in order to demonstrate that both approaches agree on their results.

Data collection

The present Tier III analysis uses the Tier II exposure and hazard database.

6.3.2 Hazard assessment

We estimated acute Derived No-effect Levels (DNEL) from the "single inhalation BMDL" estimated following the procedure in Box A4-1 (Annex 4). Estimating long-term DNEL from acute data is very uncertain. In this situation the REACH Guidelines recommend that chronic DNEL are extrapolated from acute DNEL as the latter is divided by a factor of 1-5, depending on the potency and the dose-response curve (European Chemicals Agency, 2008). Chronic DNEL calculated in this way should be given high priority for revision when long-term toxicity data become available. The process of estimating acute and chronic DNEL was informed by the REACH Guidelines and it is summarized in Box A4-2 of Annex 4. The resulting DNEL are presented in Table A4-1 (Annex 4).

6.3.3 Exposure assessment

The process of estimating exposure for the purpose of the present risk analysis is reported in Annex 5. Acute and chronic near- and far- field potential exposure doses were calculated with NanoSafer and reported in Table A5-2.

6.3.4 Risk characterization

Each acute or chronic exposure dose was contrasted to a respective acute or chronic endpoint/body system-specific DNEL to calculate a Risk Ratio (RR). These endpoint/body system-specific RR were normalized and used as inputs to the WoE model described in section 5.3.3 to calculate acute and chronic RR for a number of ES and nanomaterials (Table 6-7). If RR equal 1 it is considered that the risks are uncontrolled, while RR smaller than 1 indicate controlled risks (see equation 21, chapter 5).

Table 6-7: Normalized Risk Ratios (RR) calculated for a number of occupational exposure scenarios and nanomaterials using the Tier III risk model. The analysis considers, acute and chronic, near- and far-field exposure. NN=Near field. FF=Far field.

Material	Scenario	acute RR (NF)	acute RR (FF)	chronic RR (NF)	chronic RR (FF)
NM300	1.1: Production of nano-Ag during wet-chemistry process: opening the dryer door and transfer for grinding	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NM300	2.1: Production of Nano-Ag during wet-chemistry process: opening the grinder hatch and transfer for packaging	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NM400	3.1: Sonication of raw MWCNT	1,56E-07	8,48E-08	6,07E-07	3,37E-07
NM402	3.2: Sonication of raw MWCNT	1,49E-07	8,07E-08	5,78E-07	3,21E-07
NM400	4.1: Weighing raw MWCNT	3,47E-04	1,60E-04	1,16E-03	6,36E-04
NM402	4.2: Weighing raw MWCNT	3,30E-04	1,52E-04	1,10E-03	6,06E-04
NM400	5.1: Opening growth chamber with no exhaust and transfer of MWCNT	7,84E-11	3,18E-11	1,30E-10	7,91E-11
NM402	5.2: Opening growth chamber with no exhaust and transfer of MWCNT	7,47E-11	3,03E-11	1,24E-10	7,53E-11
NCRWE1	6.1: Manufacturer: Manual (un)loading trays inside booth	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE2	6.2: Manufacturer: Manual (un)loading trays inside booth	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE3	6.3: Manufacturer: Manual (un)loading trays inside booth	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE4	6.4: Manufacturer: Manual (un)loading trays inside booth	9,23E-01	8,57E-01	1,00E+00	1,00E+00
NCRWE1	7.1: Manufacturer: Dumping into mixing tank using focused LEV	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE2	7.2: Manufacturer: Dumping into mixing tank using focused LEV	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE3	7.3: Manufacturer: Dumping into mixing tank using focused LEV	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE4	7.4: Manufacturer: Dumping into mixing tank using focused LEV	1,00E+00	8,34E-01	1,00E+00	1,00E+00
NCRWE1	8.1: Lab: Transferring material during weighing or into vials for solution prep	3,81E-02	1,74E-02	1,18E-01	6,64E-02

NCRWE2	8.2: Lab: Transferring material during weighing or into vials for solution prep	1,15E-02	5,24E-03	3,58E-02	2,00E-02
NCRWE3	8.3: Lab: Transferring material during weighing or into vials for solution prep	2,11E-03	9,61E-04	6,56E-03	3,67E-03
NCRWE4	8.4: Lab: Transferring material during weighing or into vials for solution prep	1,81E-05	8,25E-06	5,63E-05	3,16E-05
NCRWE1	9.1: Lab: Creating stock solutions in fume hood	8,85E-08	4,72E-08	3,05E-07	2,00E-07
NCRWE2	9.2: Lab: Creating stock solutions in fume hood	5,54E-08	2,96E-08	1,91E-07	1,25E-07
NCRWE3	9.3: Lab: Creating stock solutions in fume hood	1,47E-08	7,84E-09	5,07E-08	3,32E-08
NCRWE4	9.4: Lab: Creating stock solutions in fume hood	9,11E-08	4,86E-08	3,14E-07	2,06E-07
NCRWE1	10.1: Dumping large amount of powder into vessel	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE2	10.2: Dumping large amount of powder into vessel	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE3	10.3: Dumping large amount of powder into vessel	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE4	10.4: Dumping large amount of powder into vessel	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE1	11.1: Bag/bin filling	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE2	11.2: Bag/bin filling	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE3	11.3: Bag/bin filling	1,00E+00	1,00E+00	1,00E+00	1,00E+00
NCRWE4	11.4: Bag/bin filling	8,51E-01	6,32E-01	1,00E+00	8,54E-01
NCRWE1	12.1: Laser ablation	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE2	12.2: Laser ablation	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE3	12.3: Laser ablation	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE4	12.4: Laser ablation	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE1	13.1: Weighing of TiO_2 powder	2,93E-02	1,92E-02	1,10E-01	7,20E-02
NCRWE2	13.2: Weighing of TiO_2 powder	8,85E-03	5,80E-03	3,32E-02	2,17E-02
NCRWE3	13.3Weighing of TiO_2 powder	1,62E-03	1,06E-03	6,08E-03	3,98E-03
NCRWE4	13.4: Weighing of TiO_2 powder	1,39E-05	9,13E-06	5,22E-05	3,42E-05
NM111	14.1: Preparation of inks / Preparation of nano-ZnO solution	2,90E-03	1,08E-03	6,60E-04	3,73E-04
NM110	14.2: Preparation of inks / Preparation of nano-ZnO solution	5,66E-01	4,34E-01	2,76E-01	1,58E-01
NM111	15.1: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	6,43E-08	2,14E-08	1,55E-08	8,79E-09
NM110	15.2: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	3,28E-05	1,09E-05	7,92E-06	4,49E-06

The results reported in Table 6-7 are consistent with the Tier II analysis (section 6.2.2). However, due to its higher resolution, the Tier III model proved that for some ES, labelled as "high-concern" in tier II (e.g. 3.1, 3.2, 4.1, 4.2, 6.4, 11.4, 8.1, 8.2, 8.3, 8.4, 13.1, 13.2, 13.3 and 13.4) risks are actually controlled. Risks are not controlled for scenarios 6.1, 6.2, 6.3, 6.4, 7.1, 7.2, 7.3, 7.4, 10.1, 10.2, 10.3, 10.4, 11.1, 11.2, 11.3 and 11.4, mainly because they involve handling of large quantities of dry powder, which results in significant inhalation exposure (Aitken et al., 2004; Brouwer, 2010; Hanai et al., 2009). Scenarios 7.4, 10.4 and 11.4 pose no acute risks, but in the long-term they do exhibit chronic risks. Other scenarios of comparatively high concern are 8.1, 13.1 and 14.2, mainly because they involve the relatively hazardous NCRWE1 and NM110 materials. These ES should be carefully analysed prior to decision making and if needed more data should be produced for them to reduce uncertainties and allow more informed risk analysis.

6.3.5 Uncertainty characterization

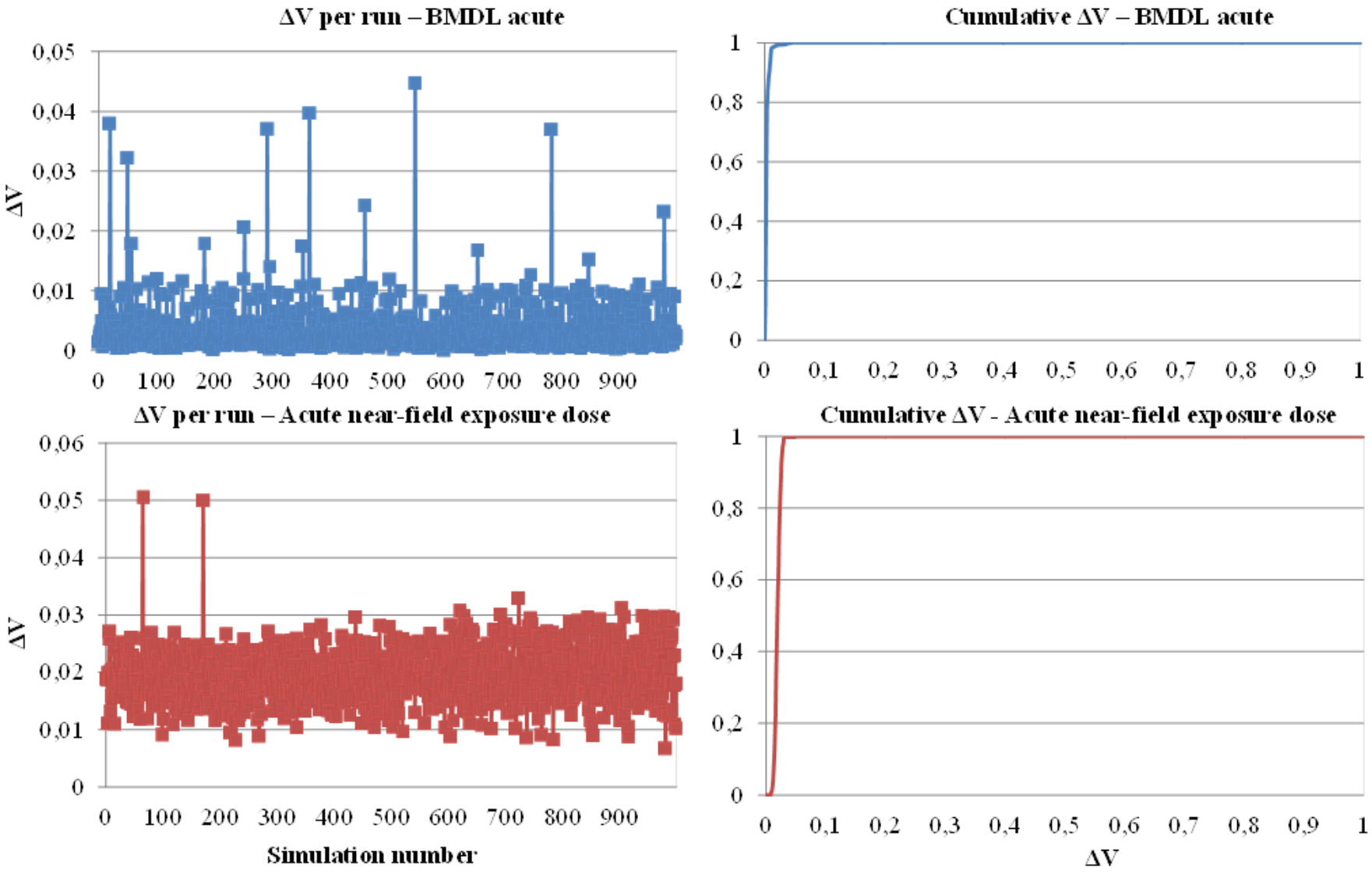
Tier III uses again a Monte Carlo probabilistic approach to analyse the uncertainties in the input data and how they affect the obtained results. The effects of variations in both BMDL and exposure doses were studied independently and the interval endpoints of the probability distributions were calculated similarly to tier II. For each exposure dose a uniform probability distribution was defined in the interval [a,b] with mean equal to the modelled estimate and with interval endpoints calculated as 1000% below and above the estimate respectively, to account for uncertainties in the data and the Nanosafer model performance. Similarly, a probability distribution was defined for each endpoint/body system-specific dose-response relationship on the interval [c,b], centred at the BMDL and interval endpoints calculated as values 100% below and above of the BMDL value respectively, to account for uncertainties in the design of the underlying toxicological experiments.

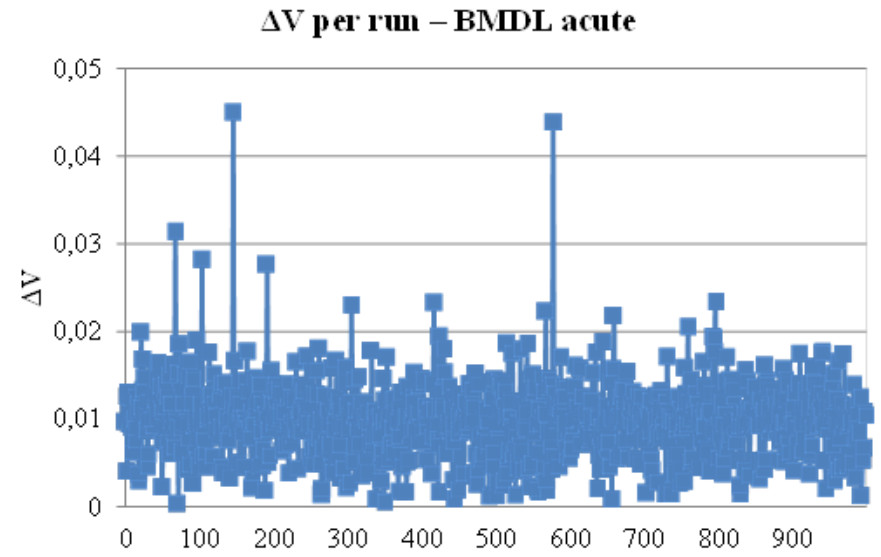
Figure 6-13 reports the (cumulative) distribution of ΔV for variations in both inputs and shows that in both cases the ΔV varies with less than 0.04 units for the exposure doses and less than 0.05 units for the BMDL in the 1000 Monte Carlo iterations. The following Table 6-8 details the average and maximum ΔV and the standard deviations due to variations in BMDL, acute and chronic NF and FF exposure.

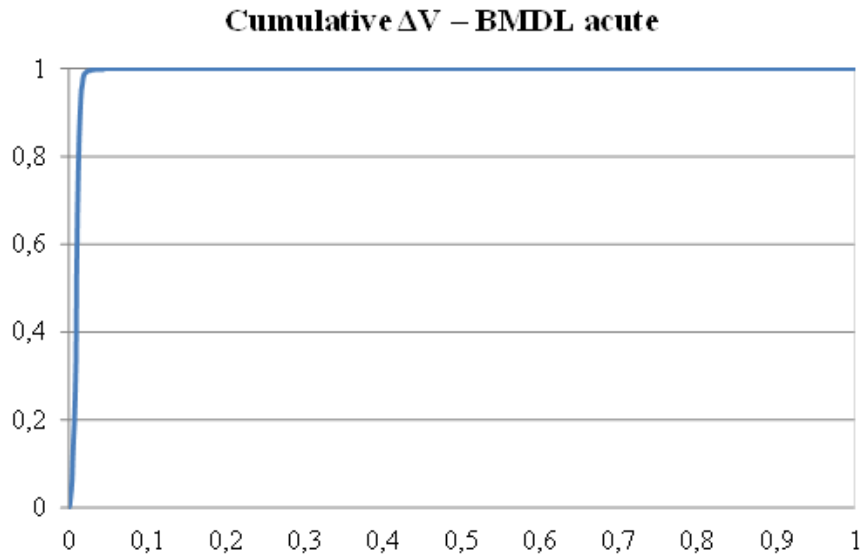
Table 6-8: Average and maximum ΔV and standard deviations due to variations in BMDL, acute and chronic NF and FF exposure in 1000 Monte Carlo simulations.

