Environmental risk assessment and management of engineered nanomaterials - The role of ecotoxicity testing

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Preface

The research contained within this PhD thesis was carried out at the Department of Environmental Engineering, at the Technical University of Denmark. It was conducted from November 2013 to November 2016 under the supervision of Professor Anders Baun with co-supervision from Associate Professor Mette Broholm and Associate Professor Steffen Foss Hansen.

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IX**.

I **Hjorth R**, Hansen SF, Jacobs M, Tickner J, Ellenbecker M, Baun A (2016). The applicability of chemical alternatives assessment for engineered nanomaterials, *Integrated Environmental Assessment and Management*. DOI: 10.1002/ieam.1762

II **Hjorth R**, Holden P, Hansen SF, Colman B, Grieger K, Hendren, CO (2016). The role of alternative testing strategies in environmental risk assessment of engineered nanomaterials. *Submitted.*

III **Hjorth R**, van Hove L, Wickson, F (2016). What can nanosafety learn from drug development? The feasibility of 'Safety by design'. *Manuscript.*

IV **Hjorth R**, Coutris C, Sevcu A, Nguyen B, Joner E, Baun A (2016). Ecotoxicity testing and environmental risk assessment of iron nanomaterials for sub-surface remediation – Recommendations from the FP7 project NanoRem. *Manuscript.*

V Wickson F, Hartmann NB, **Hjorth R**, Hansen SF, Wynne B, Baun A (2014). Balancing scientific tensions, *Nature Nanotechnology*, 9, 870

VI Hansen SF, **Hjorth R**, Skjolding LM, Bowman DM, Maynard A, Baun A (2016). A Critical and In-depth Analysis of the Dossiers from the OECD Sponsorship Programme – Has the OECD failed the nano risk community? *Submitted.*

VII Skjolding LM, Sørensen SN, Hartman NB, **Hjorth R**, Hansen SF, Baun A (2016). A Critical Review of Aquatic Ecotoxicity Testing of Nanoparticles – The Quest for Disclosing Nanoparticle Effects, *Angewandte Chemie International edition*. DOI:10.1002/anie.201604964

VIII **Hjorth R**, Sørensen SN, Olsson M, Baun A, Hartmann NB (2016). A Certain Shade of Green: Can algal pigments reveal shading effects of nanoparticles?, *Integrated Environmental Assessment and Management.* 15(1), 200-202

IX Sørensen SN, **Hjorth R**, Delgado CG, Hartmann NB, Baun A (2015). Nanoparticle ecotoxicity – Physical and/or chemical effects?, *Integrated Environmental Assessment and Management,* 11(4), 722-724

In addition, the following book chapter and ISI article, not included in this thesis, were also concluded during this PhD study:

Grieger K, Carpenter AW, Klaessig F, Lefevre E, Gunsch C, Soratana K, Landis AE, Wickson F, Hristozov D, **Hjorth R**, Linkov I (2017). Chapter 9: Sustainable Environmental Remediation using nZVI, Ed: Lowry, G and Phenrat, P. In Nanoscale Zerovalent Iron Particles for Environmental Restoration - From Fundamental Science to Field Scale Engineering Applications. Springer. *In press*.

Skjolding LM, Kern K, **Hjorth R**, Hartmann NB, Overgaard S, Ma JG, Veinot A, Baun A (2014). Uptake and depuration of gold nanoparticles in Daphnia magna, *Ecotoxicology,* 23:1172-1183

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Over the years of undertaking this study, I have had the pleasure of visiting, meeting and acquainting many researchers that have been instrumental to my research. I sincerely want to thank the people I have collaborated and coauthored with during this time. I especially wish to thank *Fern Wickson* at the GenØk Centre for Biosafety in Tromsø, for broadening my philosophical perspective on almost everything. Similarly, I owe a huge thanks to *Louise Helene Søgaard Jensen* at EPFL, *Signe Qualmann* at DHI, *Khara Grieger* at RTI International, *Andrew Maynard* at Arizona State University, *Christine Hendren* at Duke University and *Enzo Lombi* and *Erica Donner* at the University of South Australia. I also wish to thank and acknowledge *Svend Overgaard* for our collaboration on this topic during our undergraduate studies.

Finally, my gratitude goes to DTU Environment and all the good people there. It has been a joy to work with such talented colleagues.

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November 2016
Rune Hjorth

Summary

In 2004, the first article on ecotoxicity of engineered nanomaterials (ENMs) was published, subsequently giving birth to the field of nanoecotoxicity. Today, approximately a thousand peer-reviewed articles have been published on the topic albeit many challenges remain within the field. Central to these is the continued examination of the applicability of ecotoxicity testing to encompass the testing of particles, as the tests originally are developed for dissolved chemicals. Furthermore, the ability of such testing to inform environmental risk assessment and environmental risk management, including the applicability of these concepts, has been questioned.

The present thesis provides an overview of the challenges facing ecotoxicity testing of ENMs and investigates whether we can rely on such testing to inform risk assessment and eventually management of the potential environmental risk of ENMs.

Although the Organisation for Economic Co-operation and Development (OECD) launched a seven year long testing programme around the use of standardized OECD test guidelines (TGs) for ENMs, which concluded that the TGs are generally applicable to ENMs, this thesis argues that it is not possible to offer any conclusions based on their analysis. Efforts within nanoecotoxicology are focused on modifying existing TGs to improve the stability and dispersion of suspended ENMs, although it is paramount to acknowledge that the underlying assumption of the dissolved nature of the test compound is violated. Furthermore, several dilemmas - so called-double binds - should also be acknowledged as they dictate the limitations of standardization and therefore also its ability to guide risk assessment.

The paradigm of conducting *in vivo* animal toxicity testing and extrapolating the data to either humans or the environment is gradually being replaced with a focus on *in silico* and *in vitro* studies with an even greater need for and reliance on extrapolation. However, in this thesis it is argued that within ecotoxicity, whole organism models remain at the foundation of environmental risk assessment, and as such, they are likely to remain in use for nanoecotoxicology. Indeed, the use of more complex *in vivo* systems such as microcosms and mesocosms are recommended to enable and validate current risk assessment practices. But just as envisioned in human toxicology, an integrated approach must be pursued to reap the benefits of simplified as well as more complex testing systems, each fit for purpose for different tasks.

It is concluded that it is not possible to conduct environmental risk assessment of ENMs with a satisfactory level of certainty, primarily due to knowledge gaps and the uncertainty imbedded in current ecotoxicity data. Albeit with time better data will be available, it is important that tools encompassing uncertainty are utilized to facilitate decision-support. As the risk constituted by ENMs cannot be quantified, the use, need and ability of risk management options to encompass the potential risk are similarly challenged. This should invoke a precautionary stance on the use of ENMs.

Within the field of nanotoxicology the concept of creating ‘safety by design’ has received much attention, arguably both due to these risk assessment and management issues, but also in spite of them. Instead of focusing on managing complexity and uncertainty, the rise of ‘safety by design’ indicates that the field is going towards a more deterministic approach with a misplaced promise to solve these management issues scientifically.

Finally, identifying risky ENMs and safer alternatives through alternatives assessment should be encouraged. Importantly, in doing so we will also be forced to look at risk in combination with benefits, as addressing risk in isolation rarely leaves room for resolving societal issues.

Dansk sammenfatning

I 2004 blev den første artikel om nanomaterialers økotoksicitet publiceret, hvilket derved blev starten for forskningen i nanoøkotoksikologi. I dag er omkring tusind videnskabelige artikler publiceret om emnet, men mange udfordringer eksisterer forsat inden for området. Et centralt spørgsmål er anvendeligheden af de nuværende metoder til økotoksicitetstestning, da testene oprindeligt er udviklet for opløste kemikalier og ikke for partikler. Ydermere er det betvivlet, om den slags testning muliggører en vurdering og håndtering af nanomaterialers potentielle miljørisiko.

Denne afhandling giver et overblik over udfordringerme forbundet med økotoksikologisk testing af nanomaterialer og undersøger om sådanne tests kan facilitere miljørisikovurdering og -håndtering af nanomaterialer.

