2014 — 2018

Report Phase 1

MISTRA
ENVIRONMENTAL
NANOSAFETY

2014

2018

Report Phase 1

This report has been prepared by selecting research results obtained by our program scientists and presenting them within the respective Case Study of the program. Not all results from Phase 1 are able to be included here. All results can be found in the scientific publications listed at the end of this report.

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LAYOUT AND ILLUSTRATIONS
Boid AB

FÖRORD

När jag för fyra år sedan blev tillfrågad om att leda styrelsen för MISTRA Enviromental Nanosafety tyckte jag att det var ett spännande program. Fokus är att bedöma risker för miljön av nanomaterial, vilka egenskaper hos nanomaterial som är viktiga att belysa och undvika. Programmet handlar om riskbedömning och hur risk upplevs hos stakeholders och i samhället i stort. Båda är nödvändiga för en reglering av nanomaterial.

Programmet har engagerat de främsta forskarna inom området i Sverige, de flesta med kontaktnät inom EU och vidare internationellt. En mycket aktiv industripartner har ingått, Styrelsen har haft en bred sammansättning med viktiga avnämare.

Initialt såg förutsättningarna för programmet bra ut. Men en del svårigheter kom ganska snart Det tog lång tid för parterna att besluta om konsortialavtalet. Ytterligare utmaningar var att programchefen slutade och att det blev en viss omsättning i styrelsen. Självklart får man räkna med den typen av händelser när det handlar om långsiktiga program.

Efter två år var emellertid programmet i full sving. I föreliggande slutrapport redovisas resultaten.

Jag vill tacka alla de som har bidragit till de intressanta och spännande resultat som har kommit fram i programmets första fas. Jag är övertygad om att verksamheten i programmet kommer att utgöra ett bra underlag för fas 2.

ROLF ANNERBERG

Ordförande i programstyrelsen för MISTRA Environmental Nanosafety

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Chapter 1

EXECUTIVE SUMMARY

Phase 1 has shown the importance of more realistic and integrated risk assessment methods of nanoparticles

GOALS OF THE PROGRAM

The Swedish Foundation for Strategic Environmental Research (MISTRA) held an open call for nanomaterials in 2013, and chose to fund the Environmental Nanosafety program hosted by Chalmers University of Technology. The consortium is a multidisciplinary group of engineers, natural scientists, social scientists, medical and industrial representatives from the University of Gothenburg, Chalmers University of Technology, Lund University, KTH Royal Institute of Technology, Karolinska Institutet, and AkzoNobel PPC, now Nouryon. Our goal was to create a strong integrated research environment in order to develop generic methods and models that will provide a basis for risk assessments needed to promote environmentally safe development of nanotechnology in the future. Risk assessments need to be understood and accepted by societal actors who are faced with the challenge to evaluate potential hazards, as well as potential benefits, of nanomaterials. The program's natural science and technology studies are linked to its social science research by the risk assessment and societal flow studies that have been performed.

The research program has been designed around specific Case studies, chosen due to their particular relevance to existing and future Swedish industry. Case Study 1: Emission of nanoparticles from automobile application to road runoff water. Case Study 2: Systematic studies of commercially relevant nanomaterials. Case Study 3: Future nanomaterials. The prime example of a new nanomaterial of interest to the program is graphene. Labeled silica nanomaterials, that will be traceable throughout model test systems, have also been developed and studied within the program. Case studies 1 and 2 are the most substantial case studies and thread their way through the scientific workpackages of the program in a transdisciplinary manner. Research has been carried out in five work packages (WPs), each encompassing a different key aspect involved in the development of a nanoparticle risk assessment strategy.

TAKE-HOME MESSAGES FROM PHASE 1

Although the final remaining program deliverables from Phase 1 are in the process of being written up and/or submitted for peer review, it is possible to provide a few specific conclusions from Phase 1 to date. Tire studs made from tungsten carbide cobalt metal alloys may claim more lives than they save when considering the entire life cycle of the material. Nanoscale wear particles that are unintentionally produced from worn studs are detected in roadway runoff, streams and rivers where they can aggregate and preferentially dissolve, releasing cobalt ions. They are taken up by aquatic organisms and travel up the food chain. These nanoparticles show long-term toxicity in certain organisms as well as in cell culture tests in mid-to-high doses, and cobalt seems to play a key role. Biomolecules that adsorb to the materials can play a role in mitigating the toxicity of these particles. It might be possible to replace tungsten carbide cobalt with other hard materials, such as synthetic diamond, to reduce the environmental and human health impact of this product.