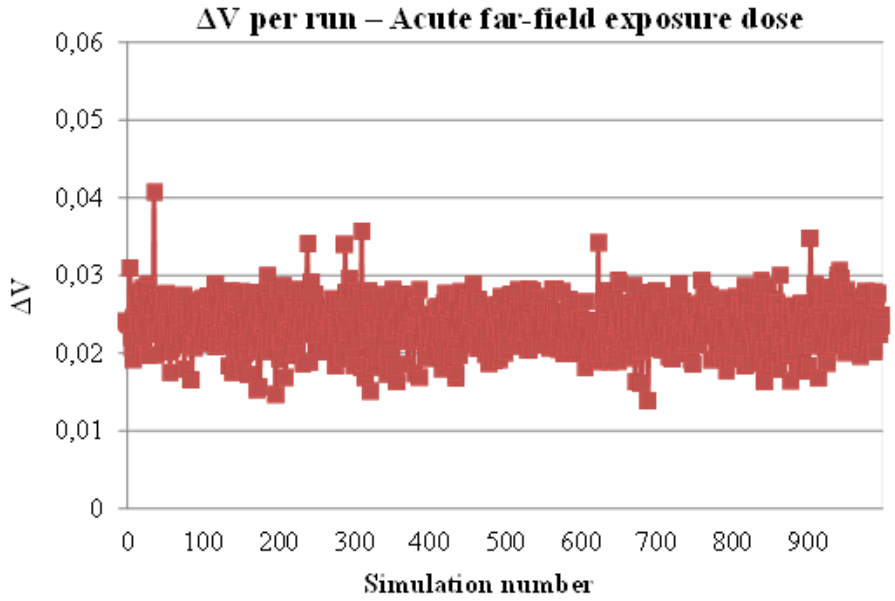
Input data type	Max ΔV	Average ΔV	Standard deviation (σ)
Acute NF exposure	0.05	0.019	0.0046
Acute DNEL	0.045	0.034	0.0039
Acute FF exposure	0.04	0.023	0.0029
Acute DNEL	0.045	0.099	0.004
Chronic NF exposure	0.037	0.025	0.0047
Chronic DNEL	0.039	0.011	0.0069
Chronic FF exposure	0.041	0.03	0.0078
Chronic DNEL	0.049	0.016	0.0011

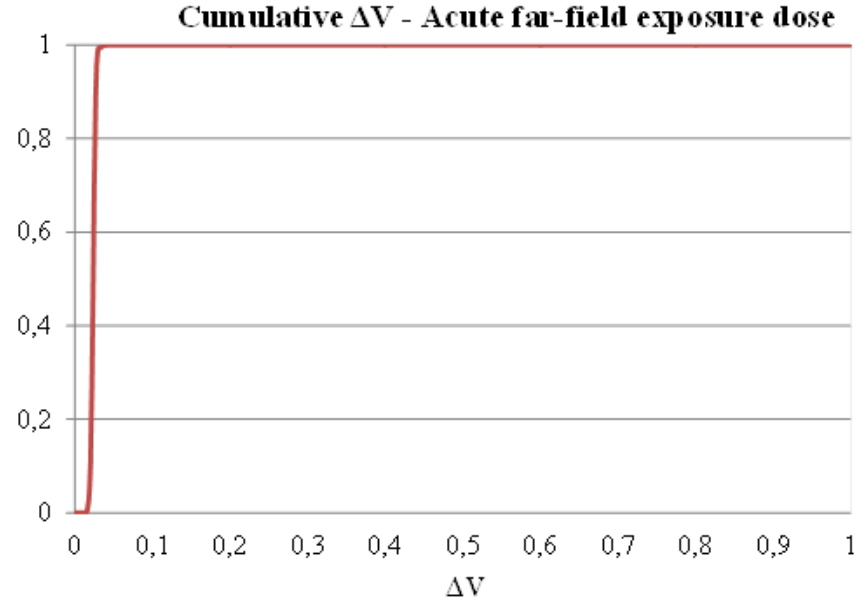
Figure 6-13: Left side: distributions of mean differences in value (ΔV) for (i) acute and chronic NF and FF exposure doses and (ii) Benchmark dose lower confidence intervals (BMDL) derived from 1000 Monte Carlo simulations changing input data; Right side: cumulative distribution of ΔV.

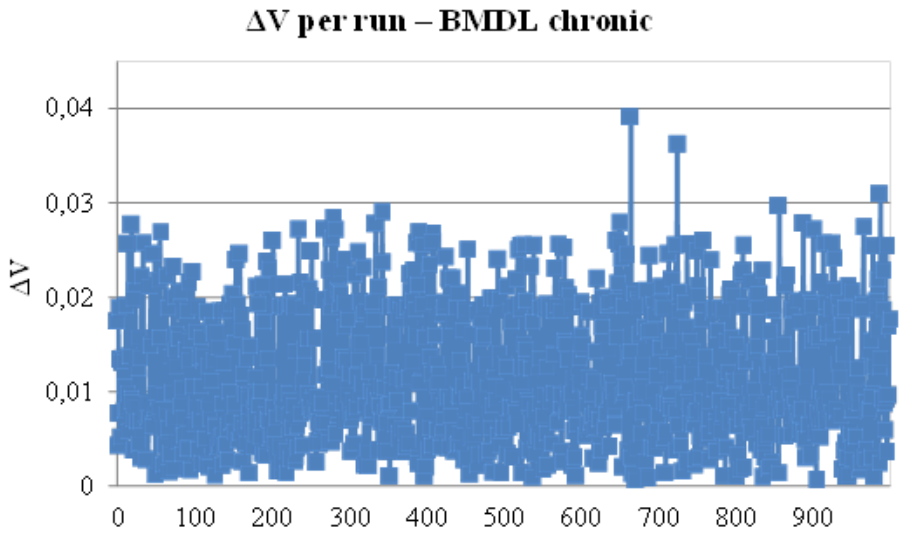


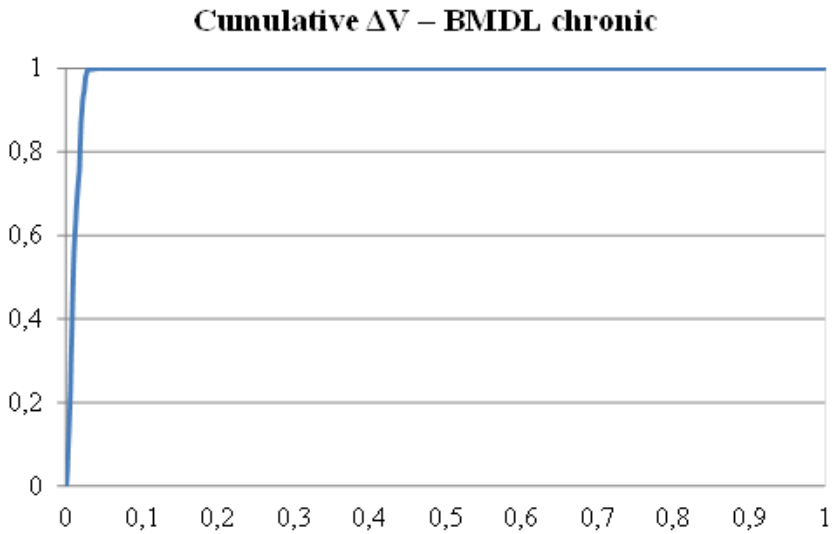

ΔV per run – BMDL acute
ΔV
0,05
0,04
0,03
0,02
0,01
0
0
100
200
300
400
500
600
700
800
900

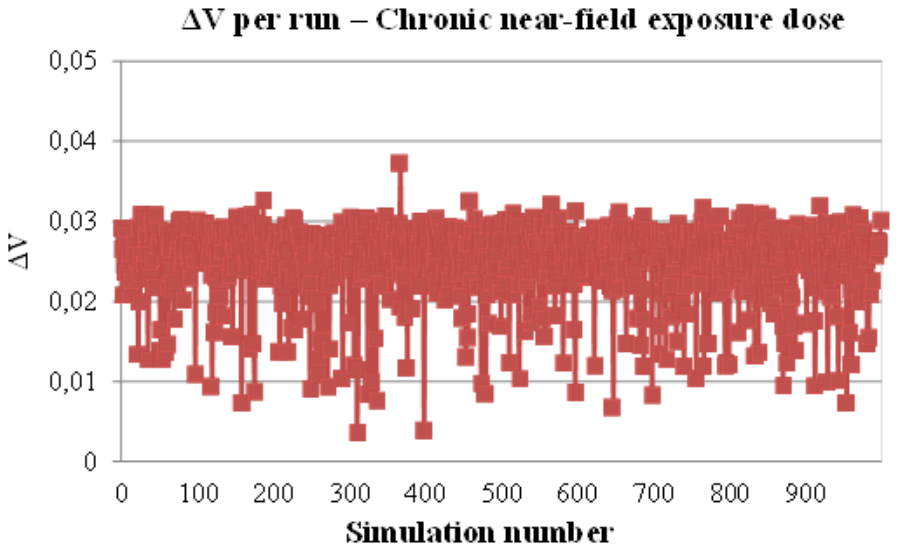

Cumulative ΔV – BMDL acute
1
0,8
0,6
0,4
0,2
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1

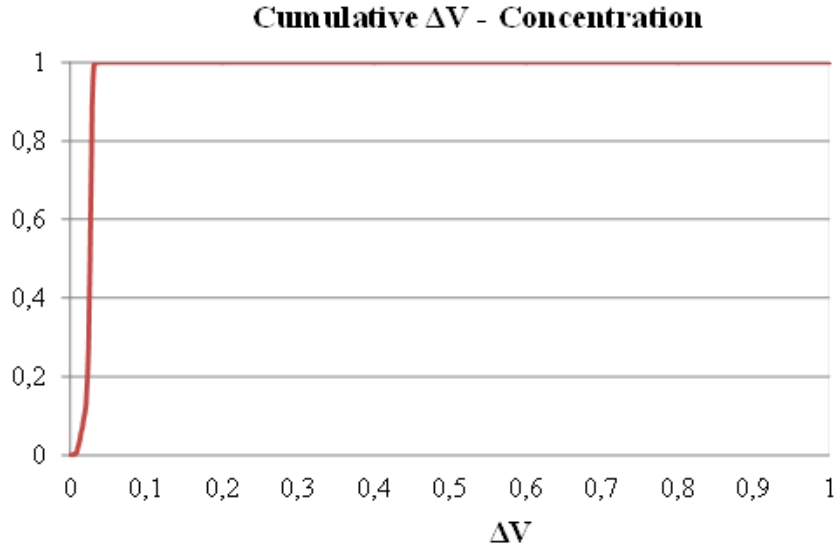

ΔV per run – Acute far-field exposure dose
ΔV
0,06
0,05
0,04
0,03
0,02
0,01
0
0
100
200
300
400
500
600
700
800
900
Simulation number

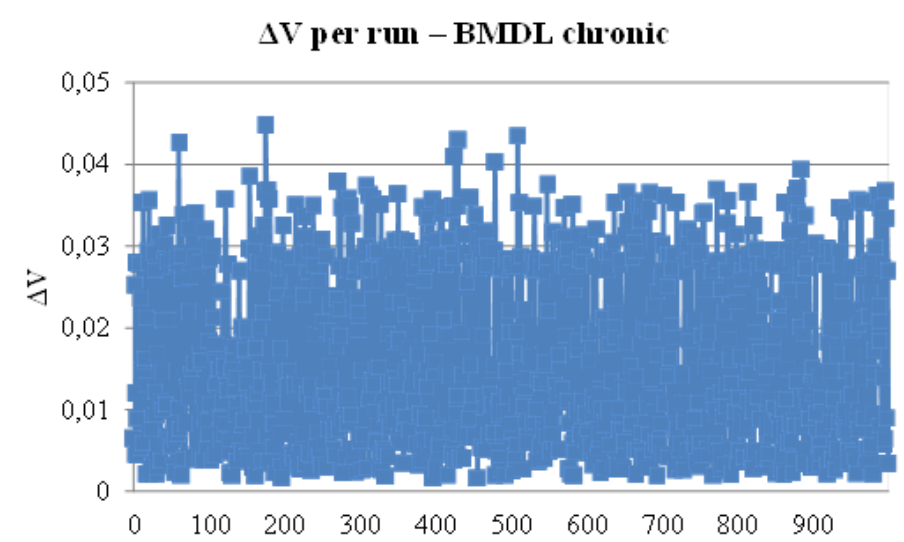

Cumulative ΔV - Acute far-field exposure dose
1
0,8
0,6
0,4
0,2
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1
ΔV

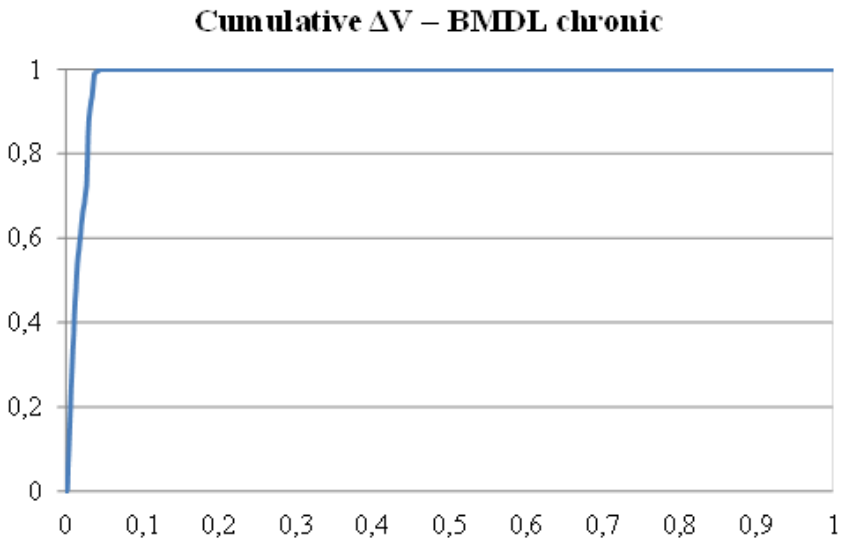

ΔV per run – BMDL chronic
ΔV
0,04
0,03
0,02
0,01
0
0 100 200 300 400 500 600 700 800 900

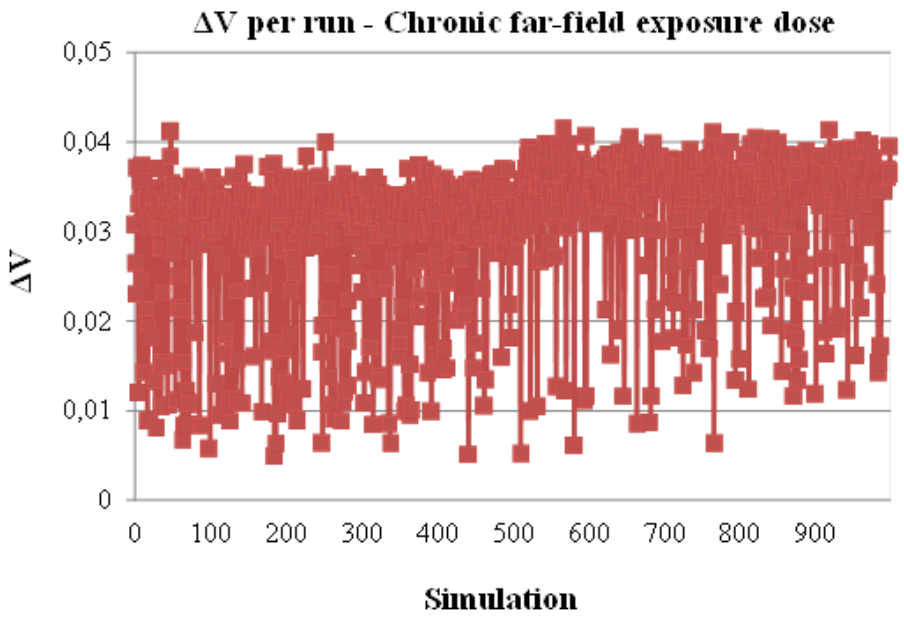

Cumulative ΔV – BMDL chronic
1
0,8
0,6
0,4
0,2
0
0 0,1 0,2 0,3 0,4 0,5 0,6 0,7 0,8 0,9 1