Selvom Organisationen for Økonomisk Samarbejde og Udvikling (OECD) på baggrund af et syv år langt testprogram har konkluderet, at OECD's retningslinjer for standardiserede tests generelt er anvendelige for nanomaterialer, argumenterer denne afhandling for, at det ikke er muligt at konkludere noget sådant udfra deres analyse. Mens bestræbelserne indenfor nanoøkotoksikologi fokuserer på at modificere de eksisterende retningslinjer for testning for at forbedre stabiliteten og dispersionen af suspenderede nanomaterialer, er det vitalt at anerkende, at den underliggende antagelse om teststoffets opløste natur er overtrådt. Yderemere eksisterer der et spændingsfelt i nanoøkotoksikologisk forskningen ift. om testing skal have et regulatorisk eller videnskabelig fokus, hvilket skaber fundamentalle dillemaer om hvad testing kan og skal undersøge.

Paradigmet om at udføre *in vivo* toksicitetstestning med dyr og derefter ekstrapolere data videre til enten mennesker eller miljøet er gradvist ved at blive erstattet med et fokus på *in silico* og *in vitro* studier med endnu længere ekstrapolering. Denne afhandling fremfører, at indenfor økotoksikologi er tests med hele organismer stadig fundamentet for miljørisikovurdering, og derfor er det forventligt, at de bibeholder deres role i nanoøkotoksikologi. Netop brugen af mere komplekse *in vivo* systemer, som mikro- og mesokosmosforsøg, anbefales for at muliggøre og validere den nuværende risikovurderingspraksis. Men præcis som for human toksikologisk testning, skal en integreret tilgang tilstræbes for at høste fordelene ved såvel de simple som de mere komplicerede testsystemer, hver med deres anvendelsesmuligheder.

Det konkluderes, at det i dag ikke er muligt at foretage miljørisikovurdering af nanomaterialer med et tilstrækkelig niveau af sikkerhed, primært grundet manglende viden og usikkerheden indlejret i nuværende økotoksicitetsdata. Omend bedre data bliver tilgængelige med tiden, er det vigtigt, at risikohåndteringsværktøjer, der kan inkorporerer usikkerhed anvendes til at fremme beslutningsstøtte. Da risikoen forbundet med nanomaterialer ikke kan kvantificeres, er brugen, nødvendigheden og anvendeligheden af risikohåndteringsmuligheder til at omfatte den potentielle risiko ligeledes svær at bedømme. Dette burde påberåbe en forsigtig brug af nanomaterialer.

Inden for nanotoksikologi har konceptet 'safety by design' modtaget meget opmærksomhed og fremgagng, hvilket indikerer at området er på vej mod en endnu mere deterministisk tilgang med et fejlplaceret løfte om at kunne løse disse håndteringsproblemer videnskabeligt. I stedet burde der fokuseres på at håndtere kompleksitet og usikkerheden i risikovurdering.

I afhandlingen tilskyndes der endeligt til identificering af risikobetonede nanomaterialer og mere sikre alternativer ved hjælp af metoden 'alternatives assessment'. Ved denne metode vil potentiel risiko blive sammenholdt med de opnåede fordele. Dette repræsentere et fremskridt ift. nuværende praksis, da adressering af risiko i isolation kun sjældent giver mulighed for at løse samfundsmæssige problemstillinger.

Table of contents

1 Background and aim

Nanotechnology is often described as the purposeful manipulation and creation of advanced materials at the nanoscale, generally termed engineered nanomaterials (ENMs). Although definitions vary, ENMs are normally defined as materials with at least one dimension below 100 nm (Boholm & Arvidsson, 2016). The study of the environmental effects of ENMs is correspondingly the study of nanoecotoxicology.

The field of nanoecotoxicology is considered a young scientific discipline as no ENMs have been tested for their potential environmental hazard prior to 2004 (Skjolding et al., 2016 Paper VII). The field has been growing rapidly since and, according to Thomson Reuters WoS, approximately 1000 peer-reviewed articles have currently been published that are identified with the terms nano* AND ecotox*. However, aquatic ecotoxicity testing of ENMs for risk assessment purposes has proven challenging, as existing test guidelines have been developed for dissolved chemicals and their applicability for ENMs and risk assessment has been questioned from the dawning of the field (OECD, 2006; Aitken et al., 2011).

ENMs are not dissolved entities as they behave as a solid phase within an aqueous phase and the stability of the suspension is influenced by colloidal forces (Petosa et al., 2010). The surface of the solid can, for instance, be charged or coated and is subject to surface forces such as van der Waals forces (Baalousha et al., 2009), which can change in different aquatic medias as well as over time. Particle aggregation and agglomeration are known issues that eventually also can lead to particle sedimentation. For dissolved chemicals a mass dose-metric is normally applied when conducting ecotoxicity testing, however for insoluble ENMs only atoms on the surface will be able to interact with an organism or the media. Other ENMs undergo partial dissolution giving rise to a more complex exposure scenario (Sørensen, 2016). For an overview of the main processes involved in these exposure issues see Figure 1.

Other concerns with testing ENMs include concentration-dependent behavior (Baalousha et al., 2016), interactions with the test container (Sekine et al., 2015), variability due to dispersion methods (Hartmann et al., 2015), the stability of the suspension (Cupi, 2015) as well as the occurrence of physical effects (Petersen et al., 2014; Sørensen et al., 2015 Paper IX). The main consequence of these issues is the creation of unstable, convoluted and time-

dependent exposure concentrations (Sørensen & Baun, 2014; Skjolding et al., 2016 Paper VII) which hampers the establishment of dose-response relationships which is at the core of aquatic ecotoxicity testing.

Uncertainty is also present throughout risk assessment (Grieger, 2011; SCENIHR, 2009; Hristozov et al., 2012) and risk management (Klaine et al., 2012). Fundamentally, each step relies on the previous steps and therefore all steps are rooted in the assumption that the generation of ecotoxicity data will facilitate the assessment of an environmental hazard which will enable decision-making further downstream in risk management. However, whether this holds true for ENMs is questioned and other approaches have been proposed (Syberg & Hansen, 2015; Miller & Wickson, 2015).

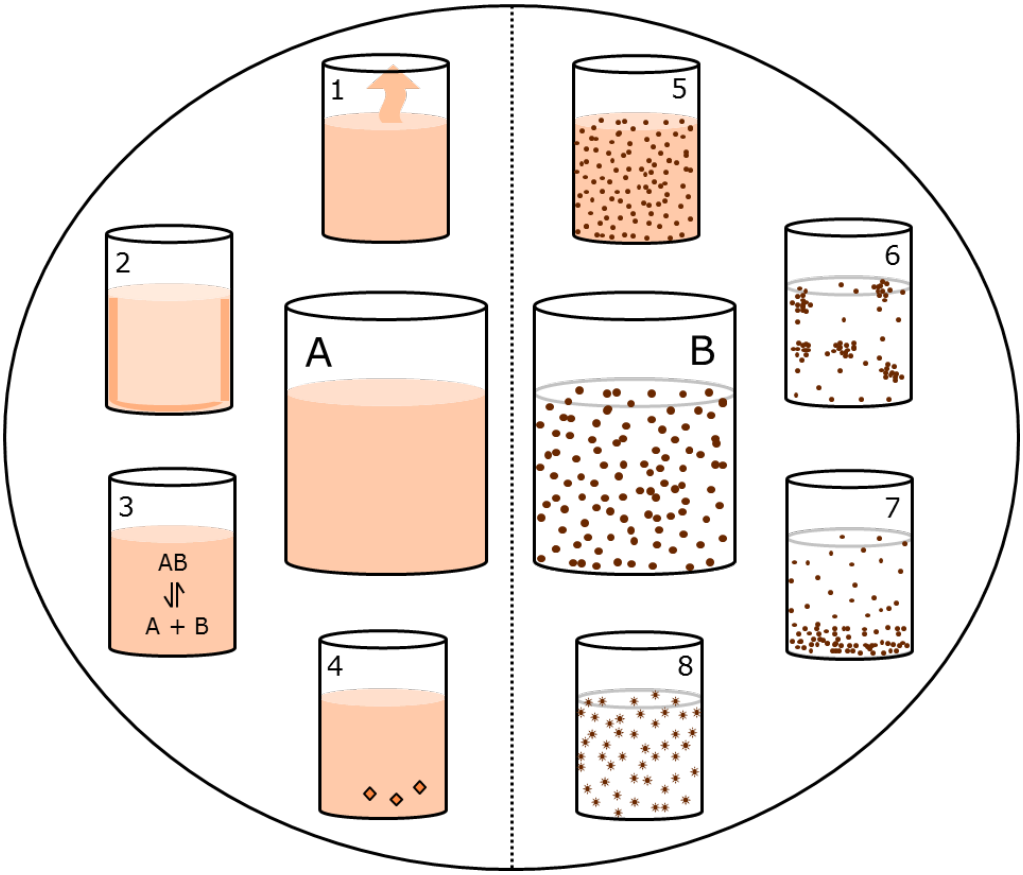

Figure 1 Overview of processes that give rise to exposure issues for aquatic testing for dissolved compounds (A) and for ENMs (B). Importantly, ENMs that partially dissolve experience issues from both (A) and (B). 1) Evaporation, 2) Adsorption, 3) Speciation, 4) Precipitation, 5) Dissolution, 6) Agglomeration and aggregation, 7) Sedimentation 8) Surface transformations. Reprinted with permission (Sørensen, 2016).