Silica nanoparticles are intentionally produced commercially in large quantities and added to paints, paper, cosmetics, foods and pharmaceuticals. They appear to be benign in our ecotoxicology and human cell toxicity models, except when given in extremely, and unrealistic, high quantities to cells in culture. The smallest silica particles tested (7 nm) show some toxic behavior in culture in high dosage, but which can be ameliorated by surface modification. The mechanisms behind these observations are still under study. Because silica nanoparticles show such low toxicity in general, they cannot be used as we had expected for developing a QSAR model for nanoparticles in our test battery. Instead, metallic nanoparticles are being used for generation of a QSAR model for nanoparticles.

Considering the multitude of application areas for nanoparticles, perceptions of risks and benefits of nanotechnology and nanomaterials differ considerably depending on area of application. Our social science studies reveal that among the public in Sweden, the level of knowledge of nanotechnology and nanomaterials is low and attitudes are formed based on other parameters such as heuristics for processing complex information. This also holds for expert stakeholders, despite a higher level of education. Experts in NGOs perceived a higher risk of nanomaterials and were more positive to regulations than their colleagues in trade associations and companies. Expert stakeholders emphasize the advantages rather than the risks of nanotechnology and nanomaterials, but that this varied slightly depending on application area. For example, they are significantly more positive to the use of nanotechnology in electronic devices compared to areas where nanoparticles come closer to the body, such as cosmetics or food. Generally, the experts support regulation of nanomaterials and nanotechnology. They are relatively negative to taxes and self-regulation as legislature, and relatively positive to selective prohibitions. However, the recent incorporation of nanomaterials in the Annexes of the European Union's chemical legislation requires manufacturers, importers and downstream users to self-regulate by registering "nanoforms" (nanomaterials) and demonstrating their safety to the environment and human health through an extensive series of material characterization and testing.

If we summarize our results so far in a broader context, we find that a complex transformation of nanomaterials occurs when released in natural aquatic environments, including stabilization, aggregation, adsorption and dissolution due to the effects of molecular interactions (from natural organic matter and proteins secreted by aquatic organisms) with nanoparticles that form a "biocorona" on the particles. The transformation gives new characteristics to the nanomaterial that affect the toxicity towards aquatic organisms. Acute (short term) tests are not enough to evaluate toxicity, including transformation effects. Classic acute Daphnia (zooplankton) and cell culture toxicity tests may not capture transformation effects either. Therefore, a deeper understanding of nanomaterial transformation and how that affects toxicity to aquatic organisms and cells in culture is needed. Relatively fast and less expensive cell culture tests with the capacity to capture the toxicity observed (or not) in whole organisms also need to be developed. Regulations on nanomaterials need to be adapted to cover the toxicity observed in natural aquatic environments. However, within a regulatory framework, innovative development of nanomaterials and products containing nanomaterials should still be possible. An efficient flow of information between experimentalists, regulators and industry is needed in order to create a suitable risk management system for current and future nanomaterials.

THE FUTURE OF THE PROGRAM

The scientific outcomes of the program so far clearly indicate the research directions needed to be addressed in the future: continued development of more realistic risk assessment methods and a strategy that incorporates the effects of biocorona formation and multiple organism microcosms, as well as the development of a risk management approach to regulate nanomaterials for the market. In Phase II, the Mistra Environmental Nanosafety program will move to Lund University under the direction of Associate Professor Tommy Cedervall. Focus will be on the fate of nanomaterials in semi-natural experimental wetland systems, at simulated realistic surface water conditions and conditions during waste handling, and how this knowledge can be implemented in nanosafety regulation. Generated data will be directly applicable for risk assessments and regulatory frameworks of relevance for both industry and society, and allow for the development of industrial processes and systems that better integrate knowledge of the particle transformation effects. In addition, and in close collaboration with our industry-network, Phase II aims to develop new frameworks that can ensure the conservation and promotion of innovation friendly environments. We wish them the best for the next four year period!

JULIE GOLD

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MISTRA Environmental Nanosafety

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Gothenburg, 31 march 2019

THE MISTRA ENVIRONMENTAL NANOSAFETY PROGRAM

Risk assessment of nanomaterials
is vital for our society

The aim of the Mistra Environmental Nanosafety program is to build knowledge to promote responsible use of nanotechnology in a sustainable society. The research focus is on assessing the environmental risks of nanomaterials, assessing which properties of nanomaterials are key to address and be avoided, and how we can protect the environment from unacceptable nanomaterial emissions in the future. As such, the program addresses risk assessment on a physical and biological level, as well as the perception of risk amongst stakeholders and society at large. Together, both are needed to update risk regulation policies for nanomaterials.