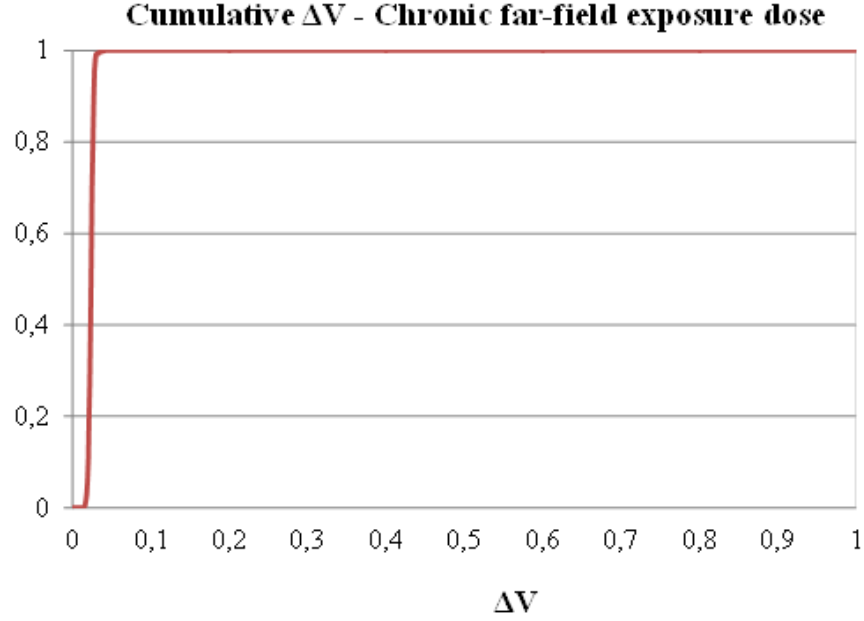

ΔV per run – Chronic near-field exposure dose
ΔV
0,05
0,04
0,03
0,02
0,01
0
0 100 200 300 400 500 600 700 800 900
Simulation number


Cumulative ΔV - Concentration
1
0,8
0,6
0,4
0,2
0
0 0,1 0,2 0,3 0,4 0,5 0,6 0,7 0,8 0,9 1
ΔV


ΔV per run – BMDL chronic
ΔV
0,05
0,04
0,03
0,02
0,01
0
0
100
200
300
400
500
600
700
800
900


Cumulative ΔV – BMDL chronic
1
0,8
0,6
0,4
0,2
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1


ΔV per run - Chronic far-field exposure dose
ΔV
0,05
0,04
0,03
0,02
0,01
0
0
100
200
300
400
500
600
700
800
900
Simulation


Cumulative ΔV - Chronic far-field exposure dose
1
0,8
0,6
0,4
0,2
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1
ΔV

6.4 Discussion

This thesis illustrates a tiered framework for RA of NOAA, in which the assessment progresses from relatively simple to more complex. The focus of the approach is the occupational risk of adverse health effects posed by exposure to NOAA via the inhalation route. The proposed framework and case studies clearly demonstrate the ease and analytical rigor of decision-analytical process and the many benefits derived from applying quantitative WoE tools. It shows how MCDA can be integrated into a larger decision framework, and suggests formal methodologies for RA of nanomaterials.

Although the requirement for iterations is not explicit in the above framework, it is relevant within each tier since the nano hazard and exposure evidence base grows fast and reassessments with new data are essential from regulatory perspective. While using a tiered RA model may reduce the need for iteration, it is not intended to entirely eliminate it.

Tier I: Quantitative risk screening

In Tier I for the first time hazard and exposure models explicitly integrate expert judgment for substituting data gaps and demonstrate that this is acceptable in real problems of practical importance. The main benefit gained from the elicitation process is that trough compensating uncertainties the applications achieved robust results. This approach adds significant flexibility to the model allowing practitioners to apply it even in situations of scarce and otherwise insufficient data. RA has been challenging for nanomaterials and emerging materials in general because of availability of multiple, often controversial, toxicity studies of different quality. This was accounted for in the Tier I hazard assessment methodology by integrating a regulatory method for data quality evaluation in terms of relevance, reliability and statistical power.

Although the application of Tier I shows robust results, they should be interpreted with caution since there are several assumptions in the proposed models that require additional validation and broader consensus from scientific community. For example, the Hazard identification approach integrates physico-chemical properties and toxicity test results into LoE-specific index values. Nominally, adding these two types of evidence instead of multiplying them can lead to overestimation of hazard. However, taking into consideration that the available toxicity data are very uncertain, often conflicting, and thus unreliable, in a conservative manner we back them up with the available physico-chemical information by using the WS aggregation operator. Second, we used MAVT method that is considered to be one of the most credible MCDA tools, but it is still prone to general problems that are characteristic of MCDA application in environmental settings, which include the use of appropriate scales and independent evaluation criteria and metrics. Even though we tried to ensure that the criteria weights reflect the relative importance of the criteria given the way in which the performance-scoring scales for the criteria have been calibrated (Steele et

al., 2009), more work is required to better understand the relationships between the criteria weights and the performance scoring scales. Third, instead of using statistical or threshold evaluation procedures, conclusions from each study are used to classify its outcomes, which may result in biases. Fourth, the expert judgment used to fill data gaps led to discrepancies between the WoE-derived and the experimental results.

Substantial input from subject matter experts is required for developing risk classes for each physico-chemical property, evaluation of statistical power and toxicological significance, and the relative weights of the components of the data quality evaluation. It is important to note, however, that eliciting information from experts is a critical and nuanced field of practice. Although a series of specific methods have been developed for this purpose (Stillwell et al. 1981; Belton and Stewart 2002) many potential biases can be introduced (Belton and Stewart, 2002; Morton et al., 2009) if proper procedures are not followed. Experts must be chosen carefully, and well-informed about the purpose of the information that is to be collected. When expert-elicited information is utilized for relative hazard identification, a formal uncertainty analysis is warranted.

In order to provide additional information to the decision maker about the confidence she should place in the assessment results we applied probabilistic Monte Carlo analysis to estimate the uncertainties associated with the model input parameters and arising from the expert elicitation process in both the hazard and the exposure models.

On hazard side the Monte Carlo uncertainty analysis showed that the model performs in a stable manner even in the context of large variations of input parameters and elicited weights. It also showed that the approach is most sensitive to changes in the toxicity evidence base, which is appropriate to the goal of the hazard assessment.

On exposure side, the analysis showed variability of exposure proportional to the number of expert assumptions and deductions used. However, variations neither in the type of the input data nor in the weights significantly influence the performance of the model and the obtained results. This indicates that inconsistencies between the WoE results and the measurements are rather due to the low quality of the dataset than to the performance of the model. This notion is confirmed by a number of scenarios (e.g. 15.2, 16.1) reporting unreliable measurements. Indeed, most measurements were derived by CPC and SMPS, which are unable to distinguish NOAA form background natural, or incidental nano aerosols or different types of NOAA (Ono-Ogasawara et al., 2009). These kinds of discrimination often require a combination of several techniques, including time-integrated sampling and offline analysis. Some approaches include comparing near-field to far-field or before-task to after-task measurements (Brouwer et al., 2011; Brouwer et al., 2009) or calculations using intrusion factors (Kuhlbusch et al., 2011). However, such methods have not been applied to the measurements reported in the NANEX database. This suggests that they often reflect background concentrations in addition to task-specific emissions. In contrast, the WoE approach was

designed to estimate activity-specific exposure. This discrepancy can be an important reason for inconsistencies between the model estimates and the corresponding measured concentrations. Another reason may be the design of the sampling campaign (Brouwer et al., 2004). Specifically, the choice of sampling locations can heavily influence measured particle number concentrations due to different NOAA agglomeration states under different operational conditions, which may lead to large variations in concentrations between sampling locations and the actual exposure sites (Maynard and Aitken, 2007).

Tier II: Quantitative relative Risk assessment

Tier 2 uses the MoE approach to perform relative ranking/prioritization of nanomaterials and ES. This is the only well-established method in the literature used for human health relative risk ranking in a regulatory context (Omenn et al., 1997). It is currently employed by the US Environmental Protection Agency (EPA) for non-carcinogens and proposed for carcinogens with non-linear dose-response characteristics (Omenn et al., 1997).

The application of the MoE approach not only removes the bias introduced by the fact that BMDL was not evaluated for humans but also acts as a statistical adjustment that allows concentrations related to different nanomaterials and scenarios to be compared on a sound ratio scale.

The normalized endpoint-specific MoE values are aggregated by means of a combined WS/OWA MCDA operator respecting the conditions that under constant exposure concentration, duration, and frequency (i) higher number of toxicity endpoints constitute higher risk; and (ii) some endpoints are more risky than others. The first condition is addressed by the WS operator through summing effects, while the second by using weights. Moreover, we adopted a conservative approach by employing the OWA operator, which assigns higher importance to higher-effect endpoints, while at the same time takes all other effects into consideration. In addition, using OWA allows controlling the level of precaution in the assessment by simply changing weights, mimicking behaviours which lie in between the maximum and average or minimum and average.

Table 6-6 shows the results of applying the Tier II model with a real dataset. Although they are more influenced by exposure rather than hazard evidence (margins among exposure doses are by orders of magnitude larger than margins among BMDL), differences in nanotoxicity do influence the ranking significantly. The results demonstrate the low resolution of the model, which is clearly a result of the adopted normalization procedure, which caused clustering of MoE estimates in the upper end of the normalization scale. Although we should interpret the results looking at clusters rather than individual scenarios as shown on Figure 6-11, the Tier II model successfully prioritises high-concern ES for further testing and risk analysis.

Tier III: Quantitative actual Risk assessment

As mentioned above and explained in section 5.2.2, endpoints can be aggregated by the OWA operator into high risk (HR) and low risk (LR) groups and rescaled by a convex and a concave *sine* function, respectively. The behaviour of the two rescaling functions with respect to the linear function is reported in Figure 6-14. When we apply those with the WS operator they change the overall behaviour of the aggregation operator as shown in Figure 6-15. It appears clearly that the convex curve tends to overestimate larger risk values in respect to smaller ones, while the concave function has the opposite behaviour. Using this property we incorporate the Precautionary principle in the model, magnifying the contribution of larger endpoint-specific RR to the assessment.

Figure 6-13: Comparison between linear (i.e. no scaling), convex and concave rescaling. In the *x* axis is the original value while in the *y* axis is the rescaled value, both are in the [0,1] domain.

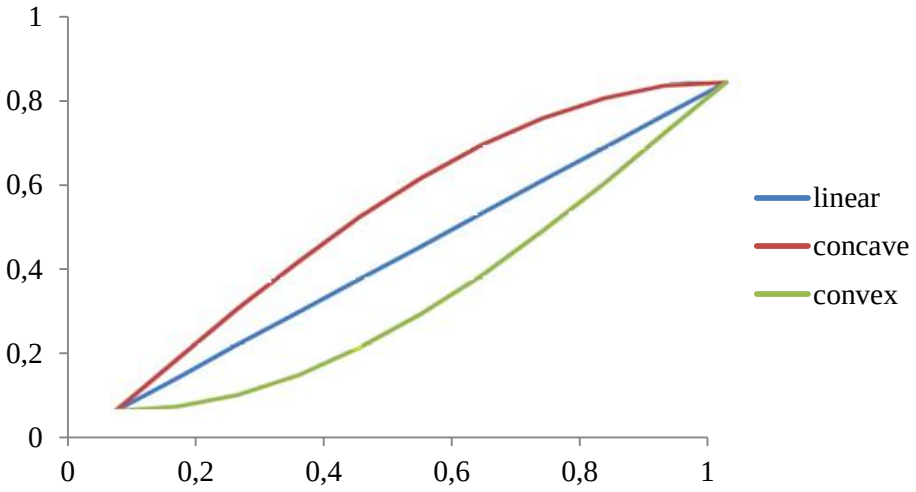
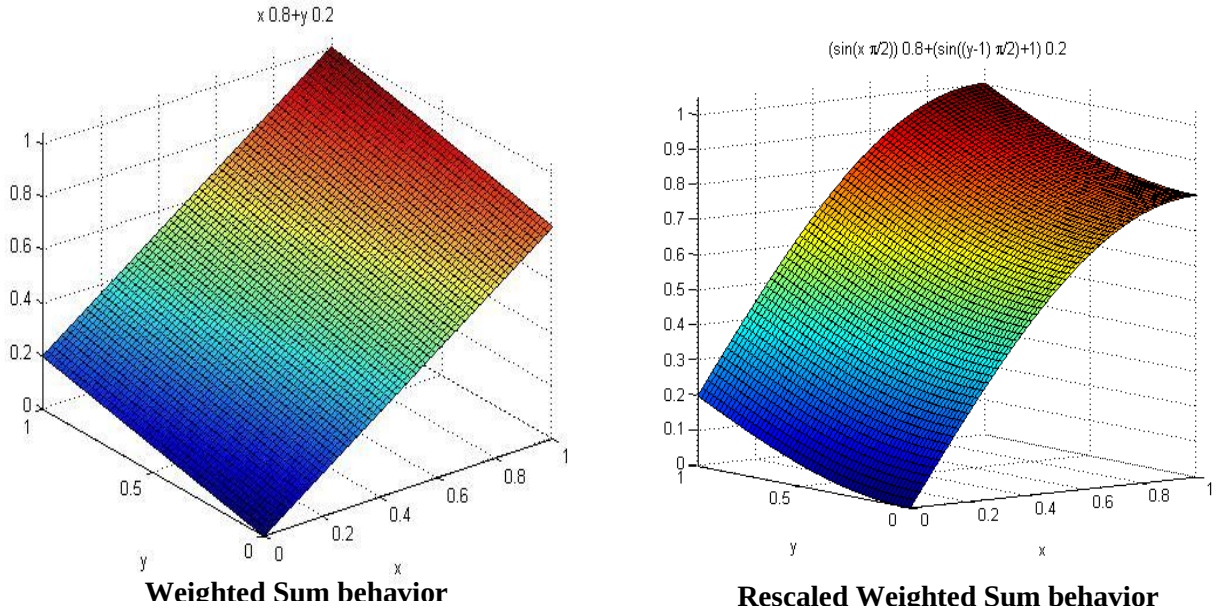


Figure 6-14: Example of ordinary (on the left) and rescaled (on the right) WS behaviour with weights $w_1 = 0.8$ and $w_2 = 0.2$. X axis: original values; Y axis: rescaled values; Z axis: weights.



In the above Tier III application uncertainties stem from the input data, expert judgment and extrapolation procedures to propagate through the whole risk analysis up to the final results.

Exposure doses used in the assessment are based solely on estimation of potential emission from workplace activities. Exposure to accidental or natural nanomaterials is not accounted for. Cumulative and aggregate exposures requires consideration (DG ENV, 2012). Failing to consider them adds uncertainty to the risk analysis.

After deposition of inhaled nanomaterials in the respiratory tract, they may translocate to the lungs, intestine, liver, spleen, brain etc. There are limited data available on the uptake and translocation kinetics of NOAA and the difference in absorption between the inhalation and instillation administration methods is not confirmed, which is a major source of uncertainty in the above risk analysis.

In the above RA we used default AF to extrapolate effects between species. Similarly, an intraspecies default AF of 5 was used to account for differences between workers and the general population. These AF are established based on historical knowledge on the mechanisms influencing dose and toxicity of conventional chemicals. It is largely unclear whether the same factors are appropriate for nanomaterials, and if not, then how they should be adapted (DG ENV, 2012). Although the Scientific Committee of the European Food Safety Authority concluded that the current scientific literature does not imply a need for different assessment factors for nanomaterials (EFSA 2011), we deem that the uncertainties caused by interspecies and intraspecies variability are yet to be documented.

Time scaling is often used for extrapolation from sub-acute to sub-chronic or sub-chronic to chronic effects in RA, however extrapolation from acute to chronic effects is generally avoided due to high level of uncertainty. Since we lacked data to perform chronic RA we did derive chronic DNEL out of acute data, which added significant uncertainty to the risk analysis.