Overall, there is a need to address the applicability and current challenges present in ecotoxicity testing of ENMs as well as the ability to handle ENM risks through environmental risk assessment and management. The present thesis aims at covering these aspects primarily within a European context. Specially, the aim of the thesis is to:

- Evaluate the applicability of standardized aquatic ecotoxicity testing for environmental risk assessment of ENMs **(Paper IV - IX)**

- Provide an outlook for how ecotoxicity testing of ENMs can be improved to provide a better foundation for environmental risk assessment **(Paper II)**

- Analyze and discuss the feasibility of current risk management options to ensure the environmental safety of ENMs **(Paper I & III)**

2 The applicability of standardized ecotoxicity testing for ENMs

In 1983, the U.S. National Research Council (NRC) at the National Academy of Science published the so-called ‘Red Book’ entitled ‘Risk assessment in the Federal Government: Managing the process’ (National Research Council, 1983). Risk assessment was defined as ‘the use of the factual base to define the health effects of exposure of individuals or populations to hazardous materials and situations’ and incorporates hazard identification, dose response assessment, exposure assessment and risk characterization. The Red Book is considered the original foundation for current risk assessment schemes, including environmental risk assessment, and consequently environmental risk assessment consists of the same four steps (ECHA, 2008; Syberg & Hansen, 2015). Through hazard identification, a dose response relationship should be established, with the aim to estimate a Predicted No-Effect Concentration (PNEC), below which no environmental harm is expected. Risk characterization is then considered the ratio between a PNEC and the Predicted Environmental Concentration (PEC).

2.1 Hazard identification

Within ecotoxicology, testing is performed for two reasons, as described by Calow (1997):

1. To *anticipate* how toxicants are likely to impact ecological systems
2. To *assess* what changes are taking place in ecological systems under the influence of released toxic substances

Generally anticipatory tests comprise laboratory testing whereas assessment tests typically are performed in the field or in ‘near-field’ conditions. Hazard identification of chemicals and ENMs is the first step in risk assessment and relies on anticipatory testing. To ensure reproducibility and reliability, these tests have been harmonized to a large extent through standardized methods and standardized test guidelines (TGs). These are developed by international organizations, such as the Organisation for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO). In this thesis, standardized tests and standardized testing refer to these.

As mentioned in Section 1, TGs have never been developed specifically for the testing of ENMs; instead there has been a reliance on TGs developed for dissolved chemicals to also cover ENMs, although ENMs do not adhere to the test requirements and assumptions in the TGs. Today, uncertainty remains regarding the applicability of the TGs for ENMs however various stakeholders have formulated recommendations and offered preliminary as well as more decisive conclusions (Rasmussen et al., 2016; Petersen et al., 2015; Skjolding et al., 2016 Paper VII; Hund-Rinke et al., 2016; Sørensen, 2016; Bondarenko et al., 2016; ECHA, 2016a; OECD, 2015, Hansen et al., 2016 Paper VI).

2.2 Modifying OECD test guidelines

To address the applicability of current TGs to encompass ENMs, international efforts have been directed towards the OECD and their Working Party on Manufactured Nanomaterials (WPMN). In 2007, the WPMN launched a Sponsorship Programme to pool expertise and fund the safety testing of selected ENMs. In 2012, the WPMN concluded that the preliminary analysis of the Sponsorship Programme showed that the current OECD tests are ‘in general appropriate for assessing the safety of nanomaterials, but [they] may have to be adapted to the specificities of nanomaterials’ (OECD, 2012). Two years later, the OECD seemed to specify this by deeming TGs on alga growth inhibition (TG 201), *D. magna* reproduction (TG 211), earthworm reproduction (TG 222) and *L. variegatus* toxicity (TG 225) applicable to ENMs (OECD, 2014). The reasoning behind this was elaborated on by Kühnel & Nickel (2014), who state that participants at an OECD workshop found these tests generally applicable but also identified numerous issues that needed to be addressed.

Finally, in 2016, the OECD released the outcome of the seven-year Sponsorship Programme, in the form of 11 dossiers, along with statements supporting the continued use of the standardized tests, with the caveat that modifications and adaptions would be needed (OECD, 2015; Rasmussen et al., 2016). Rasmussen et al. (2016), specify that TG 201, 202 (acute immobilization of daphnids), 211, 222 and 225 are ‘generally applicable’, but emphasize again that adaptions might be needed for some of them.

However, a comprehensive analysis of the 11 dossiers shows that it is difficult to conclude much from the collected data and that the conclusions of the OECD are unsupported (Hansen et al., 2016 Paper VI). As seen in Figure 2, only a few TGs have been tested across a diverse number of ENMs, which would be needed to evaluate their general applicability. This concern is even greater for the guidelines on environmental fate and behavior. Nevertheless, the main issue is that the reporting in the dossiers largely is insufficient to enable an analysis of their validity. For this reason, even for the most used tests, e.g. TG 201, 202 and 203, it is difficult to offer any conclusions on the applicability of the tests (Hansen et al., 2016 Paper VI).