A main goal of the research program is to contribute to the development of generic methods and models for making *integrated* risk assessments, particle- and material flow analyses and life-cycle assessments of nanotechnology. This image illustrates our integrated approach to studying the distribution and hazard assessment of nanoparticles in aquatic environments. We study the distribution and fate of nanoparticles (e.g. aggregation, molecular adsorption, dissolution), as well as their uptake and eco-toxicological effect on a range of aquatic organisms at various trophic levels, including effects on cell lines. Certain studies are performed in real environments (e.g. collection and detection of nanoparticles in stream beds), while other studies involve laboratory-based test models, such as standard toxicity tests as well as new models that aim to mimic natural environments and ecosystems.

The main questions addressed in this program correspond to different stages of the environmental fate of nanoparticles emitted into the aquatic environment:

- How are nanoparticles emitted into the aquatic environment?
- What happens to the nanoparticles when they are emitted into the aquatic environment?
- How toxic are the nanoparticles to the relevant organisms at different levels of the food chain?

WP1 WP2 WP3 WP4 WP5

CASE 1

CASE 2

CASE 3

THE SCIENTIFIC WORK PACKAGES

Research has been carried out in five work packages (WPs), each encompassing a different key aspect involved in the development of a nanoparticle risk assessment strategy:

WP1 - EXPOSURE, FATE AND LIFE-CYCLE ASSESSMENTS
...provides new insights on flows and transformations of nanomaterials through the technosphere, their release into the biosphere and the fate of the nanomaterials in the environment. Analytical methods are developed for both metallic and non-metallic nanoparticles (NPs). Furthermore, innovation close and NM-adaptedlife-cycle assessment methods will be developed and applied to case-studies, giving new knowledge on the environmental performance of some NMs. Work Package Leaders: Martin Hassellöv, Inger Odnevall-Wallinder

WP2 - THE MOLECULAR NANO-BIOINTERFACE
...provides new understanding on how the formation of a primary corona of environmental organic material on nanoparticles influences subsequent interactions between nanoparticles and organisms and biomolecules. Work Package Leaders: Sofia Svedhem (2014-16), Tommy Cedervall

WP3 - INTEGRATED HAZARD ASSESSMENTS
...provide specific details on the relation between physico-chemical properties and the environmental hazards of selected NPs, but will go beyond the state of the art by broadening the scope of such QSAR-analyses with respect to biological complexity. The experimental results will be for the first time systematized and condensed into a tiered scheme for an integrated hazard assessment of NPs. Contributions to an improved (eco)toxicological hazard assessment of nanoparticles, provides specific details on the relation between physico-chemical properties and the environmental hazards of selected nanoparticles, but will also broaden the scope of such QSAR-analyses with respect to biological complexity. Work Package Leaders: Thomas Backhaus, Bengt Fadeel

WP4 - SOCIETAL DIMENSIONS OF NANOSAFETY
...studies the perceptions of societal actors including industry, government, experts, and non-government organisations together with their interpretations and standpoints regarding potential risks and benefits of nanotechnology. Views on the challenging issues concerning the regulation of nanotechnology and its scientific underpinning in terms of expert based risk assessment will also be explored. Furthermore a screening level method for assessing risks of engineered NMs building on present available methods and data from the program will be developed nano – risk regulation: perception, assessment and management of risk in relation to nanomaterials, addresses the perceptions of societal actors including industry, government, experts, and non-government organisations regarding potential risks and benefits of nanotechnology. Work Package Leaders: Åsa Boholm, Sverker Molander

WP5 - NANOTECHNOLOGICAL SOLUTIONS TO ENVIRONMENTAL PROBLEM
...involves the design of novel silica nanoproducts aimed for environmental applications. The synthesis of tracker-silica nanoparticles is proposed to allow detection of silicia nanoparticles under environmentally and biologically relevant conditions. Such methodology could be an important tool to map the fate of NPs in the environment. Work Package Leader: Michael Persson

THE CASE STUDIES

The research program has been designed around specific Case studies, chosen for their particular relevance to Swedish industry. Case studies 1 and 2 are the most substantial case studies and thread their way through the scientific workpackages of the program in a multidisciplinary manner.