Chemical-Specific Adjustment Factors (CSAF) should be used instead of the default assumptions described above (WHO, 2005), however, the definition of CSAF requires reliable physico-chemical data and good understanding of the mode of action behind the observed biological responses. Current limitations in data and mechanistic knowledge cause significant uncertainty in the derivation of CSAF, which prevented us from using such values in the above RA process.

At present there is no information on the validity of using the NanoSafer exposure model and it does not account for agglomeration and sedimentation processes, which are important factors influencing the fate of nanomaterials in occupational settings. Unfortunately aerosol dynamic modelling applied to nanomaterials is strongly constrained by the sparse data on source strengths, workplace measurements and contextual information, and is therefore still beyond the state of the art. The above considerations are additional sources of uncertainty, which should be accounted for.

In the toxicity data used for the above risk analysis, doses were reported only in mass metrics. In fact, for some nanomaterials the actual metric that best describes the distribution and the observed effects in test organisms may not be mass, but for example, particle number, surface area, or another metric (Aitken et al., 2011; Hankin et al., 2011). Using the wrong metric may result in failure to identify dose-response relationships important for the risk analysis. In order to avoid/reduce this type of uncertainty knowledge about the mechanisms underlying the observed effect (and determining fate) would be required to make a decision on the scientifically most appropriate dose metrics on a case-by-case basis or for defined groups on nanomaterials (DG ENV, 2012).

RA of nanomaterials can be strengthened is bottom-up production of *in vitro* and *in vivo* data and modelling activities are informed by top-down recommendations from risk assessors and regulators. Some important issues such as characterisation, comparable/standardised protocols were addressed in ENPRA and are reflected in the risk analysis.

In order to evaluate the above uncertainties, we suggested a sound probabilistic method, which effectively incorporates known experimental/analytical errors and uses expert judgment to account for unknown uncertainties (see section 5.3.2.2). The application of this approach clearly shows that the performance of the proposed Tier III risk model is stable against changes in the input variables. However, its reliance on expert judgment to transform unknown uncertainties into confidence intervals is a source of uncertainty itself. In order to manage this, it is essential to establish a formalized elicitation process.

Risk management in the face of uncertainty

The need for Risk management of nanomaterials in the context of high uncertainty stems from historical experience with other emerging pollutants (e.g. chlorofluorocarbons, polychlorinated biphenyls), which have had unexpected adverse health and ecological consequences, resulting in huge costs for society and impeding innovation (Koehler and Som, 2008). Therefore, it remains in the interest of all stakeholders to apply prudent Risk management approaches to nanomaterials based on the best available evidence.

Taking preventive action in the absence of scientific certainty is embodied by the Precautionary Principle. Although it is generally applied "where there are threats of serious or irreversible damage or harm, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent this potential damage or harm" (UN, 1992), the interpretation of the Precautionary Principle in terms of "threats of serious or irreversible harm" presents a challenge in the field of nanomaterials, mainly due to the paucity of information linking properties and effects (DG ENV, 2012). Preventive actions should consider all available information and allow read across from other substances with similar physico-chemical characteristics and biological identities in making informed decisions about hazard and risk. Although this thesis proposed a sound strategy for achieving this, in many instances the results will include significant uncertainties. When evaluated risks are deemed uncertain, additional controls should be

warranted as a primary prevention measure until further data become available to allow more informed risk analysis (Schulte and Salamanca-Buentello, 2007).

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CHAPTER 7

Conclusions

Contents included in:

Hristozov D, Gottardo S, Cinelli M, Isigonis P, Zabeo A, Critto A, Van Tongeren M, Tran L, Marcomini A. (2012). Application of a quantitative Weight of evidence approach for ranking and prioritization of occupational exposure scenarios for Titanium dioxide and Carbon nanomaterials. Nanotoxicology (in press)

and

Hristozov D, Zabeo A, Linkov I, Critto A, Isigonis P, Marcomini A. (2012b). A Weight of evidence approach for hazard screening of engineered nanomaterials. Nanotoxicology (in press)

This thesis was triggered by the need for criteria, methodologies and tools to inform Risk management and regulation of engineered nano-objects and their aggregates and agglomerates in the near term. It involves the development of a tiered approach for quantitative occupational risk analysis and its application to a set of commercially available nanomaterials. Recognized regulatory approaches such as Margin of exposure and Derived No-effect Level were embedded into the proposed framework thus providing enhanced functionality in performing both relative and absolute risk analysis. In order to target the most recent scientific research outcomes to every day needs of end-users and to ensure updating over time we used Multi-criteria decision analysis (MCDA), a quantitative Weight of evidence (WoE) approach, allowing integration of heterogeneous information and tailoring various models and tools to specific decisional contexts.

The main benefit arising from using MCDA for early stage nanosafety evaluations is that its framework allows incorporation of heterogeneous information in combination with expert judgement to make informed decisions in the face of uncertainty. On the hazard side the framework is flexible enough to incorporate a system for data quality evaluation, which is essential given the high number of unreliable toxicity results in the literature (Hristozov et al., 2012b). On exposure side the framework incorporates a system for filling data gaps by means of expert opinion, which is essential given the paucity of the available exposure evidence base (Hristozov et al., 2012a). The call for quantitative, robust decision making tools in the nanosafety area is unlikely to wane, and the importance placed on them will increase with time. The production of new information will naturally move modelling activities towards the quantitative region of the WoE spectrum. MCDA can potentially overcome many limitations of the existing qualitative WOE approaches, while at the same time provide a flexible framework applicable to various needs.

The application of MCDA for risk analysis of nanomaterials showed that they are most risky in exposure scenarios concerned with handling of dry powders in large quantities (e.g. bag/bin filling, manual

un/loading, dumping), which is confirmed by the state-of-the-art literature (Brouwer, 2010) as well as by the results of the Japanese NEDO project (Hanai et al., 2009). For such scenarios risks are often not controlled and suitable risk mitigation measures need to be implemented. Scenarios, where risks are controlled, but are still relatively high involve handling of dry powders in smaller amounts (e.g. transferring of materials for solution preparation, weighing). In contrast, synthesis wet chemistry and laser ablation scenarios were shown to pose negligible risks due to very low emissions. The analysis clearly shows that the differences in the risk estimates among scenarios are mainly due to differences in exposure potential. The reason is that the investigated nanomaterials have similar toxic potency, while exposure levels differ among processes with orders of magnitude.

We applied a probabilistic Monte Carlo approach to analyse the uncertainties in the above results and the sensitivity of the proposed hazard, exposure and risk models. The results showed that the outputs of the above models do not vary significantly due to variations in the input parameters, confirming that they are stable and reliable. However, the considerable uncertainties in the input parameters imply that the above results should be interpreted with caution and the above analysis should be repeated in an iterative process as better data become available. Such data may include high-resolution exposure estimates based on aerosol dynamic modelling (considering agglomeration/aggregation or particle loss by deposition) in combination with chronic inhalation toxicity studies. The production of these data will significantly reduce the present uncertainties and will ensure more informed regulatory decision making.

Acknowledgements

I first and foremost thank my supervisor, Prof. Antonio Marcomini, for his enormous support and to Prof. Jürgen Ertel for introducing me to this fascinating subject.

I also thank Stefania Gottardo for her encouragement and invaluable suggestions on how to improve my work. She was great in commenting on the drafts I handed in and giving very constructive feedback and critics.

I am also very thankful to my colleagues from the Ca' Foscari University and the Venice Research Consortium for their great support in carrying out the ENPRA project work related to this thesis. Special thanks to Alex Zabeo and Panos Isigonis for helping me with mathematics and to Andrea Critto for supervising the activities.

I am grateful to the EU FP7 ENPRA Project (NMP4-SL-2009-228789) for funding my doctoral work. I also thank to all project partners who contributed to my work with their data. Special thanks to Martie van Tongeren and Lang Tran from IOM and to Ilse Gosens, Jos Bessems and Wout Slob from RIVM for their invaluable advices on aspects of the performed risk analyses.

I am deeply grateful for all the support my colleague and friend Igor Linkov gave me during my stay in Boston. He always sent me articles that I often did not appreciate until some weeks later, when they gave me new valuable ideas. I would also like to thank Prof. Jeffery Keisler, Christy Foran and Matthew Bates for all discussions we had and for giving me inputs and comments on my work.

I am also very thankful to Phil Sayre who gave me the great opportunity to spend some time at the US Environmental Protection Agency and experience Washington DC. I would also like to send my thanks to Michael Tsang , Edward Fallon, and Joy Murphy at the EPA Office of Research and Development for giving me support and advices on where to go, dragging me for coffee breaks and lunches and taking me to picnics and kayaking.

Last, but not least I thank my family and my girlfriend Stella who stood by my side in all good and bad moments in the last years and provided me with immense emotional support.

ANNEX 1

Definitions of "nanomaterial" and related terms

Table A1-1: Definitions of "nanomaterial" and related terms by international organizations

International Organization for Standardization (ISO) CEN/ISO/TS 27687/2008
Nanoscale: Size range from approximately 1 nm to 100 nm.
Nanoobject: Material with one, two or three external dimensions in the nanoscale.
Particle: Minute piece of matter with defined physical boundaries (from ISO 14644-6/2007).
Nanoparticle: Nanoobject with all three external dimensions in the nanoscale.
Agglomerates: Collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components.
Aggregates: Particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.
Nanoplate: Nanoobject with one external dimension in the nanoscale and the two other external dimensions significantly larger.
Nanofibre: Nanoobject with two similar external dimensions in the nanoscale and the third dimension significantly larger.
Nanotube: Hollow nanofibre.
Nanorod: Solid nanofibre.
Nanowire: Electrically conducting or semiconducting nanofibre.
Quantum dot: Crystalline nanoparticle that exhibits size-dependent properties due to quantum confinement effects on the electronic states.
International Organization for Standardization (ISO) TS 80004-1/2010
Nanomaterial: Material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.
Working Party on Manufactured Nanomaterials (WPMN) under the OECD Joint Chemicals Programme
Nanoscale: Size range typically between 1 nm and 100 nm.
Nanomaterial: Material which is either a nanoobject or is nanostructured.
Nanoobject: Material confined in one, two, or three dimensions at the nanoscale.
Nanostructured: Having an internal or surface structure at the nanoscale.
Manufactured nanomaterials: Nanomaterials intentionally produced to have specific properties or specific composition.

Table A1-2: Definitions of "nanomaterial" and related terms proposed by European organizations

EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)
Nanoscale: A feature characterized by dimensions of the order of 100 nm or less.
Nanostructure: Any structure that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less.
Nanomaterial: Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.
Nanoparticle: A discrete entity which has three dimensions of the order of 100 nm or less.
Nanosheet: A discrete entity which has one dimension of the order of 100 nm or less and two long dimensions.
Nanorod: A discrete entity which has two dimensions that are of the order of 100 nm or less, and one long dimension.
Nanotube: A discrete hollow entity which has two dimensions of the order of 100 nm or less and one long dimension.
Nanoparticulate matter: A substance comprising of particles, the substantial majority of which have three dimensions of the order of 100 nm or less.
EU Scientific Committee on Consumer Products (SCCP)
Nanoscale: Having one or more dimensions of the order of 100 nm or less.
Nanomaterial: Material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features.
Nanoparticle: Particle with one or more dimensions at the nanoscale.
European Commission (EC)
Nanomaterial: natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials
Regulation (EU) No 528/2012 on Biocide Products
Nanomaterial: natural or manufactured active substance or non-active substance containing particles, in an unbound state or as an aggregate or as agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm. Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials.
Regulation (EU) No 1169/2011 on Food Information
Engineered nanomaterial: any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale.

Table A1-3: Definitions of "nanomaterial" proposed by national authorities.

Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
Industrial nanomaterial: Industrial nanomaterials are those industrial materials intentionally produced, manufactured or engineered to have specific properties or specific composition, and one or more dimensions typically between 1 nm and 100 nm. This size range refers to individual particle size, and does not take into account agglomeration of particles.
Health Canada
Nanomaterial: Any manufactured product, material, substance, ingredient, device, system or structure to be nanomaterial if: (a) it is at or within the nanoscale in at least one spatial dimension, or; (b) it is smaller or larger than the nanoscale in all spatial dimensions and exhibits one or more nanoscale phenomena.
Danish Ministry of the Environment
Nanomaterials: Materials which are less than 100 nanometres in length along the shortest side or have structures which have such small dimensions but are built into larger materials (i.e. nanostructured surfaces). A nanometre is a millionth of a millimetre. Nanomaterials can be produced from existing chemical substances or completely new chemical compounds, and can be made from one or more substances. The small size of the materials is reason for their special characteristics.
UK Department for Environment, Food and Rural Affairs, DEFRA
Nanoscale material: Having two or more dimensions up to 200 nm.
US Environment Protection Agency (EPA) Stewardship Programme for nanoscale materials under the Toxic Substances Control Act (TSCA)
Engineered nanoscale material: Any particle, substance, or material that has been engineered to have one or more dimensions in the nanoscale. **Nanoscale:** The size range between the atomic/molecular state and the bulk/macro state. This is generally, but not exclusively, below 100 nm and above 1 nm.

ANNEX 2

Recommendations for inclusion of nano-specific data and information in the chemical safety data sheet (SDS)

#	SDS chapter description	Need for nano-specific information/ data	Type of necessary data
1	Identification of the substance/mixture and of the company/undertaking	Necessary	Under "purpose“, a declaration of the specific properties of the nanosized components should be made. Examples: • The nanoparticles contained increase the antibacterial properties of the coat of paint. • The nanoparticles alter the surface structure and make cleaning easier.
2	Hazards identification	Necessary	Data regarding the assessment of potential sources of risk should be included in this chapter for the purposes of a general assessment, since specific data on damage to health and the environment are only available at the moment from individual cases. When these are available, they should be cited. Where no specific dangers are known, general information will be recommended.
3	Composition/ information on ingredients	Necessary	It is strongly recommended that the type and amount of the nanomaterials present in the product are provided in this chapter, including also information on coating or fictionalization.
4	Description of first aid measures	Preferable	N/A
5	Fire fighting measures	Important	Data on the increased risk of fire or explosion should always be provided in a substance specific way.
6	Accidental release measures	Preferable	N/A
7	Handling and storage	Important	In order to systematically minimize exposure, the available various control measures should be prioritised based on the "TOP Procedure Principal" and set out in chapter 7 of the SDS.
8	Exposure	Important	N/A

	controls/personal protection		
9	Physical and chemical properties	Necessary	Compared with larger particles of the same chemical composition, nanoparticles often have differing mechanical, electrical, optical, chemical, magnetic or biological properties. A minimum required physicochemical dataset should be included in this chapter.
10	Stability and reactivity	Preferable	
11	Toxicological information	Preferable	N/A
12	Ecological information	Preferable	
13	Disposal considerations	Important	In this chapter information should be included on possible nano-specific properties which during the disposal process of nanomaterials could lead to the release of nano-objects and potential human and/or environmental exposure.
14	Transport information	Preferable	N/A
15	Regulatory information	Preferable	N/A
16	Other information	Preferable	N/A

Legend:
Necessary: the necessary minimum data for evaluation and safe handling of nanomaterials. Without this data, the necessity of protective measures for employees, consumers and the environment cannot be fully evaluated.
Important: it is important to provide nano-specific information and recommendations for safe handling wherever possible in these SDS chapters.
Preferable: data on these chapters is currently available for very few nanomaterials. However any available data should be included.