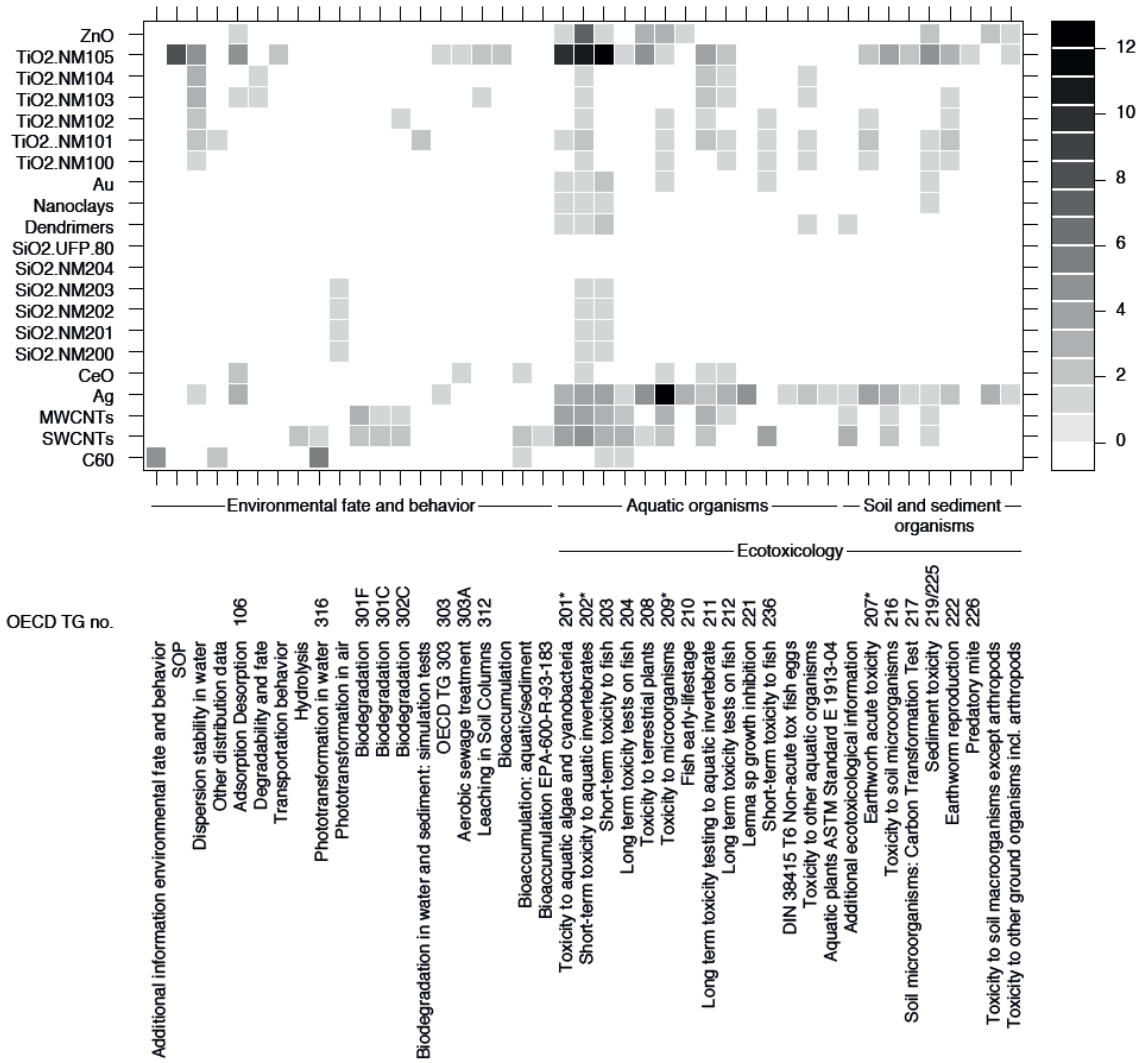

Figure 2 Heatmap of the number of tests carried out for each endpoint for each material in the OECD Sponsorship Programme (Hansen et al., 2016 Paper VI).

Besides from the OECD WPMN, other stakeholders have reached similar conclusions on the applicability of standardized testing of ENMs as well as suggested modifications to the current guidelines. Deeming the TGs applicable to ENMs seemed expected early on (Crane et al., 2008), and today there is a good understanding of the challenges that creates, primarily around the overarching issue of adequately determining exposure and separating indirect physical effects from chemical toxicity (Handy et al., 2012a, 2012b; Sørensen et al., 2015 Paper IX; Sørensen, 2016). It is important to note that similar issues can be present in the testing of e.g. metals (OECD, 2000) or turbid solutions containing soil or dyes (Cleuvers & Weyers, 2003). As reviewed by Hjorth et al. (2016 Paper IV), Fe salts give rise to concerns regarding the validity of the tests to such a degree that their use is discouraged, primarily due to issues with precipitation, speciation and indirect physical effects.

Recently, Petersen et al. (2015) discussed possible modifications to OECD TGs including adjusting medium composition, standardizing testing vessels and ENMs dispersion methods to address the issue of exposure control. Likewise, the FP 7 project MARINA also proposed modifications to OECD TG 201 and 202 among others (Hund-Rinke et al., 2016), however, these primarily tackle specific issues, e.g. the introduction of artifacts during measurements, and do not aim at remediating the underlying issue with the testing paradigm (i.e. that particles are not dissolved). Even with careful considerations on optimizing test conditions - such as adjusting medium composition, dispersion methods and pH levels - stable ENMs suspensions are hard to maintain in testing media (Cupi et al., 2015; Cupi, 2015), effectively ruining a stable exposure level during the test incubation. In general, significant progress has been made on how to characterize ENMs and describe their behavior during testing - however, good characterization aside - describing the inherent toxicity of a specific material is still challenging if said material is undergoing change during testing and furthermore violates the premise of the test. Minimizing the influence of time, as well as accounting for its effect, has also been proposed as a potential solution to increase the quality of data obtained in e.g. TG 201 (Sørensen & Baun, 2014).

However, not all seem to agree on the severity of the challenges facing standardized testing. For instance, within the European FP7 project NANOVALID, which aimed at providing methods for reliable hazard identification of ENMs, a test battery consisting of 15 bioassays were used to

assess seven ENMs (Bondarenko et al., 2016). The authors concluded that the standardized tests 'proved efficient' for ENMs and they especially praised the unmodified use of TG 201 and 202 and toxicity testing with *V. fischeri* (ISO21338:2010) to accurately pinpoint hazardous ENMs. Notably TG 201 and 202 were found to be the most sensitive and were described as 'supposedly easily adapted for the testing of ENMs' (Bondarenko et al., 2016). This is in contrast to findings by Hjorth et at. (2016a Paper IV) that specifically addresses the difficult use and interpretation of results from these three tests, which also finds support from other studies (Sørensen et al., 2015 Paper IX; Cupi et al., 2015; Hjorth et al., 2016b Paper VIII; Skjolding et al., 2016 Paper VII).

The European Chemicals Agency (ECHA) is currently modifying their guidance document on aquatic ecotoxicity testing of ENMs (ECHA, 2016a). Based on the current draft, dated May 2016, the revisited guidance will primarily be based on publications that affirm the applicability of the tests, e.g. Petersen et al. (2015), Rasmussen et al. (2016) and OECD (2012; 2014). For TG 201 and 202, however, ECHA specifically refer to Hjorth et al. (2016b Paper VIII), Sørensen et al. (2015 Paper IX) and Cupi et al. (2015), indicating that ECHA acknowledges, at least, part of the challenges facing aquatic ecotoxicity testing.

2.3 The three double-binds of nanoecotoxicology

The effort to modify existing guidelines reflects a need for a set of standardized ecotoxicity tests applicable for ENMs, which can feed into regulatory risk assessment and ensure mutual acceptance of data (MAD). As noted by Wickson et al. (2014 Paper V) the quest for standardization, however, is not unequivocal in the best interest of nanoecotoxicology – rather it is a mixed blessing. Specifically, standardization produces three double-binds or dilemmas with no good outcome (i.e. 'you are damned if you do and damned if you don't').

2.3.1 It is too early and too late

The first double-bind emerges as standardization in nanoecotoxicology is both too early and too late. It is too early as the field is still young and working towards gaining an understanding of which endpoints are relevant and whether or not we can adapt current test guidelines as covered above. But in a sense it is also too late, as we needed the tests when nanomaterials first

entered the marketplace. Since new products containing nanomaterials are continually being released onto the market, knowledge is needed for regulatory purposes right away, limiting the possibility of studying the adequacy of current methods, leaving standardization facing a temporal double-bind. This only compounds the temporal challenges already facing regulatory agencies (Linkov & Satterstrom, 2008).

2.3.2 Realism vs control

A more fundamental issue in toxicology is the tension between pursuing testing with a high degree of realism at the expense of control and vice versa. This is part of the general challenge for ecotoxicity testing, as tests ideally should be relevant, reproducible, reliable, robust and repeatable, however testing always incorporate a compromise and trade-offs between these five Rs (Calow, 1997), giving rise to the second double-bind. In standardized testing emphasis is given to achieve testing with a high degree of control. However, controlling experimental conditions to enhance comparison and reproducibility tends to undermine environmental realism – i.e. what is being tested under highly controlled and standardized conditions often has little connection with the complex and varied real world conditions. This 'realism-control' double-bind, which is widespread for risk-related science, is particularly salient for regulators who are looking for knowledge that is reliable, reproducible and environmentally relevant.

2.3.3 Selective ignorance

The last double-bind originates from a more universal dilemma within the philosophy of science, as pursuing knowledge according to any research paradigm unavoidably creates 'selective ignorance'. All scientific research is structured or framed by its own paradigm, which includes theoretical assumptions, research questions and criteria for defining 'adequate evidence', with a corresponding use of particular instruments or methods. This means that selecting a particular way to generate knowledge inclines researchers toward partial understandings of complex phenomena. Standardizing nanoecotoxicity testing early on, can therefore be seen as irresponsible as one cannot assume that a fuller understanding is not relevant, in particular as the field is characterized by high levels of uncertainty (Grieger, 2011). By not paying due respect to fundamental differences in the behaviour of ENMs in existing testing systems, flawed test methods may fail to capture impacts that are relevant for ENMs.

3 Testing needs for environmental risk assessment

In REACH, ecotoxicity data are needed for two overall purposes: 1) Classification and labelling of chemicals, and 2) PNEC derivation (ECHA, 2016c). In the first case the aim is to rank ecotoxicity and compare it to well-defined criteria and cut-off values – i.e., a relative metrics that can be used for e.g. regulation and hazard communication. In the second case the purpose is to derive a deterministic value for the concentration below which no toxic effects are likely to occur, i.e. an absolute metrics for a protective concentration. This fits well with the prior distinction between anticipatory laboratory testing and field-scale assessment testing, as some tests are suited for hazard identification and others for the assessment of environmental impact (see Section 2.1.1).

3.1 Feasibility of environmental risk assessment for engineered nanomaterials

Generally all steps through hazard identification to risk characterization have been deemed applicable in principle for ENMs and establishing PNEC-values have similarly been assessed feasible although with similar caveats as for ecotoxicity testing (Grieger et al., 2010; Aitken et al., 2011; Palmqvist et al., 2015). However, the issues from testing carry over to risk assessment as the assessment only is ‘as good as the quality of the data’ (Som et al., 2013).

Recently Lüftzhøft et al. (2015) set out to determine the PNEC values of nine ENMs, deemed regulatory relevant. They identified 1,200 scientific papers on nanoecotoxicology, with less than half of them providing data that could be relevant for PNEC derivation. However, only few of these were found adequate for risk assessment purposes, rendering PNEC derivation through assessment factors the only feasible approach. In REACH, species sensitivity distribution (SSD) is an alternative option for PNEC derivation, but as concluded by Lüftzhøft et al. (2015), high quality No Observed Effect Concentrations (NOECs) values from multiple taxonomic groups, needed for this approach, do not exist for ENMs. In general, the authors identified a lack of long-term studies, studies at different trophic levels and in different environmental compartments, but the unreliability and potential inadequacy of nanoecotoxicology testing was also stressed (Lüftzhøft et al., 2015).

This work was part of the NanoDen project commissioned by the Danish EPA. Based on these findings, the final NanoDen report concluded that there is little environmental concern for ENMs (Kjølholt et al., 2015) and corresponding conclusions have also been reported by e.g. Voelker et al. (2015) for nanoscale silver. Critically, due to data gaps, Kjølholt et al. (2015) was only able to perform risk assessment for the freshwater compartment, and since sediments have been deemed a possible sink for ENMs, more data on the long-term impacts in this compartment is needed (Lüftzhøft et al., 2015; Mouneyrac et al., 2015; Wang et al., 2016; Coll et al., 2016). Furthermore, the validity of the results are heavily questioned and an emphasis was put on the dubious quality of ecotoxicity data feeding into risk assessment, which was identified as 'the major gap' for estimation of PNEC values. As stated by Kjølholt et al. (2015) '…despite a wide range of tests have been performed according to accepted international guidelines (or modification thereof), they cannot be fully trusted to yield accurate and conservative estimates of the toxicity of an ENM'. Additionally, extrapolation from e.g. EC_{50} or NOEC-values to PNEC values involves a new set of assumptions that have not been examined for ENMs. The premise of PNEC derivation is that through the establishment of a dose-response relationship it is possible to estimate a dose at which no effect, or only an acceptable effect will occur. However, as described in Chapter 1 & 2, the confidence in dose-response relationships is limited, especially for far-reaching extrapolations due to, for instance the concentration-dependent behavior of ENMs (Baalousha et al., 2016). As reviewed by Syberg and Hansen (2015), even the extrapolation for dissolved chemicals is based more on convention than scientific evidence.

Importantly, a confounding factor is the severe lack of fate and exposure data within nanotoxicology, meaning even with accurate PNEC values, risk characterization would still suffer due to a lack of predicted environmental concentrations (PECs). This leaves modelling based on production estimates as the current approach to predict environmental concentrations (Mueller & Nowack, 2008; Sun et al., 2014).

Modelling is similarly used to derive PNEC estimates. Recently Coll et al. (2016) utilized probabilistic species sensitivity distributions (PSSD) to establish PNEC values for nanoscale titanium dioxide, silver, zinc oxide, carbon nanotubes and fullerenes. Missing NOEC values are estimated through assessment factors and the combined data are used to generate PSSDs through a Monte Carlo based model described by Gottschalk &

Nowack (2013). Again, none of the estimated PNEC values were higher than the PEC values for both the freshwater and the soil compartment. Wang et al. (2016) reached a similar conclusion for iron based ENMs. However, both Coll et al. (2016) and Wang et al. (2016) could not estimate the risk in sediments due to lack of ecotoxicity data.

Others challenge whether environmental risk assessment is even applicable for ENMs or whether it should be the risk analysis tool of choice (Syberg & Hansen, 2015; Miller & Wickson, 2015) and alternative methods or frameworks for decision support and risk analysis have been proposed (Grieger, 2011; Grieger et al., 2012; Rio-Echevarria & Rickerby, 2015; Arvidsson et al., 2016). To various degrees, these all try to embrace the uncertainty present in risk assessment to facilitate decision-support.

3.2 Testing in the 21st century

Due to the current constraints in nanoecotoxicology testing and the inability to adequately assess risk, the development of new testing strategies has received much attention. These primarily address the need to fill knowledge gaps but also aim towards identifying patterns in nanotoxicology that will allow for grouping, read-across and predictive testing needed to avoid case-by-case assessments (Villeneuve & Garcia-Reyero, 2011; Godwin et al., 2015). To achieve this, prioritization needs within nanotoxicology, in both the short term and long term, have been formulated by e.g. Stone et al. (2014). These deliberations are part of a bigger trend within toxicology clearest articulated by the NRC in 2007, where they published 'Toxicity Testing in the 21st Century: A Vision and a Strategy' (National Research Council, 2007). In this report - and in a report leading up to it (National Research Council, 2006) - they envisioned a radical paradigm shift needed to improve toxicity testing by:

i. Providing broader coverage of chemicals and their mixtures, end points, and life-stage vulnerabilities.

ii. Reducing the cost and time of testing, increase efficiency and flexibility, and make it possible to reach a decision more quickly.

iii. Using fewer animals and cause minimal suffering to animals that are used.

iv. Developing a more robust scientific basis of risk assessment by providing detailed mechanistic and dosimetry information and by encouraging the integration of toxicologic and population-based data.

In other words, a shift from 'time-consuming' and 'resource-intensive' *in vivo* testing to integrated and fast *in vitro* and *in silico* testing to address the 'depth' and 'breadth' needs in risk assessment in conjunction with animal welfare and resource considerations. The focus of the NRC was clearly on human toxicology, but it has likewise shaped the vision for ecotoxicology partly as similar thoughts also are present in the REACH (Hartung, 2009). The '21st–century vision' is correspondingly a major driver in nanotoxicology (Walker & Bucher, 2009; Lai, 2012; Nel et al., 2013a; Nel, 2013; Savolainen et al., 2013). However, moving away from the traditional *in vivo* testing towards a more integrated testing approach based on modelling and screening rests on the predictive power of these new test systems, collectively referred to as alternative testing strategies (ATS).

3.2.1 Alternative testing strategies

Today, alternative testing or ATS is used synonymously with non-animal testing as it is seen as a response to the pressure to replace, reduce and refine animal testing in toxicology (Shatkin & Ong, 2016). However in ecotoxicology animal models still remain at the foundation of environmental risk assessment. In nanoecotoxicology *in vivo* models, such as crustaceans, dominate the literature and more testing with higher trophic level species, such as fish, is requested to facilitate better risk assessment of ENMs (Juganson et al., 2015; Lüftzhøft et al., 2015). In contrast to human toxicology, ecotoxicology is therefore not looking to substitute the current *in vivo* testing with corresponding *in vitro* models. Consequently, the role of ATS in nanoecotoxicology is conflicted. This is also the premise of Paper II (Hjorth et al., 2016c Paper II), which sets out to discuss and define the use of ATS in the field of nanoecotoxicology, based on its ability to feed into ERA. It is clear that a single *in vitro* study is not sufficient for ERA but it is instead likely that a holistic approach between high throughput screening and higher-tier testing, including suitable standardized tests, could provide useful answers for risk assessment. Specifically, Hjorth et al. (2016c Paper II) propose that interplay between simple low-tier tests and validation through higher-tier tests along the so-called 'ecotoxicity complexity continuum'

(Figure 3) should be pursued to advance the development of faster and more accurate risk analysis.