ANNEX 3

List of toxicity studies used for Tier I hazard assessment

Citation	Type of study
Barlow P, Clouter-Baker A, Donaldson K, MacCallum J, Stone V. (2005). Carbon black nanoparticles induce type II epithelial cells to release chemotaxins for alveolar macrophages. Particle and Fibre Toxicology 2: 11.	In vitro
Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. (2004). Pulmonary Responses of Mice, Rats, and Hamsters to Subchronic Inhalation of Ultrafine Titanium Dioxide Particles. Toxicological Sciences 77: 347-357.	In vivo (inhalation)
Chen H-W, Su S-F, Chien C-T, Lin W-H, Yu S-L, Chou C-C, Chen JJW, Yang P-C. (2006). Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. The FASEB Journal 20: 2393-2395.	In vivo (inhalation)
Churg A, Gilks B, Dai J. (1999). Induction of fibrogenic mediators by fine and ultrafine titanium dioxide in rat tracheal explants. American Journal of Physiology - Lung Cellular and Molecular Physiology 277: L975-L982.	In vivo (inhalation)
Ferin J, Oberdörster G, Penney DP. (1992). Pulmonary Retention of Ultrafine and Fine Particles in Rats. American Journal of Respiratory Cell and Molecular Biology 6: 535-542.	In vivo (inhalation)
Grassian VH, Adamcakova-Dodd A, Pettibone JM, O'Shaughnessy PI, Thorne PS. (2007). Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes. Nanotoxicology 1: 211-226.	In vivo (inhalation)
Gurr J-R, Wang ASS, Chen C-H, Jan K-Y. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213: 66-73.	In vitro
Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levsen K. (1995). Chronic Inhalation Exposure of Wistar Rats and two Different Strains of Mice to Diesel Engine Exhaust, Carbon Black, and Titanium Dioxide. Inhalation Toxicology 7: 533-556.	In vivo (inhalation)
Helfenstein M, Miragoli M, Rohr S, Müller L, Wick P, Mohr M, Gehr P, Rothen-Rutishauser B. (2008). Effects of combustion-derived ultrafine particles and manufactured nanoparticles on heart cells in vitro. Toxicology 253: 70-78.	In vitro
Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. (2005). In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicology in Vitro 19: 975-983.	In vitro
Inoue K-i, Takano H, Yanagisawa R, Koike E, Shimada A. (2009). Size effects of latex nanomaterials on lung inflammation in mice. Toxicology and Applied Pharmacology 234: 68-76.	In vivo (inhalation)
Inoue K, Takano H, Ohnuki M, Yanagisawa R, Sakurai M, Shimada A, Mizushima K,	In vivo

Yoshikawa T. (2008). Size effects of nanomaterials on lung inflammation and coagulatory disturbance. International Journal of Immunopathology and Pharmacology 21: 197-206	(inhalation)
Kobayashi N, Naya M, Endoh S, Maru J, Yamamoto K, Nakanishi J. (2009). Comparative pulmonary toxicity study of nano-TiO_2 particles of different sizes and agglomerations in rats: Different short- and long-term post-instillation results. Toxicology 264: 110-118.	In vivo (inhalation)
L'Azou B, Jorly J, On D, Sellier E, Moisan F, Fleury-Feith J, Cambar J, Brochard P, Ohayon-Courtes C. (2008). In vitro effects of nanoparticles on renal cells. Particle and Fibre Toxicology 5: 22.	In vivo (inhalation)
Lee KP, Trochimowicz HJ, Reinhardt CF. (1985). Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicology and Applied Pharmacology 79: 179-192.	In vivo (inhalation)
Linnainmaa K, Kivipensas P, Vainio H. (1997). Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells. Toxicology in Vitro 11: 329-335.	In vitro
Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. (2006). Titanium Dioxide (P25) Produces Reactive Oxygen Species in Immortalized Brain Microglia (BV2): Implications for Nanoparticle Neurotoxicity†. Environmental Science & Technology 40: 4346-4352.	In vitro
Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, Hester S, Lowry GV, Veronesi B. (2007). Nanosize Titanium Dioxide Stimulates Reactive Oxygen Species in Brain Microglia and Damages Neurons in Vitro. Environ Health Perspect 115	In vitro
Muhle H, Bellmann B, Creutzenberg O, Dasenbrock C, Ernst H, Kilpper R, MacKenzie JC, Morrow P, Mohr U, Takenaka S et al. (1991). Pulmonary Response to Toner upon Chronic Inhalation Exposure in Rats. Toxicological Sciences 17: 280-299.	In vivo (inhalation)
Oberdoerster G, Ferin J, Gelein R, Soderholm AC, Finkelstein J. (1992). Role of the alveolar macrophage in lung injury: studies with ultrafine particles. Environ Health Perspect 97: 193-199.	In vivo (inhalation)
Oberdörster G, Ferin J, Lehnert BE. (1994). Correlation between Particle Size, In Vivo Particle Persistence, and Lung Injury. Environmental Health Perspectives 102: 173-179.	In vivo (inhalation)
Park E-J, Yi J, Chung K-H, Ryu D-Y, Choi J, Park K. (2008). Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. Toxicology Letters 180: 222-229.	In vitro
Peters K, Unger R, Kirkpatrick C, Gatti A, Monari E. (2004). Effects of nanoscaled particles on endothelial cell function in vitro: Studies on viability, proliferation and inflammation. Journal of Materials Science: Materials in Medicine 15: 321-325.	In vitro
Rehn B, Seiler F, Rehn S, Bruch J, Maier M. (2003). Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicology and Applied Pharmacology 189: 84-95.	In vivo (inhalation)
Renwick LC, Brown D, Clouter A, Donaldson K. (2004). Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occupational and Environmental Medicine 61: 442-447.	In vivo (inhalation)

Sager T, Kommineni C, Castranova V. (2008). Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: role of particle surface area. Particle and Fibre Toxicology 5: 17.	In vivo (inhalation)
Wang J, Chen C, Liu Y, Jiao F, Li W, Lao F, Li Y, Li B, Ge C, Zhou G et al. (2008a). Potential neurological lesion after nasal instillation of TiO_2 nanoparticles in the anatase and rutile crystal phases. Toxicology Letters 183: 72-80.	In vivo (inhalation)
Wang J, Liu Y, Jiao F, Lao F, Li W, Gu Y, Li Y, Ge C, Zhou G, Li B et al. (2008b). Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles. Toxicology 254: 82-90.	In vivo (inhalation)
Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM. (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: Differential responses related to surface properties. Toxicology 230: 90-104.	In vivo (inhalation)
Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL. (2006). Pulmonary Instillation Studies with Nanoscale TiO_2 Rods and Dots in Rats: Toxicity Is not Dependent upon Particle Size and Surface Area. Toxicological Sciences 91: 227-236.	In vivo (inhalation)

ANNEX 4

Dose-response analysis

All *in-vivo* data produced in ENPRA have been sent to the Institute of Occupational Medicine (IOM) in an agreed reporting format. The data has been extracted from these data files in order to get it into the required format for the PROAST (http://www.rivm.nl/en/foodnutritionandwater/foodsafety/proast.jsp software. Using the data that has been supplied to date dose-response modelling has been undertaken in order to determine the 5% or the 10% Benchmark dose (BMD) for each endpoint/organ system/particle combination for which there is a dose-response relationship. These BMD values, and associated confidence intervals, for the data that have been received to date have been stored to use for risk analysis. Since the lower confidence limits of the BMD (i.e. BMDL) values corresponded to instillation bolus doses delivered to the test animals they were "corrected" to inhalation BMDL following the two "extreme" approaches reported in Box A4-1. Because the acute inhalation BMDL were more certain (since estimated from acute instillation data) they were used as the "starting point" to estimate acute and chronic Derived no-effect levels (DNEL) using the procedure reported in Box A4-2. The original and the converted BMDL as well as the DNEL values are reported in Table A4-1.

Box A4-1: Two methods to extrapolate instillation BMDL to inhalation BMDL.

Correcting acute instillation BMDL to acute inhalation BMDL

Convert mouse instillation BMDL (mg/animal) into mouse inhalation BMDL (in mg/m^3) by dividing it by the default mouse tidal volume (2.2 x 10^{-7} m^3/animal).

$$\text{inhalation BMDL}(mg/m^3) = \frac{\text{instillation BMDL (mg/animal)}}{\text{animal tidal volume } (m^3/\text{animal})}$$

This approach assumed no difference in absorption between inhalation and instillation.

Correcting acute instillation BMDL to 8-hour averaged mouse inhalation BMDL (high uncertainy!)

Convert mouse instillation BMDL (mg/animal) into mouse inhalation BMDL (in mg/m^3) by dividing it by the default mouse tidal volume (2.2 x 10^{-7} m^3/animal) and by the total number of inhalations of a mouse for 8 hours (i.e. 78240), which represents a work shift.

$$\text{inhalation BMDL}(mg/m^3) = \frac{\frac{\text{instillation BMDL}(mg/\text{animal})}{\text{animal tidal volume } (m^3/\text{animal})}}{\text{total number of animal inhalations for 8 hours}}$$

This approach assumed no difference in absorption between inhalation and instillation.

Box A4-1: Estimation of acute and chronic DNEL from inhalation BMDL.

Stepwise procedure for estimating acute and chronic DNEL

Step 1: Select the relevant dose-descriptor(s).

Endpoint/body system-specific instillation BMDL values from the ENPRA *in vivo* toxicity dataset are the dose descriptors to use for calculation of DNEL.

Step 2: Modify the relevant dose descriptor(s) per endpoint to the correct starting point (in a few situations, the effects assessment is not directly comparable to the Exposure assessment in terms of exposure route, units and/or dimensions and the dose descriptor needs to be converted).

The instillation BMDL were "corrected" to "single inhalation BMDL" as described in the above Box A4-1. Because the exposure duration in the *in vivo* toxicity studies (i.e. instillation bolus dose delivered within a tenth of a second) differed from that of the target workers population (i.e. 15 minutes for acute exposure as recommended by the REACH guidelines), time scaling was performed following the modified Haber's law ($C^n \times t = k$), where "C" is the concentration, "n" is a regression coefficient, "t" is the exposure time and "k" is a constant. Since the scaling is from shorter to longer exposure time (t), n was set to 1 as advised by the REACH guidelines (European Chemicals Agency, 2008).

Step 3: Apply assessment factors (AF) to the correct starting point to account for uncertainties in the extrapolation of experimental data to the real human exposure situation.

Preferably, the value for each individual AF is based on substance-specific information. In the lack of such data we used the following default AF recommended by the REACH Guidelines:

- for interspecies differences: value of 10;
- for intraspecies differences: value of 5.

The DNEL values resulting from the extrapolation procedure are presented in the following Table A4-1.

Table A4-1: BMDL and DNEL values for different NOAA, organ systems and endpoints.

Material type	Material	Test method	Organ system	Endpoint	BMDL (ug/animal)	Single inhalation BMDL (mg/m^3)	Daily averaged inhalation BMDL (mg/m^3)(8 h)	DNELacute (mg/m^3)	DNELchronic (mg/m^3)
TiO_2	NCRWE1	Intratracheal instillation	Pulmonary	Inflammation and immune response	6,48E+00	2,95E+04	3,80E-01	4,39E-02	8,78E-03
	NCRWE1	Intratracheal instillation	Pulmonary	Cytotoxicity	5,70E+00	2,59E+04	3,30E-01	3,86E-02	9,65E-03
	NCRWE1	Intratracheal instillation	Hepatic	Organ pathology	7,00E-01	3,20E+03	4,00E-02	4,77E-03	9,54E-04
	NCRWE1	Intratracheal instillation	Renal	Organ pathology	4,27E+01	1,94E+05	2,48E+00	2,89E-01	7,23E-02
	NCRWE2	Intratracheal instillation	Cardiovascular	Organ pathology	1,20E+00	5,43E+03	7,00E-02	8,09E-03	1,62E-03
	NCRWE2	Intratracheal instillation	Pulmonary	Oxidative stress	1,14E+01	5,16E+04	6,60E-01	7,68E-02	1,54E-02
	NCRWE3	Intratracheal instillation	Pulmonary	Genotoxicity	8,25E+00	3,75E+04	4,80E-01	5,59E-02	1,40E-02
	NCRWE4	Intratracheal instillation	Pulmonary	Organ pathology	6,80E-01	3,11E+03	4,00E-02	4,62E-03	9,24E-04
	NCRWE4	Intratracheal instillation	Cardiovascular	Inflammation and immune response	3,40E-01	1,52E+03	2,00E-02	2,27E-03	7,57E-04
ZnO	NM111	Intratracheal instillation	Pulmonary	Inflammation and immune response	1,93E+00	8,79E+03	1,10E-01	1,31E-02	6,55E-03
	NM111	Intratracheal instillation	Pulmonary	Cytotoxicity	3,70E-01	1,70E+03	2,00E-02	2,53E-03	5,06E-04
	NM111	Intratracheal instillation	Cardiovascular	Organ pathology	2,37E+00	1,08E+04	1,40E-01	1,61E-02	8,05E-03
	NM111	Intratracheal instillation	Cardiovascular	Inflammation and immune response	8,96E+00	4,08E+04	5,20E-01	6,07E-02	6,07E-02

	NM110	Intratracheal instillation	Pulmonary	Oxidative stress	4,23E+00	1,93E+04	2,50E-01	2,87E-02	1,44E-02
	NM110	Intratracheal instillation	Pulmonary	Genotoxicity	3,00E-03	1,33E+01	0,00E+00	1,98E-05	6,60E-06
	NM110	Intratracheal instillation	Lymphatic	Organ pathology	2,00E+00	9,11E+03	1,20E-01	1,36E-02	1,36E-02
	NM300	Intratracheal instillation	Cardiovascular	Organ pathology	1,23E+01	5,60E+04	7,20E-01	8,34E-02	1,67E-02
Ag	NM300	Intratracheal instillation	Pulmonary	Oxidative stress	7,40E-01	3,37E+03	4,00E-02	5,02E-03	1,67E-03
	NM300	Intratracheal instillation	Renal	Organ pathology	1,92E+01	8,71E+04	1,11E+00	1,30E-01	6,50E-02
	NM400	Intratracheal instillation	Pulmonary	Inflammation and immune response	5,79E+00	2,63E+04	3,40E-01	3,92E-02	1,31E-02
MWCNT	NM400	Intratracheal instillation	Pulmonary	Genotoxicity	7,76E+00	3,51E+04	4,50E-01	5,22E-02	1,04E-02
	NM400	Intratracheal instillation	Lymphatic	Organ pathology	2,20E+01	9,98E+04	1,28E+00	1,49E-01	7,45E-02
	NM402	Intratracheal instillation	Pulmonary	Oxidative stress	2,62E+01	1,19E+05	1,52E+00	1,77E-01	3,54E-02

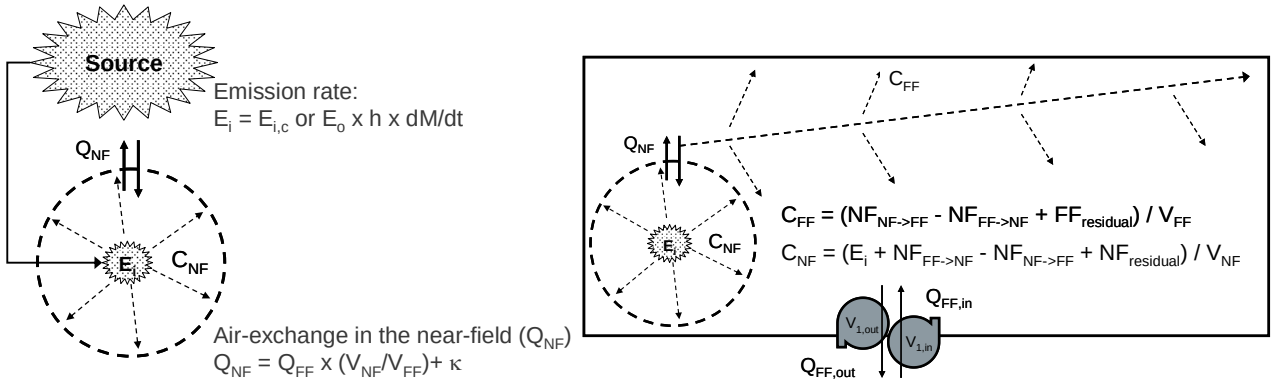
ANNEX 5

Exposure assessment

A5.1 NanoSafer model

To evaluate exposure, we performed first order modelling of the nanoparticles dust concentrations in the near- and far-field using the background model in NanoSafer control banding tool (Jensen et al., in preparation). Figure A5-1 gives a schematic overview of the model.