However, as also accentuated earlier by Wickson et al. (2015 Paper V) there is a considerable need for diversity in testing. For that reason, exploratory testing has value in itself, and as such conducting testing 'without necessarily assuring regulatory relevance should be encouraged to improve the understanding of a variety of factors (e.g. toxic mode(s) of action of ENMs) which indirectly will highlight what should be emphasized for risk assessment' (Hjorth et al., 2016c Paper II).

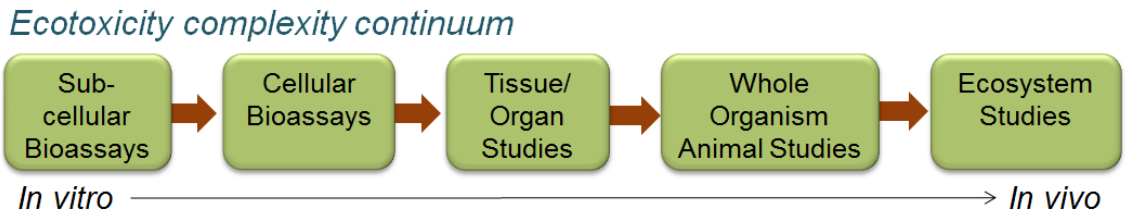

Figure 3 The 'Ecotoxicity complexity continuum'. Ecotoxicity testing span from anticipatory sub-cellular *in vitro* assays to assessment testing in complex ecosystems, either in mesocosm or in the field (Hjorth et al., 2016c Paper II).

ATS of course also face many of the same overall testing issues as reviewed in Chapter 1 & 2 and whereas field and mesocosm data already can feed directly into risk assessment (e.g. ECHA, 2008), the use of *in vitro* tests needs to be validated (Nel et al., 2013b; Shatkin & Ong, 2016). The proposed changes to ecotoxicity testing challenge the current way of incorporating and assessing ecotoxicity data in environmental risk assessment and the regulatory readiness can be questioned (Hjorth et al., 2016c Paper II; Malloy & Beryt, 2016). Partly, this is caused by a regulatory focus on standardized anticipatory testing, highlighted by the use of Klimisch scores for data quality evaluation, which ranks studies performed with standardized guideline testing under good laboratory practices (GLP) above all other studies (Klimisch et al., 1997). This is in contrast to the needs for environmental risk assessment which calls for more long-term assessment testing, i.e. testing with an assessment scope instead of testing for hazard identification.

As ENMs do not uphold the exposure requirements in current laboratory testing, assessing their environmental impact and fate in more complex environments (e.g. micro- and mesocosms), will provide a more realistic exposure regime (Shaw & Kennedy, 1996; Baalousha et al., 2016). The case

for more environmentally relevant testing is also advocated by e.g. Holden et al. (2016). As such testing is resource intensive, only selected materials with a high production, hazard potential or exposure potential should be prioritized. A similar tiered/concern driven testing is described by Oomen et al. (2014), Hund-Rinke et al. (2015) or found within the data requirements in REACH. Currently, micro- and mesocosms experiments remain substantially unexplored for ENMs (Bour et al., 2015a, 2015b; Minetto et al., 2016), but would be relevant for the assessment of the long term impact of ENMs as well as to validate or examine NOECs or PNEC derivations. As the use of assessment factors in environmental risk assessment to facilitate extrapolation from dose descriptors is disputed for ENMs, more environmental realistic testing can validate and/or circumvent their use. In general, a better understanding of ecotoxicity is obtained by using laboratory studies in conjunction with field-based studies (Chapman, 1995). As described earlier, laboratory testing of Fe salts faces many of the same challenges as ENMs, and for the derivation of PNECs and environmental quality standards, field or near-field studies have been useful to provide better estimates than what can be achieved through conventional laboratory testing (Hjorth et al., 2016a Paper IV).

4 Environmental risk management

As the ‘ultimate goal of assessing risk is managing risk’ (Klaine et al., 2012), facilitating environmental risk management of ENMs is consequently the eventual purpose of ecotoxicity testing. The Red book (see Section 2.1) introduced and recommended a distinction between the scientific aspects (risk assessment) and policy aspects (risk management) - which consequently, also have had a significant impact on the structure of European regulation, although relatively later (Lofstedt, 2003). Risk management is defined as the ‘process of weighing policy alternatives and selecting the most appropriate regulatory action, integrating the results of risk assessment with engineering data and with social, economic, and political concerns to reach a decision’ (National Research Council, 1983). In other words, whereas risk assessment addresses how risky something is, risk management asks ‘what are we willing to accept’ and ‘what shall we do about it’. Risk management normally is comprised of four steps: risk classification, risk benefit-analysis, risk reduction and finally, monitoring and review (van Leeuwen, 2007).

As shown in the previous chapters, ecotoxicity testing has challenges in providing suitable data for risk assessment, which also only is applicable to ENMs with caveats, and consequently the environmental risk posed by ENMs is difficult to estimate with certainty. A similar conclusion is reached by Klaine et al. (2012) who state: ‘A consensus view exists that the paucity of usable data on the environmental hazard of nanomaterials has created unacceptable uncertainty in risk analysis from the regulatory decision-making perspective’. This both highlights the need for effective tools for risk management of ENMs, but also the uncertainty in which risk managers have to navigate. Understandably, if testing and assessment is compromised, risk management consequently becomes a steep uphill battle. Classifying risk (i.e. determining whether a risk is acceptable) is arguable not possible as the risk cannot be quantified as per the consensus view above, or can only be done to some degree as seen in Section 3.1.1 The risk benefit-analysis is seen as a tool to identify cost-effective risk reduction options, if risk reduction is deemed necessary. However, again it relies on a good quantification of the risk (van Leeuwen, 2007). Furthermore, tools for environmental monitoring of ENMs are not available (Gottschalk et al., 2013; Coll et al., 2016) hampering the use of environmental quality standards (Baun et al., 2009).

The point being that although risk management still has risk reduction options to consider, the need and ability of these options to reduce risk is hard to assess.

4.1 ‘Safety by design’

Generally risk management options can be ranked according to their efficiency and preferability, with risk elimination (i.e. completely removing a hazardous chemical) and substitution (i.e. replacing a hazardous chemical with a safer alternative) as the highest ranked, respectively (Oksel et al., 2016). Whereas the focus for environmental risk management normally is on exposure control (e.g. how high of a concentration is allowed in the environment), for ENMs it has arguable primarily been on hazard control as the risk management option that has received the most attention has been the creation of safer ENMs (Lynch, 2016). This is a derivative of predictive testing that enables rapid screening and identification of ENMs with low or lower intrinsic hazard potential, which has spurred considerable funding as well as regulatory interest. This has especially been true for the purposeful manipulation of ENMs based on high throughput screenings to achieve ‘safe-’, ‘safer-’ or ‘safety by design’ (SbD). Table 1 provides an overview of current European research projects which strive towards SbD and as described by Hjorth et al. (2016d Paper III), the paradigm of designing for safety is emphasized throughout current nanosafety research.

Although the term SbD can imply otherwise, true elimination of risk is only possible through no exposure, practically implying no use of a material, which seems outside the scope of SbD. However, what the actual concept of SbD entails is unclear. Indeed, despite having had a noticeable presence in past research projects and a dominating role in current ones, SbD appears superficial, unevaluated and un-conceptualized with little to no scientific literature attached to it (Hjorth et al., 2016d Paper III).

What can be achieved through SbD therefore remains to be seen. Nonetheless, Hjorth et al. (2016d Paper III) point out that conceptually perceiving ‘safety’ as an inherent material property is fundamentally problematic as it tend to neglect exposure considerations and marks a return to the hazy lines between the scientific task of risk assessment and the policy task of risk management.

Table 1 Overview and focus of the projects in the 2016 Nanosafety Cluster Compendium that address safety by design (SbD). The exes indicate whether the projects are involved in the conceptual idea behind SbD and whether safety is approached through hazard, exposure and/or fate considerations (Hjorth et al., 2016d Paper III).