Figure A5-1: Schematic illustration of the NanoSafer exposure model. The left-hand illustrates the definition of the emission rate (Ei), its dilution in the near-field (NF) and air-exchange (Q_{NF}) between NF and the far-field (FF), where κ can be considered a thermal diffusion constant. The right-hand side illustrates the transfer of air between the NF and the FF and ventilation exchange with outdoor air (Q_{FF}). The theoretical imission values are calculated at a time-resolution of 1 minute and based on the E_i and dust transfer with air-exchange functions for NF to FF ($NF_{NF->FF}$); FF to NF ($NF_{FF->NF}$); Q_{FF}, and the residual polluted air in the NF ($NF_{residual}$) and FF ($FF_{residual}$) normalized for the volumes of the NF (V_{NF}) and FF (V_{FF}), respectively.



In brief, the model is a two-compartment instantaneous mixing model consisting of a Near-Field (NF; activity zone) with a radius of 1.35 m and a Far-Field (FF; general work-room) volume. The activity is always located in the NF and diluted first in the NF and then the FF zone. The emission rate (E_i) is determined or assessed from constant release values ($E_{i,c}$) or the dustiness index (E_o) multiplied with an activity energy index (h) and the mass-flow (dM/dt), where the time-resolution currently is set to 1 minute. The activity indices (Table A5-1) have been inspired from the Dutch Stoffenmanager and has been slightly modified based on judgement of comparability between traditional powders and nano-powders. E_i is immediately diluted into the volume (V_{NF}) of the near-field (NF), a sphere with a radius of 1.35 m. The concentrations are then modelled by iterated transfer of air-masses between the NF and Far-Field (FF) workroom and dilution therein every 1 minute. Particle removal and clean air is ensured by ventilation exchange with outdoor air ($Q_{FF,out}$). No additional engineered local exposure ventilation control,

agglomeration or particle loss by sedimentation is considered. Consequently the calculated NF and FF dust concentrations (C_{NF} and C_{FF}), should normally be considered as worst case concentrations. Yet, since the h-factors have not been validated for nanoparticle powders, a larger uncertainty may exist than for conventional powders. Still, the values have been selected as conservative to not immediately underestimate reality.

Table A5-1: Activity energy factors for modifying estimating emission rates from dustiness indices.

Activity energy level	Relative energy scale	Descriptor	Example
h_0	0.0	zero energy	transport of clean drums, containers, bags etc.
h_1	0.1	very low energy	weighing material with a small laboratory spoon etc.
h_2	0.25	low energy	drop height < 5 cm; handling leaky or contaminated bags etc.
h_3	0.50	moderate energy	drop height 5 – 30 cm; mixing powder into liquid medium etc.
h_4	0.80	high energy	drop height 30 – 100 cm; pouring, bagging, emptying big-bags etc.
h_5	1.00	high energy	drop height >100 cm; dry mixing, sweeping, use of pressurized air etc.

A5.2 Analysis

As described above, data on the emission rate ($E_{i,c}$) or emission potential (E_0) are essential for the exposure modelling in NanoSafer. In the current assessment, we are forced to assess the exposure during handling of both powders and dispersions (NM300K as well as potential intermediate manufacturing and process stages for the powders).

For powders, ENPRA Work Package 3 established dustiness data using down-scaled EN15051 drum with on-line measurements of size distributions (5.6 nm to 20 µm) and selective filter collection for gravimetrical measurement of respirable or inhalable dust. For both respirable and inhalable dustiness, the levels varied from “very low” to “high” according to the EN15051 dustiness categories. High dustiness was found for NRCWE1, NRCWE2, NRCWE3. Low to Moderate dustiness was found for NM-101, NM-110, and NM-111, and very low dustiness for NRCWE-004.

Online size-distributions, measured with a FMPS (Fast Mobility Particle Sizer, TSI Inc.) and APS (Aerodynamic Particle Sizer, TSI Inc.) showed that all powders released small particles with a primary peak size-mode between 150 and 200 nm and secondary size-modes in both the nm and µm size range (Figure A5-2). As have been observed in numerous powder dustiness tests, the rotating drum testing did not result in major concentrations of particles smaller than 100 nm. In these powders, the numbers of small nanoparticles constitute 5 to 10% of the total particle number concentration and the respirable particles highly dominate

the measured size-range (Figure A5-3). The only sample falling outside of this general trend is NM111 with a mode around 6 µm, which is above the 4.7 µm d_{50} value of respirable dust.

Figure A5-2: a) Inhalable and b) respirable dustiness indexes. Dashed lines indicate limit values for EN 15051 ranking of powder dustiness.

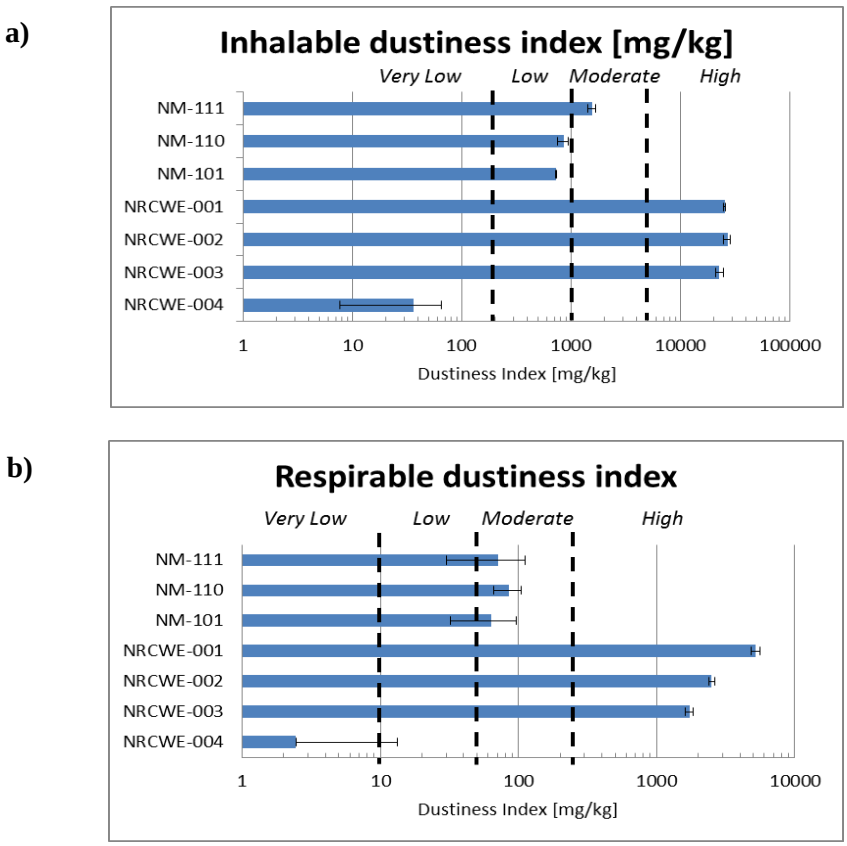
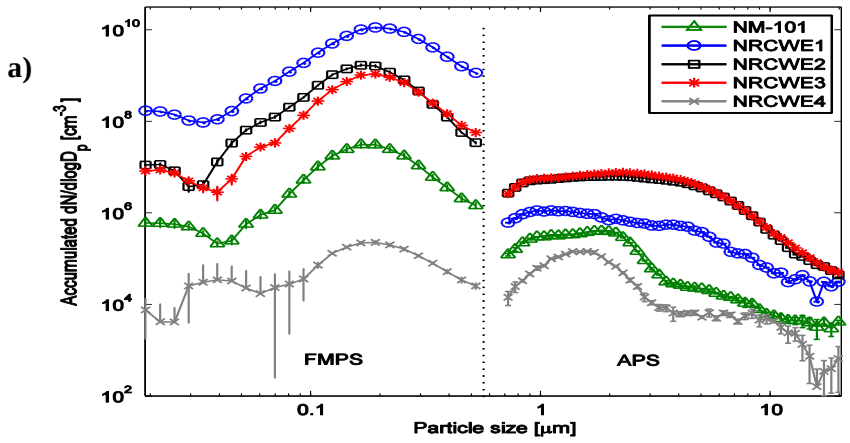
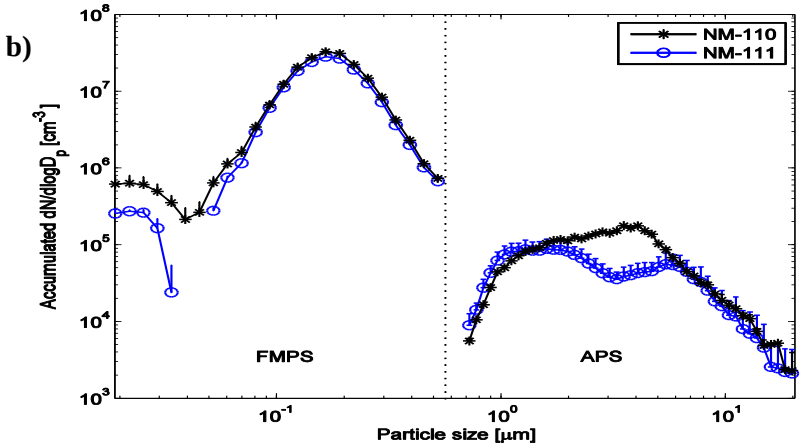


Figure A5-3: Combined accumulated particle size-distribution measured by the FMPS and APS. a) Accumulated size distribution for all five titanium dioxide powders. b) Accumulated size distribution for the two zincite samples.





NM-300 was only available as dispersion. Currently, there has not been established any release potential for wet-chemical synthesis nor handling of liquids with dispersed nanoparticles for NanoSafer. As a conservative worst case estimate, it is assumed that the airborne particle emission is 0.1 wt% of the time-resolved process concentration during high energy processes such as probe sonication and spraying. However, for wet chemical synthesis involving no and very low-level mechanical energy input such as gentle stirring, the airborne release rate is assumed "0". Some authors reported high elevations in particle number during top hatch inspections in tanks large scale synthesis of nano-Ag (Park et al. 2009), but there is a very high likelihood that these measured particles in reality are condensated process liquids rather than silver nanoparticles. Opening of CVD reactor has not shown evidence of CNT release during this process either, which should be expected as CNT would grow on catalysts fixed to a substrate (Han et al., 2008). Synthesis using floating catalysts could be associated with higher risk of particle release. Therefore, for calculation of emission rate potentials (E_0) in these non-dustiness related scenarios, we used the assumed release percentage, a high energy factor of 1, and the average mass-flow rate. This was the process-related material input into the near-field.

A5.3 Results

Table A5-2 summarizes the scenarios, input data and estimated potential exposure doses. In a few cases, the work process was unclear and a conservative assumption was made to enable an assessment. For all cases, the acute NF exposure generally only moderately exceeded the acute FF exposure. This is due to the generally extended durations of the work coupled with relatively large work-rooms and high air-exchange rates ($\leq$ 8 h^{-1}), which enable efficient dilution and diffusion from the NF and filtering out through the general ventilation.

However, in response to the wide variation in the use rates and volumes applied in the work scenarios, there is a very wide range in the actual estimated exposure potential levels. Of course, no exposure was assessed for the inspection and transfer of the liquid phase synthesis Ag in NM300 (scenarios 1.1 and 2.1).

This was also the case for the laser ablation scenario for the five TiO_2 samples, where complete enclosure was assumed (scenarios 12.0 to 12.4).

In contrast, large scale use of highly dusty powders (NRCWE1, NRCWE2, and NRCWE3) resulted in high to very high exposure level potentials. The highest values were found for the TiO_2 scenario groups 6, 7, 10 and 11. In these cases the exposure level potential reaches several tens to hundreds of mg/m^3 levels. In one case (scenarios 10.0 to 10.4) repeatedly dumping of 560 kg over 10 minutes 16 times, the exposure potential even reach g/m^3 levels. Such high concentrations will surely trigger a range of aerosol dynamic processes resulting in extensive agglomeration and sedimentation, which is neglected in this model.

The time-resolved evolution of all work scenarios emitting particles are shown in Figures A5-2 a to j. As seen, all scenarios were modelled using even distribution of the repeated activities starting at time = 1 minute. In all cases, the dust rapidly builds up within the first or first few minutes. Due to the logarithmic plotting, most of the initial concentration build up is not plotted as the "zero-value" is not plotted. Scenarios 14.1, 14.2, 15.1 and 15.2 are plotted in natural numbers on the Y-axis, so these can be visited to understand the particle evolution in the initial stage of the dust build-up.

Due to the recirculation of dust between the NF and FF, the highest acute NF and FF concentrations are always found in association with the last cycle in the scenario. The modelled build-up of dust is best seen in scenario groups 6, 9, 10 and 11 (Figures A5-2b, e, f and g).

Finally, it should be emphasized that these exposure estimates do not consider efficiency of mechanical exhaust ventilation equipment and partial enclosure. Therefore the exposure level concentrations in scenario groups 6 (Booth), 7 (focused LEV), and 9 (Fume-hood) are not considering the efficiency of these protective measures. Final evaluation needs to consider this.

Table A5-2: Selected primary physicochemical and dustiness characteristics of the nanomaterials evaluated in the Risk assessment exercise.

Sample	XRD size [nm]	sigma	major phase	DLS d_{peak}* [nm]	Resp. DI€ [mg/kg]	Inh. DI£ [mg/kg]	Tot N [n/kg]	N100 [%]	Resp [n/kg]	Resp [%]	SSA [m^2/g]
NM-101 (TiO_2)	7	-	anatase	-	64±32	728±10	2.04E+09	6.87	2.02E+09	99.28	322
NRCWE-001 (TiO_2)	10	1	rutile	-	5204±362	25303±546	8.08E+11	7.26	8.03E+11	99.37	99.0
NRCWE-002 (+TiO_2)	10	1	rutile	-	2506±117	26883±1936	1.06E+11	8.85	1.06E+11	99.27	84.3
NRCWE-003 (-TiO_2)	10	1	rutile	-	1730±117	22707±1856	6.87E+10	4.91	6.80E+10	99.07	84.2
NRCWE-004 (TiO_2)	94	-	rutile	-	2.4±10.7	36±29	2.69E+07	10.25	2.58E+07	96.10	5.1
NM-110 (ZnO)	71	-	zincite	-	85±19	854±96	2.04E+09	8.46	2.03E+09	99.18	14
NM-111 (silane-ZnO)	58	-	zincite	-	71±40	1546±112	2.59E+09	4.89	1.74E+09	67.13	18
NM-300 (Ag) dry	14	2	metallic	-	NA	NA	-	-	-		NA
NM-300 (Ag) liquid	7	1	-	59	NA	NA	-	-	-		NA
NM-300K (Ag) liquid	-	-	-	-	NA	NA	-	-	-		NA
NM-400 (PC-MWCNT)	NA	-	MWCNT	-	375$	NA	-	-	-	All$	298
NM-402 (MWCNT	NA	-	MWCNT	-	375$	NA	-	-	-	All$	225

Legend: * Dominant number peak size of dispersion from Dynamic Light Scattering analyses.
€ Respirable dustiness index.
£ Inhalable dustiness index.
$ Default high dustiness level when data does not exist

Table A5-3: Acute and daily (8-hour) inhalation doses (D_{inh}) calculated for a number of occupational exposure scenarios using the NanoSafer model. NF=Near-field. FF=Far-field.