	Projects	Statement	Concept	Hazard	Exposure	Fate
Horizon 2020	**SmartNanoTox**	'By scanning main groups of engineered NMs, we will identify the NM properties that might be responsible for causing a particular toxic effect and lead to a particular AO, and thus should be modified or avoided. This will provide means of grouping and read- across characterization of NMs and enable development of materials that are **safe by design'**		X		
	NanoFase	[activities in NanoFase] 'will aid **Safe by Design** and Benign by Design Concepts, as it will inform on how basic ENM properties will affect their final environmental form(s) and distribution following environmental release, allowing this to be a relevant consideration in the design phase'				X
	NANOGENTOOLS	'Conduct research and training on biophysical techniques and mathematical models for accurate and fast nanotoxicity prediction linked to **safety-by-design concepts'**		X		
	ProSafe	[one objective is to] 'Acceptance and further elaboration of the NANoREG safe innovation and **safe-by-design concept**'	X			
	NANoREG II	'The NANoREG II project, built around the challenge of coupling **SbD** to the regulatory process, will demonstrate and establish new principles and ideas based on data from value chain implementation studies to establish **SbD** as a fundamental pillar in the validation of a novel MNM'	X	X	X	X
FP7	**eNanoMapper**	'…we will develop resources, tools and standards for a scientifically sound risk assessment of ENMs that will support the **design of new safe** and environment- friendly ENMs as well as the assessment of existing materials'		X		
	FutureNanoNeeds	[one objective is] 'To develop an understanding of the relationships between nanoparticle (pristine) structure, its properties (including in situ), and its biological and environmental activity (that is, structure and 'identity' broadly defined) thereby giving early support to the science of 'new nanomaterials**, safe by design''**		X		
	GUIDEnano	'**SbD strategies** were intended to : re-design relevant physicochemical properties of NM to mitigate their hazardous potential, while maintaining their characteristic functionality within the NM enabled product, avoid or reduce the release of NM during different life cycle stages of the nano-enabled products by improving compatibility between NM and matrix, to lower the possibility of environmental and/or human exposure to NM, avoid or reduce the environmental and/or human exposure to NM by designing and synthesizing less reactive and/or less persistent NM'		X	X	
	NanoMILE	'NanoMILE intends to revolutionise nanosafety research through its robust and novel approaches to the selection and development of the test nanomaterials, its technically and computationally advanced integration of systems biology, its thoughtfully balanced toxicological / ecotoxicological approaches, its development of novel high throughput platforms for screening and its feedback loops for development of nanomaterials that are **safer by design'**		X		
	NanoToxClass	'NanoToxClass also enhances our understanding of modes of action for NM and can give guidance to the large set of possible toxicity endpoints for NM by selecting the most predictive ones (which will be then used as a basis for grouping). Omics techniques will enable to assess NM hazards on a mechanistic basis and will enable the determination of adverse outcome pathways (AOP). Finally, this knowledge may be applied as a tool to create safer nanoparticles (so called **"safe-by-design" approaches**)'		X		
	SUN	'The SUN approach has covered the entire lifecycles of real nanoproducts, aiming at developing **safer by design strategies** in order to open new possibilities for innovators to design greener nanotechnologies'		X	X	X
	NANoREG	'An integrated research strategy which addresses **product/material design and the safety aspects** for humans and the environment will be developed'	X	X	X	

Ensuring safety, and correspondingly accepting risk, is primarily a political task. SbD therefore represents a reduction and a recasting of risk management issues into technical problems. However, early identification of risk and corresponding focus on risk mitigation and safer alternatives is of course desired. These elements are also a central part of 'chemical alternatives assessment', which addresses the second best option in risk management – substitution.

4.2 Alternatives assessment

Alternatives assessment offers a concrete framework of steps to identify and evaluate potentially safer alternatives to a chemical of concern (see Figure 4). The use of alternatives assessment has witnessed tremendous growth in the last number of years driven by the increased demand to eliminate or replace known hazardous chemicals in consumer products and manufacturing processes (Lavoie et al., 2010; Malloy et al., 2015). As argued by Hjorth et al. (2016e Paper I), ENMs may not easily fit into existing frameworks even though they can be considered both emerging "chemicals" of concern, as well as potentially safer alternatives to currently used chemicals. Nevertheless, the authors conclude that the frameworks could be adapted to better embrace ENMs. The pivot of the issue is once again, the challenges in adequately assessing the hazard and exposure of ENMs, needed to compare alternatives. However, as the decision-making in alternatives assessment is based on relativity (i.e. is this material safer than this one), quantitative measurements of, for instance, hazard might not be needed to facilitate identification of safer alternatives (Hjorth et al., 2016e Paper I). Therefore many of the aspirations of predictive testing and SbD, as covered earlier, could potentially find a framework in alternatives assessment to provide near-term decision-making about material design choices to ultimately enable the generation of safer ENMs. Hjorth et al. (2016e Paper I) conclude that case studies with ENMs are encouraged as well as required to further study the methodological needs of nano-specific tools for alternatives assessment to adequately cover ENMs.

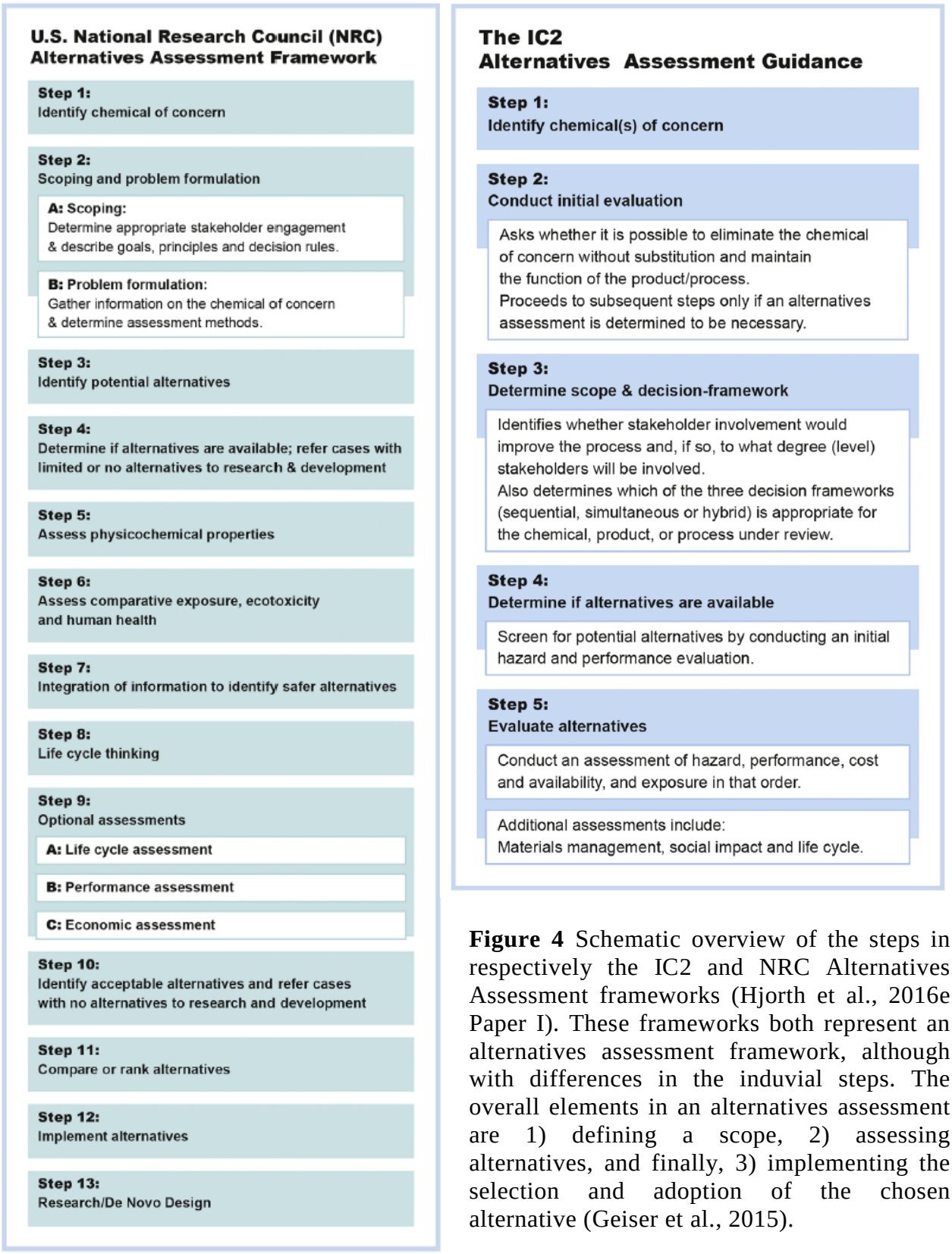

Figure 4 Schematic overview of the steps in respectively the IC2 and NRC Alternatives Assessment frameworks (Hjorth et al., 2016e Paper I). These frameworks both represent an alternatives assessment framework, although with differences in the induvial steps. The overall elements in an alternatives assessment are 1) defining a scope, 2) assessing alternatives, and finally, 3) implementing the selection and adoption of the chosen alternative (Geiser et al., 2015).

Importantly, the first question in alternatives assessment is ‘why is the chemical or material present?’, ‘what function should be retained or be present in an alternative?’ This explicitly makes it clear that chemicals and materials should have a purpose or provide an added benefit to a product. Focusing on material function allows for a wider consideration of alternatives but also includes directing attention to the necessity of that function (Tickner et al., 2015). As stated by Hjorth et al. (2016e paper I) ’if […] a nanomaterial serves no necessary function or is completely redundant and is potentially risky for human or environmental health, it may be easiest to simply avoid using that material’.

With few viable risk management options, the benefit, necessity and use of ENMs need to be addressed. Gellert et al. (2015) emphasize that such considerations are part of a precautionary stance as ‘determining the necessity of a risky course of action [is] crucial to assess safer alternatives’. This also reflects the proposal by Tickner it al. (2015) or e.g. Finkel (2011) and Malloy et al. (2016) to focus on possible solutions and the function of a risky behaviour, instead of determining risk in isolation. I.e. whether or not a risk can be tolerated should resonate with whether or not the introduction of a risk is needed.

4.3 Invoking the precautionary principle

The profound scientific uncertainty present throughout risk assessment has repetitively been mentioned as a factor hampering risk management of ENMs. When the assessment of a potentially irreversible risk is impossible due to scientific uncertainty, the precautionary principle (PP) has been used to justify regulatory measures (Harremoës et al., 2001). European legislators, however, have thus far been reluctant with taking recourse to the PP for ENMs, although it is acknowledged as a guiding element in EU legislation and also enshrined in the Maastricht Treaty (European Commision, 2000). According to Sandin (1999) the PP can be condensed to four dimensions: ‘If there is (1) a threat, which is (2) uncertain, then (3) some kind of action (4) is mandatory’. Generally the PP is recognized to be a broader concept (Raffensperger & Tickner, 1999; Kriebel et al., 2001; Hansen et al., 2007) and to incorporate:

- Taking preventive action in the face of uncertainty
- Shifting the burden of proof to the proponents of an activity
- Exploring a wide range of alternatives to possibly harmful actions
- Increasing public participation in decision making

Perhaps the most prominent definition of PP in international law has been the Rio Declarations Principle 15 which state:

> ‘In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation’.

In European legal text the PP is less defined; it is for instance not defined in the Maastricht Treaty – its use is only described or ‘prescribed’ and ‘adumbrated’ as stated by the European Commission (European Commision, 2000). In general it is still debated to what degree the PP is part of international law (Garnett & Parsons, 2016). In 2000, The European Commission aimed to clarify the use of PP and establish guidelines for applying it. They concluded that PP covers circumstances where: (1) scientific evidence is insufficient, inconclusive or uncertain, and (2) there are reasonable grounds for concern that the potentially dangerous effects on the environment, human, animal or plant health may be inconsistent with the chosen level of protection.

The European Commission furthermore stated that recourse to the PP presupposes: (a) identification of potentially negative effects resulting from a phenomenon, product or process and (b) a scientific evaluation of the risk which because of the insufficiency of the data, their inconclusive or imprecise nature, makes it impossible to determine with sufficient certainty the risk in question (European Commision, 2000). Point (b) adds an extra dimension to point (1), meaning that scientific evidence is insufficient, inconclusive or uncertain to such a degree that a scientific evaluation of the risk is rendered impossible.

This interpretation describes recourse to precautionary actions to ‘presuppose’ and ‘cover’ some circumstances, which therefore ‘can’ justify recourse to the PP to ensure a certain level of protection. But it does not dictate that the PP should be invoked if these circumstances are present,

which is fundamentally different from ‘uncertainty shall not excuse inaction’ in the Rio Declaration or ‘action is mandatory’ in Sandins (1999) condensation. Critic of the interpretation by the European Commission and its vagueness has also been articulated by e.g. Hansen et al. (2007) and in a recent review on the application of the PP within the EU, Garnett and Parsons (2016) concluded that despite the European Commision Communication (European Commission, 2000), when to invoke the PP is still poorly defined.

In 2008, the European Commission acknowledged that risk management measures are necessary for ENMs and that they should be based on the PP (European Commision, 2008), but also specified that recourse to the PP could merely entail e.g. initiation of more research. This series of events was commented on by Gellert (2015), who concluded that the EU attitude on ENMs does not ‘fit with a precautionary stance’ and ‘lack of information is often used as an excuse not to act’. This is in line with the report ‘Late lessons from early warnings: the precautionary principle 1896-2000’, which specifically warns against this behaviour (Harremoës et al., 2001) which also is present in nanotoxicology (Hansen et al., 2008; Gee et al., 2013). For instance, the biocidal use of nanoscale silver is the most used ENMs application in consumer products and a call for regulation has been proposed for years (Aitken et al., 2009; Hjorth et al., 2010; Hansen & Baun, 2015). However, any decision-making on nanoscale silver appears stuck in evaluation (Hansen & Baun, 2012) and currently silver and nanoscale silver are awaiting review in Europe under the Biocidal Product Regulation expected to be completed by 2024 (ECHA, 2016b; Mackevica et al., 2016).

5 Conclusion

As presented in this thesis, the challenges for ENMs are numerous with issues at most, if not all, levels of risk analysis – from ecotoxicity testing to hazard identification and risk management. In contrast to the conclusions offered by the OECD on the general applicability of ecotoxicity testing guidelines for ENMs, this thesis emphasize that although modifying the guidelines can alleviate testing issues, it should be acknowledged that the underlying premise of the tests is violated. Arguably, meaningful - albeit potentially flawed - data can still be obtained through ecotoxicity testing however it requires modification of the tests, transparent and detailed reporting of how the testing was conducted as well as description of the particle behavior during test incubation and finally, expert scrutiny of the outcome.

Even though the 21st toxicity testing paradigm is calling for further reliance on extrapolation in risk analysis, nanoecotoxicology would do well by proceeding with caution. As the field still is facing fundamental challenges in testing, emulating the same general level of ambition does not seem recommendable. Furthermore, even the current use of extrapolation factors in environmental risk assessment is questionable and their validation and/or circumvention are needed through more complex testing supported by a better mechanistic understanding of the potential environmental impacts of ENMs. In general, there appears to be a lack of assessment testing aiming at providing data for PNEC derivation, which forces the use of data from standardized anticipatory testing.

Despite improvements in data generation, collection and evaluation – risk assessment is still currently hampered and decision-making is required in spite of scientific uncertainty. The academic exercise of trying to overcome the testing and assessment challenges does not seem to be generating the demanded solutions for risk management, although with time it potentially could. While current estimates conclude that ENMs pose little to no environmental risk, these estimates are riddled with uncertainty which should invoke a precautionary stance towards the use of ENMs.

Wide-spread uncertainty regarding testing, assessment and management of ENMs is likely to persist and decision-support tools encompassing this should be applied as well as further developed. These conclusions are not aligned with the current trend within nanotoxicology as the rise of ‘safety by

design’ represents a more deterministic approach with a misplaced promise to solve these management issues scientifically.

Both alternatives assessment and a precautionary approach to risk management highlight that the concept of necessity and risk-benefit considerations need to be addressed on a management and societal level. This seems especially crucial for ENMs as the role of ecotoxicity testing to provide reliable ecotoxicity data that enables risk assessment and management is currently hampered.

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7 Papers

I **Hjorth R**, Hansen SF, Jacobs M, Tickner J, Ellenbecker M, Baun A (2016). The applicability of chemical alternatives assessment for engineered nanomaterials, *Integrated Environmental Assessment and Management*. DOI: 10.1002/ieam.1762

II **Hjorth R**, Holden P, Hansen SF, Colman B, Grieger K, Hendren, CO (2016). The role of alternative testing strategies in environmental risk assessment of engineered nanomaterials. *Submitted.*

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