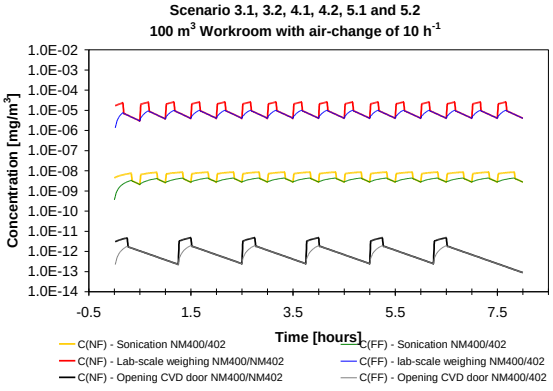
Material	Scenario	Repirable dustiness of powder (mg/kg)	Handling activity energy (H)	Notes on H factor and process	Total mass of material handled in each work cycle (kg)	Duration of the work cycle (min)	Pause between work cycles (min)	Number of work cycles per day (n)	Amount of nanomaterial handled in each transfer (kg)	Volume of the work room (width x length x height) (m3)	Air exchange rate (h-1)	Acute NF (mg/m3)	Acute FF (mg/m3)	8-hour NF (mg/m3)	8-hour FF (mg/m3)
NM300	1.1: Production of nano-Ag during wet-chemistry process: opening the dryer door and transfer for grinding	0	0	emission of Ag NP assumed negligible	1,50E+02	6,00E+01	4,20E+02	1,00E+00	1,50E+02	1,00E+02	4,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NM300	2.1: Production of Nano-Ag during wet-chemistry process: opening the grinder hatch and transfer for packaging	0	0	emission of Ag NP assumed negligible	1,50E+02	7,50E+01	4,05E+02	1,00E+00	1,50E+02	1,00E+02	4,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NM400	3.1: Sonication of raw MWCNT	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	2,00E+01	1,00E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	8,10E-09	4,40E-09	6,30E-09	3,50E-09
NM402	3.2: Sonication of raw MWCNT	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	2,00E+01	1,00E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	8,10E-09	4,40E-09	6,30E-09	3,50E-09
NM400	4.1: Weighing raw MWCNT	375	0,1	lab scale handling	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	1,80E-05	8,30E-06	1,20E-05	6,60E-06
NM402	4.2: Weighing raw MWCNT	375	0,1	lab scale handling	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	1,80E-05	8,30E-06	1,20E-05	6,60E-06
NM400	5.1: Opening growth chamber with no exhaust and transfer of MWCNT	0,01	1	not related to dustiness; assumed worst case release 0.1%	2,00E-07	1,50E+01	6,00E+01	6,00E+00	2,00E-07	1,00E+02	8,00E+00	4,07E-12	1,65E-12	1,35E-12	8,21E-13
NM402	5.2: Opening growth chamber with no exhaust and transfer of MWCNT	0,01	1	not related to dustiness; assumed worst case release 0.1%	2,00E-07	1,50E+01	6,00E+01	6,00E+00	2,00E-07	1,00E+02	8,00E+00	4,07E-12	1,65E-12	1,35E-12	8,21E-13
NCRWE1	6.1: Manufacturer: Manual (un)loading	5204	0,25	mechanical extraction?; energy	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	4,32E+01	4,00E+00	8,27E+01	6,54E+01	7,20E+01	5,88E+01

	trays inside booth			assumed to be low											
NCRWE2	6.2: Manufacturer: Manual (un)loading trays inside booth	2506	0,25	mechanical extraction?; energy assumed to be low	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	4,32E+01	4,00E+00	3,98E+01	3,15E+01	3,47E+01	2,83E+01
NCRWE3	6.3: Manufacturer: Manual (un)loading trays inside booth	1730	0,25	mechanical extraction?; energy assumed to be low	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	4,32E+01	4,00E+00	2,75E+01	2,17E+01	2,39E+01	1,95E+01
NCRWE4	6.4: Manufacturer: Manual (un)loading trays inside booth	2,4	0,25	mechanical extraction?; energy assumed to be low	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	4,32E+01	4,00E+00	3,82E-02	3,02E-02	3,32E-02	2,71E-02
NCRWE1	7.1: Manufacturer: Dumping into mixing tank using focused LEV	5204	0,8	drop height > 0.3 m	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	1,00E+02	8,00E+00	1,17E+02	5,97E+01	9,80E+01	5,46E+01
NCRWE2	7.2: Manufacturer: Dumping into mixing tank using focused LEV	2506	0,8	drop height > 0.3 m	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	1,00E+02	8,00E+00	5,65E+01	2,88E+01	4,72E+01	2,63E+01
NCRWE3	7.3: Manufacturer: Dumping into mixing tank using focused LEV	1730	0,8	drop height > 0.3 m	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	1,00E+02	8,00E+00	3,90E+01	1,99E+01	3,26E+01	1,82E+01
NCRWE4	7.4: Manufacturer: Dumping into mixing tank using focused LEV	2,4	0,8	drop height > 0.3 m	1,00E+01	1,00E+01	1,00E+01	2,40E+01	1,00E+01	1,00E+02	8,00E+00	5,41E-02	2,75E-02	4,52E-02	2,52E-02
NCRWE1	8.1: Lab: Transferring material during weighing or into vials for solution prep	5204	0,1	drop-height assumed low and low energy	2,00E-04	5,00E+00	2,50E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	2,64E-04	1,20E-04	1,64E-04	9,21E-05
NCRWE2	8.2: Lab: Transferring material during weighing or into vials for solution prep	2506	0,1	drop-height assumed low and low energy	2,00E-04	5,00E+00	2,50E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	1,27E-04	5,80E-05	7,91E-05	4,43E-05
NCRWE3	8.3: Lab: Transferring material during weighing or into vials for solution prep	1730	0,1	drop-height assumed low and low energy	2,00E-04	5,00E+00	2,50E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	8,78E-05	4,00E-05	5,46E-05	3,06E-05
NCRWE4	8.4: Lab: Transferring material during weighing or into vials for solution prep	2,4	0,1	drop-height assumed low and low energy	2,00E-04	5,00E+00	2,50E+01	1,60E+01	2,00E-04	1,00E+02	8,00E+00	1,22E-07	5,55E-08	7,58E-08	4,25E-08

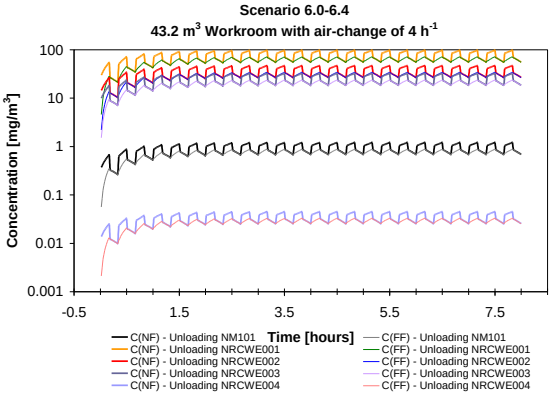
NCRWE1	9.1: Lab: Creating stock solutions in fume hood	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	4,00E+00	6,13E-10	3,27E-10	4,23E-10	2,77E-10
NCRWE2	9.2: Lab: Creating stock solutions in fume hood	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	4,00E+00	6,13E-10	3,27E-10	4,23E-10	2,77E-10
NCRWE3	9.3: Lab: Creating stock solutions in fume hood	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	4,00E+00	6,13E-10	3,27E-10	4,23E-10	2,77E-10
NCRWE4	9.4: Lab: Creating stock solutions in fume hood	0,001	1	not related to dustiness; assumed worst case release 0.1%	2,00E-04	1,00E+01	2,00E+01	1,60E+01	2,00E-04	1,00E+02	4,00E+00	6,13E-10	3,27E-10	4,23E-10	2,77E-10
NCRWE1	10.1: Dumping large amount of powder into vessel	5204	0,8	drop height assumed > 0.3 m	5,60E+02	1,00E+01	2,00E+01	1,60E+01	5,60E+02	7,50E+01	4,00E+00	8,26E+03	5,09E+03	5,91E+03	4,24E+03
NCRWE2	10.2: Dumping large amount of powder into vessel	2506	0,8	drop height assumed > 0.3 m	5,60E+02	1,00E+01	2,00E+01	1,60E+01	5,60E+02	7,50E+01	4,00E+00	3,98E+03	2,45E+03	2,85E+03	2,04E+03
NCRWE3	10.3: Dumping large amount of powder into vessel	1730	0,8	drop height assumed > 0.3 m	5,60E+02	1,00E+01	2,00E+01	1,60E+01	5,60E+02	7,50E+01	4,00E+00	2,75E+03	1,69E+03	1,97E+03	1,41E+03
NCRWE4	10.4: Dumping large amount of powder into vessel	2,4	0,8	drop height assumed > 0.3 m	5,60E+02	1,00E+01	2,00E+01	1,60E+01	5,60E+02	7,50E+01	4,00E+00	3,81E+00	2,35E+00	2,73E+00	1,96E+00
NCRWE1	11.1: Bag/bin filling	5204	0,8	drop height assumed > 0.3 m	2,50E+02	4,80E+02	0,00E+00	1,00E+00	2,50E+02	7,06E+02	4,00E+00	6,40E+01	1,42E+01	6,27E+01	1,29E+01
NCRWE2	11.2: Bag/bin filling	2506	0,8	drop height assumed > 0.3 m	2,50E+02	4,80E+02	0,00E+00	1,00E+00	2,50E+02	7,06E+02	4,00E+00	3,08E+01	6,86E+00	3,02E+01	6,23E+00
NCRWE3	11.3: Bag/bin filling	1730	0,8	drop height assumed > 0.3 m	2,50E+02	4,80E+02	0,00E+00	1,00E+00	2,50E+02	7,06E+02	4,00E+00	2,13E+01	4,73E+00	2,09E+01	4,30E+00
NCRWE4	11.4: Bag/bin filling	2,4	0,8	drop height assumed > 0.3 m	2,50E+02	4,80E+02	0,00E+00	1,00E+00	2,50E+02	7,06E+02	4,00E+00	2,95E-02	6,57E-03	2,89E-02	5,96E-03
NCRWE1	12.1: Laser ablation	0	0	full enclosure assumed	3,00E-03	8,00E+00	5,20E+01	8,00E+00	3,00E-03	1,50E+02	8,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE2	12.2: Laser ablation	0	0	full enclosure assumed	3,00E-03	8,00E+00	5,20E+01	8,00E+00	3,00E-03	1,50E+02	8,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE3	12.3: Laser ablation	0	0	full enclosure	3,00E-03	8,00E+00	5,20E+01	8,00E+00	3,00E-03	1,50E+02	8,00E+	0,00E+00	0,00E+00	0,00E+00	0,00E+00

				assumed							00				
NCRWE4	12.4: Laser ablation	0	0	full enclosure assumed	3,00E-03	8,00E+00	5,20E+01	8,00E+00	3,00E-03	1,50E+02	8,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
NCRWE1	13.1: Weighing of TiO_2 powder	5204	0,1	low amount and low energy assumed	1,00E-04	3,00E+00	1,20E+01	2,40E+01	1,00E-04	7,00E+01	8,00E+00	2,03E-04	1,33E-04	1,52E-04	9,99E-05
NCRWE2	13.2: Weighing of TiO_2 powder	2506	0,1	low amount and low energy assumed	1,00E-04	3,00E+00	1,20E+01	2,40E+01	1,00E-04	7,00E+01	8,00E+00	9,78E-05	6,41E-05	7,34E-05	4,81E-05
NCRWE3	13.3 Weighing of TiO_2 powder	1730	0,1	low amount and low energy assumed	1,00E-04	3,00E+00	1,20E+01	2,40E+01	1,00E-04	7,00E+01	8,00E+00	6,75E-05	4,43E-05	5,07E-05	3,32E-05
NCRWE4	13.4: Weighing of TiO_2 powder	2,4	0,1	low amount and low energy assumed	1,00E-04	3,00E+00	1,20E+01	2,40E+01	1,00E-04	7,00E+01	8,00E+00	9,37E-08	6,14E-08	7,03E-08	4,61E-08
NM111	14.1: Preparation of inks / Preparation of nano-ZnO solution	85	0,5	process unclear; estimated moderate H-factor powder dust release	5,00E-04	5,00E+00	4,75E+02	1,00E+00	5,00E-04	1,00E+02	8,00E+00	4,67E-05	1,73E-05	2,13E-06	1,20E-06
NM110	14.2: Preparation of inks / Preparation of nano-ZnO solution	71	0,5	process unclear; estimated moderate H-factor powder dust release	5,00E-04	5,00E+00	4,75E+02	1,00E+00	5,00E-04	1,00E+02	8,00E+00	3,90E-05	1,45E-05	1,78E-06	1,01E-06
NM111	15.1: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	0,001	1	not related to dustiness; assumed worst case release 0.1%	1,00E-04	1,00E+01	4,70E+02	1,00E+00	1,00E-04	1,00E+02	8,00E+00	1,04E-09	3,45E-10	5,00E-11	2,83E-11
NM110	15.2: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	0,001	1	not related to dustiness; assumed worst case release 0.1%	1,00E-04	1,00E+01	4,70E+02	1,00E+00	1,00E-04	1,00E+02	8,00E+00	1,04E-09	3,45E-10	5,00E-11	2,83E-11

Figure A5-4: Modelled time-series of the NF and FF exposure level potentials for the scenarios listed in Table A5-3. a) Inspection of CVD chamber, sonication, and weighing CNT in scenario groups 3, 4, and 4. b) Unloading TiO_2 from trays in scenario group 6. c) Dumping TiO_2 into a wet mixer in scenario group 7. d) Laboratory scale weighing of TiO_2 in scenario group 8. e) Laboratory scale sonication of dispersions with TiO_2 in scenario group 9. Similar values are estimated for all NMs, because this is considered a process specific emission rate. f) Large-scale dumping of TiO_2 in scenario group 10. g) Slowly filling TiO_2 into a big-bag in scenario group 11. h) Small scale weighing-out TiO2 in scenario group 13. i) Small scale weighing out and preparing ZnO dispersions in scenario group 14. j) Small-scale spraying of ZnO dispersions in scenario group 15.



a)



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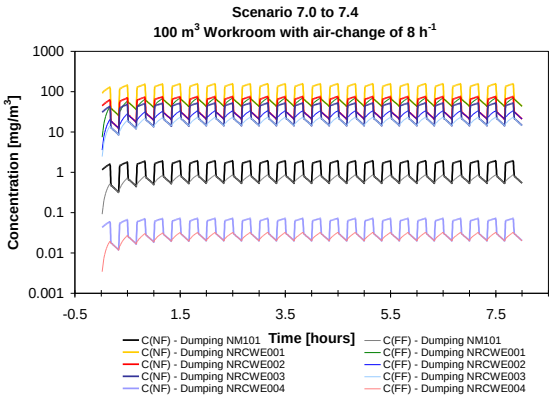

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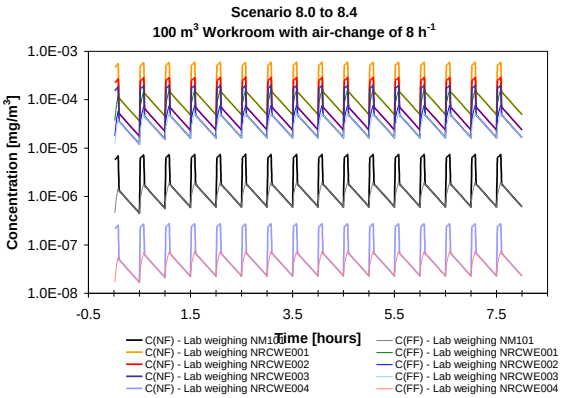

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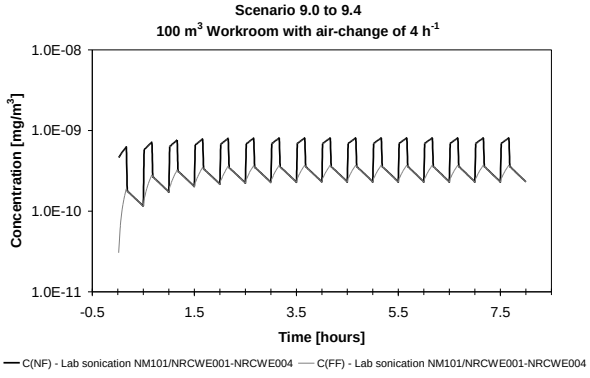
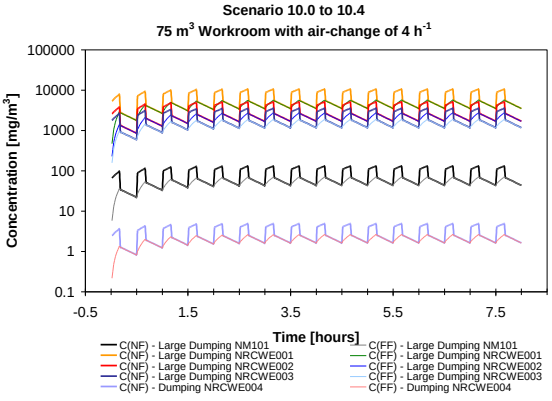
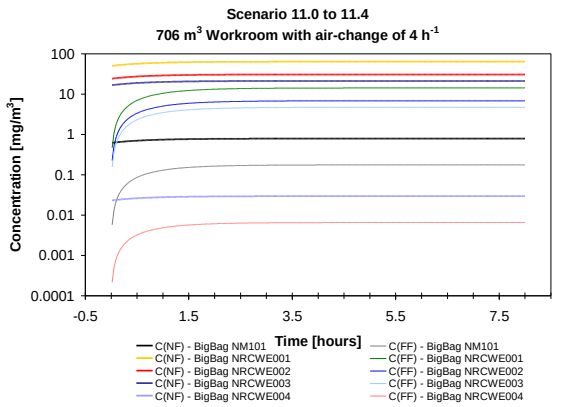


e)



f)

g)



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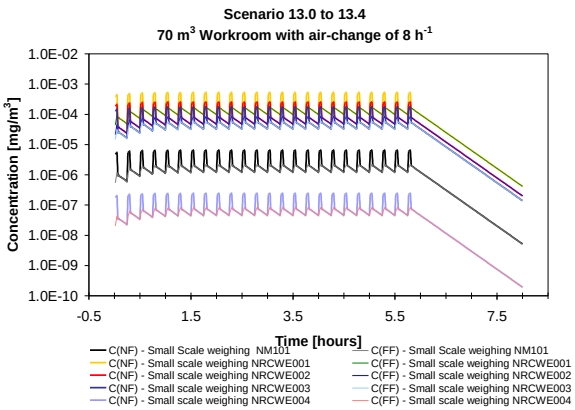

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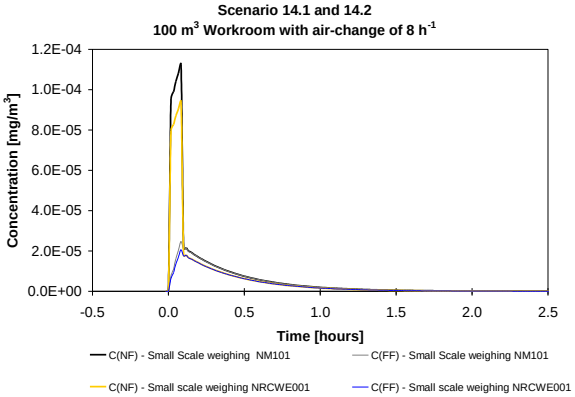

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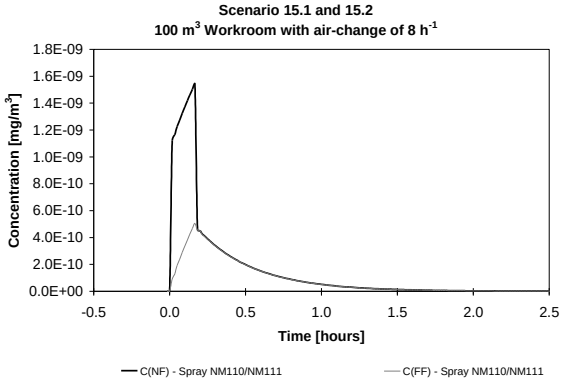

A5.4 References

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Jensen KA, Saber AT, Kristensen HV, Koponen IK, Wallin H. NanoSafer: Web-based precautionary risk evaluation of dust exposure to manufactured nanomaterials using first order modelling (in preparation).

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ANNEX 6

Endpoint/body system-specific Margins of exposure

Table A6-1: Endpoint-specific MoE values for a number of ES and NOAA calculated based on daily averaged inhalation BMDL (mg/m^3) and daily inhalation doses corresponding to 8-hour worker shifts.

Material	Scenario	Organ system	Endpoint	Single inhalation BMDL (mg/m^3)	Daily inhalation dose (D_{inh}) (mg/m^3)	Endpoint-specific MoE
NCRWE1	6.1: Manufacturer: Manual (un)loading trays inside booth	Pulmonary	Inflammation and immune response	3,80E-01	8,27E+01	4,59E-03
		Pulmonary	Cytotoxicity	3,30E-01	8,27E+01	3,99E-03
		Pulmonary	Organ pathology	4,00E-02	8,27E+01	4,83E-04
		Cardiovascular	Inflammation and immune response	2,00E-02	8,27E+01	2,42E-04
NCRWE1	7.1: Manufacturer: Dumping into mixing tank using focused LEV	Pulmonary	Inflammation and immune response	3,80E-01	1,17E+02	3,24E-03
		Pulmonary	Cytotoxicity	3,30E-01	1,17E+02	2,81E-03
		Pulmonary	Organ pathology	4,00E-02	1,17E+02	3,41E-04
		Cardiovascular	Inflammation and immune response	2,00E-02	1,17E+02	1,70E-04
NCRWE1	8.1: Lab: Transferring material during weighing or into vials for solution prep	Pulmonary	Inflammation and immune response	3,80E-01	2,64E-04	1,44E+03
		Pulmonary	Cytotoxicity	3,30E-01	2,64E-04	1,25E+03
		Pulmonary	Organ pathology	4,00E-02	2,64E-04	1,52E+02
		Cardiovascular	Inflammation and immune response	2,00E-02	2,64E-04	7,58E+01
NCRWE1	9.1: Lab: Creating stock solutions in fume hood	Pulmonary	Inflammation and immune response	3,80E-01	6,13E-10	6,20E+08
		Pulmonary	Cytotoxicity	3,30E-01	6,13E-10	5,38E+08
		Pulmonary	Organ pathology	4,00E-02	6,13E-10	6,53E+07
		Cardiovascular	Inflammation and immune response	2,00E-02	6,13E-10	3,26E+07

		Pulmonary	Inflammation and immune response	3,80E-01	8,26E+03	4,60E-05
NCRWE1	10.1: Dumping large amount of powder into vessel	Pulmonary	Cytotoxicity	3,30E-01	8,26E+03	3,99E-05
		Pulmonary	Organ pathology	4,00E-02	8,26E+03	4,84E-06
		Cardiovascular	Inflammation and immune response	2,00E-02	8,26E+03	2,42E-06
		Pulmonary	Inflammation and immune response	3,80E-01	6,40E+01	5,93E-03
NCRWE1	11.1: Bag/bin filling	Pulmonary	Cytotoxicity	3,30E-01	6,40E+01	5,15E-03
		Pulmonary	Organ pathology	4,00E-02	6,40E+01	6,25E-04
		Cardiovascular	Inflammation and immune response	2,00E-02	6,40E+01	3,12E-04
		Pulmonary	Inflammation and immune response	3,80E-01	0,00E+00	0,00E+00
NCRWE1	12.1: Laser ablation	Pulmonary	Cytotoxicity	3,30E-01	0,00E+00	0,00E+00
		Pulmonary	Organ pathology	4,00E-02	0,00E+00	0,00E+00
		Cardiovascular	Inflammation and immune response	2,00E-02	0,00E+00	0,00E+00
		Pulmonary	Inflammation and immune response	3,80E-01	2,03E-04	1,87E+03
NCRWE1	13.1: Weighing of TiO_2 powder	Pulmonary	Cytotoxicity	3,30E-01	2,03E-04	1,62E+03
		Pulmonary	Organ pathology	4,00E-02	2,03E-04	1,97E+02
		Cardiovascular	Inflammation and immune response	2,00E-02	2,03E-04	9,84E+01
NCRWE2	6.2: Manufacturer: Manual (un)loading trays inside booth	Hepatic	Organ pathology	4,00E-02	3,98E+01	1,00E-03
		Renal	Organ pathology	2,48E+00	3,98E+01	6,22E-02
NCRWE2	7.2: Manufacturer: Dumping into mixing tank using focused LEV	Hepatic	Organ pathology	4,00E-02	5,65E+01	7,08E-04
		Renal	Organ pathology	2,48E+00	5,65E+01	4,39E-02
NCRWE2	8.2: Lab: Transferring material during weighing or into vials for solution prep	Hepatic	Organ pathology	4,00E-02	1,27E-04	3,15E+02
		Renal	Organ pathology	2,48E+00	1,27E-04	1,95E+04
NCRWE2	9.2: Lab: Creating stock solutions in fume hood	Hepatic	Organ pathology	4,00E-02	6,13E-10	6,53E+07
		Renal	Organ pathology	2,48E+00	6,13E-10	4,05E+09
NCRWE2	10.2: Dumping large amount	Hepatic	Organ pathology	4,00E-02	3,98E+03	1,01E-05

	of powder into vessel	Renal	Organ pathology	2,48E+00	3,98E+03	6,23E-04
NCRWE2	11.2: Bag/bin filling	Hepatic	Organ pathology	4,00E-02	3,08E+01	1,30E-03
		Renal	Organ pathology	2,48E+00	3,08E+01	8,04E-02
NCRWE2	12.2: Laser ablation	Hepatic	Organ pathology	4,00E-02	0,00E+00	0,00E+00
		Renal	Organ pathology	2,48E+00	0,00E+00	0,00E+00
NCRWE2	13.2: Weighing of TiO_2 powder	Hepatic	Organ pathology	4,00E-02	9,78E-05	4,09E+02
		Renal	Organ pathology	2,48E+00	9,78E-05	2,53E+04
NCRWE3	6.3: Manufacturer: Manual (un)loading trays inside booth	Cardiovascular	Organ pathology	7,00E-02	2,75E+01	2,55E-03
NCRWE3	7.3: Manufacturer: Dumping into mixing tank using focused LEV	Cardiovascular	Organ pathology	7,00E-02	3,90E+01	1,79E-03
NCRWE3	8.3: Lab: Transferring material during weighing or into vials for solution prep	Cardiovascular	Organ pathology	7,00E-02	8,78E-05	7,98E+02
NCRWE3	9.3: Lab: Creating stock solutions in fume hood	Cardiovascular	Organ pathology	7,00E-02	6,13E-10	1,14E+08
NCRWE3	10.3: Dumping large amount of powder into vessel	Cardiovascular	Organ pathology	7,00E-02	2,75E+03	2,55E-05
NCRWE3	11.3: Bag/bin filling	Cardiovascular	Organ pathology	7,00E-02	2,13E+01	3,29E-03
NCRWE3	12.3: Laser ablation	Cardiovascular	Organ pathology	7,00E-02	0,00E+00	0,00E+00
NCRWE3	13.3: Weighing of TiO_2 powder	Cardiovascular	Organ pathology	7,00E-02	6,75E-05	1,04E+03
NCRWE4	6.4: Manufacturer: Manual (un)loading trays inside booth	Pulmonary	Oxidative stress	6,60E-01	3,82E-02	1,73E+01
		Pulmonary	Genotoxicity	4,80E-01	3,82E-02	1,26E+01
NCRWE4	7.4: Manufacturer: Dumping into mixing tank using focused LEV	Pulmonary	Oxidative stress	6,60E-01	5,41E-02	1,22E+01
		Pulmonary	Genotoxicity	4,80E-01	5,41E-02	8,87E+00
NCRWE4	8.4: Lab: Transferring material during weighing or into vials for solution prep	Pulmonary	Oxidative stress	6,60E-01	1,22E-07	5,42E+06
		Pulmonary	Genotoxicity	4,80E-01	1,22E-07	3,94E+06

NCRWE4	9.4: Lab: Creating stock solutions in fume hood	Pulmonary	Oxidative stress	6,60E-01	6,13E-10	1,08E+09
		Pulmonary	Genotoxicity	4,80E-01	6,13E-10	7,83E+08
NCRWE4	10.4: Dumping large amount of powder into vessel	Pulmonary	Oxidative stress	6,60E-01	3,81E+00	1,73E-01
		Pulmonary	Genotoxicity	4,80E-01	3,81E+00	1,26E-01
NCRWE4	11.4: Bag/bin filling	Pulmonary	Oxidative stress	6,60E-01	2,95E-02	2,23E+01
		Pulmonary	Genotoxicity	4,80E-01	2,95E-02	1,63E+01
NCRWE4	12.4: Laser ablation	Pulmonary	Oxidative stress	6,60E-01	0,00E+00	0,00E+00
		Pulmonary	Genotoxicity	4,80E-01	0,00E+00	0,00E+00
NCRWE4	13.4: Weighing of TiO_2 powder	Pulmonary	Oxidative stress	6,60E-01	9,37E-08	7,04E+06
		Pulmonary	Genotoxicity	4,80E-01	9,37E-08	5,12E+06
NM400	3.1: Sonication of raw MWCNT	Pulmonary	Inflammation and immune response	3,40E-01	8,10E-09	4,20E+07
		Pulmonary	Genotoxicity	4,50E-01	8,10E-09	5,56E+07
		Pulmonary	Oxidative stress	1,52E+00	8,10E-09	1,88E+08
NM400	4.1: Weighing raw MWCNT	Pulmonary	Inflammation and immune response	3,40E-01	1,80E-05	1,89E+04
		Pulmonary	Genotoxicity	4,50E-01	1,80E-05	2,50E+04
		Pulmonary	Oxidative stress	1,52E+00	1,80E-05	8,44E+04
NM400	5.1: Opening growth chamber with no exhaust and transfer of MWCNT	Pulmonary	Inflammation and immune response	3,40E-01	4,07E-12	8,35E+10
		Pulmonary	Genotoxicity	4,50E-01	4,07E-12	1,11E+11
		Pulmonary	Oxidative stress	1,52E+00	4,07E-12	3,73E+11
NM402	3.2: Sonication of raw MWCNT	Lymphatic	Organ pathology	1,28E+00	8,10E-09	1,58E+08
NM402	4.2: Weighing raw MWCNT	Lymphatic	Organ pathology	1,28E+00	1,80E-05	7,11E+04
NM402	5.2: Opening growth chamber with no exhaust and transfer of MWCNT	Lymphatic	Organ pathology	1,28E+00	4,07E-12	3,14E+11
NM111	14.1: Preparation of inks / Preparation of nano-ZnO	Pulmonary	Inflammation and immune response	1,10E-01	4,67132E-05	2,35E+03
		Pulmonary	Cytotoxicity	2,00E-02	4,67132E-05	4,28E+02

	solution	Pulmonary	Oxidative stress	2,50E-01	4,67132E-05	5,35E+03
		Pulmonary	Genotoxicity	0,00E+00	4,67132E-05	0,00E+00
NM111	15.1: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	Pulmonary	Inflammation and immune response	1,10E-01	1,03581E-09	1,06E+08
		Pulmonary	Cytotoxicity	2,00E-02	1,03581E-09	1,93E+07
		Pulmonary	Oxidative stress	2,50E-01	1,03581E-09	2,41E+08
		Pulmonary	Genotoxicity	0,00E+00	1,03581E-09	0,00E+00
NM110	14.2: Preparation of inks / Preparation of nano-ZnO solution	Cardiovascular	Organ pathology	1,40E-01	3,90193E-05	3,59E+03
		Cardiovascular	Inflammation and immune response	5,20E-01	3,90193E-05	1,33E+04
		Lymphatic	Organ pathology	1,20E-01	3,90193E-05	3,08E+03
NM110	15.2: Preparation of inks /Deposit of the "nano-ZnO ink" on a silicon substrate	Cardiovascular	Organ pathology	1,40E-01	1,03581E-09	1,35E+08
		Cardiovascular	Inflammation and immune response	5,20E-01	1,03581E-09	5,02E+08
		Lymphatic	Organ pathology	1,20E-01	1,03581E-09	1,16E+08
NM300	1.1: Production of Nano-Ag during wet-chemistry process: opening the dryer door and transfer for grinding	Cardiovascular	Organ pathology	7,20E-01	0,00E+00	0,00E+00
		Pulmonary	Oxidative stress	4,00E-02	0,00E+00	0,00E+00
		Renal	Organ pathology	1,11E+00	0,00E+00	0,00E+00
NM300	2.1: Production of Nano-Ag during wet-chemistry process: opening the grinder hatch and transfer for packaging	Cardiovascular	Organ pathology	7,20E-01	0,00E+00	0,00E+00
		Pulmonary	Oxidative stress	4,00E-02	0,00E+00	0,00E+00
		Renal	Organ pathology	1,11E+00	0,00E+00	0,00E+00