CASE STUDY 1: EMISSION OF NANOPARTICLES FROM AUTOMOBILE APPLICATION TO ROAD RUNOFF WATER
In roadway runoff waters during the winter season, there is high abundance of tungsten carbide (WC, often cobalt-containing, Co), which is believed to originate from the wear of tire studs. The program has developed a method for the analysis of WC particles in urban stream water, sediments and aquatic organisms, for the purpose of tracking engineered NMs in the environment, including uptake in biota. Furthermore, the tire studs product chain was used to understand how the transformation and release of WC and WC-Co NPs can be investigated and described.

Partners: Chalmers University of Technology, Karolinska Institute, Lund University, Royal University of Technology (KTH) and University of Gothenburg

CASE STUDY 2: SYSTEMATIC STUDIES OF COMMERCIALLY RELEVANT NANOMATERIALS
The program has conducted studies of systematically varying nanomaterials selected based on their industrial importance, in this case silica nanoparticles used in paper and paint products. Typically, such materials undergo product development towards new large-scale applications, a process that needs to be accompanied by safety considerations. AkzoNobel has produced a library of silica nanoparticles of varying size and surface modification for study in a variety of model test systems of environmental and human cell toxicity. The aim has been to develop a quantitative structure-activity relationship (QSAR) model for integrated model test systems, including in vitro and in vivo tests.

Partners: Chalmers University of Technology, Karolinska Institute, Lund University, Nouryon, Royal University of Technology (KTH) and University of Gothenburg

CASE STUDY 3: FUTURE NANOMATERIALS
The prime example of a new nanomaterial of interest to the program is graphene. It is also interesting as an example of a carbon-based material that is more demanding to detect in complex samples compared to, e.g., metals. This case study has been limited to life cycle analysis of nanomaterial production methods. Labeled silica nanomaterials, that will be traceable throughout model test systems, have also been developed and studied within the program.

Partners: Chalmers University of Technology

RESULTS

Chapter 2

SOCIAL DIMENSIONS OF NANOSAFETY

The view on nanotechnology and nanomaterials among the Swedish public and expert stakeholders

Potentially harmful exposure of the environment and humans to nanoparticles (NPs) and nanomaterials (NMs) is today a growing concern, engaging societal actors such as regulatory authorities, non-governmental organizations (NGOs), expert scientific bodies, market agents and industry. Due to the fact that toxicity of chemicals in nanoform is not always predictable from the toxicity of "the same material" in bulk or molecular forms, risk assessment of NMs is surrounded by considerable uncertainty. Furthermore, public understanding of risk tends to be vague regarding nanotechnology, both in the USA and in Europe [1]. Studies shows that lay audiences have limited knowledge about NPs and nanotechnology (NT), and that attitudes depend on the area of application; ambivalence combined with technological optimism and hopes of benefits can be said to dominate public opinion on nanotechnology [2][3]. Difficulties to arrive at agreed upon definitions, standardizations and protocols for risk assessment [4][5] pave the way for substantial challenges to risk communication addressing the public [6]. The future modes in which humans and objects can be refined and rearranged in accordance with NT imply that socially and culturally significant promises, threats, doubts and anxieties are incorporated in new and unexpected ways. Reflection on "current and future ethical, legal and social aspects" of NT [7] is therefore strongly called for; see e.g. [8][2], on social, cultural, historical and ethical issues of nanotechnology.

Risks to the environment and humans associated with NMs pose challenges to society for several reasons. Firstly, there is an uncertainty about causal mechanisms and probability in risk assessment of engineered NPs and NT. Secondly, methods for description, characterization and testing of NMs are still partly lacking, particularly for environmental risk assessment (ERA) [9][5], but the scientific community is learning about hurdles for NM risk assessment and how to overcome them, which is reflected in the quickly growing number of publications and collaborations in the field . For instance, assessment of toxicity of NMs is highly demanding due to the systemic and structural complexities involved in the physical-chemical-biological interactions taking place at the nano scale for any specific NM. Demanding risk assessments and high level of uncertainty make it difficult for scientists to produce policy-relevant information. At the same time, regulatory bodies ask for robust scientific risk assessment that can serve as decision support for regulatory action [9]. Although public concern about risks of NMs is generally low, except for some specific issues such as sun screens and antibacterial silver in clothes [10], public awareness might increase in the future, which then might lead to increased concerns about safety and use of NPs and NMs. The volume of NT innovation is fast growing globally and more areas of application and use emerge (including food, textiles, health, care, drugs, diagnostics, coatings, electronics, etc.), causing environmental impact through the product chains (from raw material extraction, manufacturing, consumer use to waste handling). A growing awareness of risk on the part of workers, consumers, patients, and citizens, can therefore be expected, posing increasing pressure on governments and industry regarding regulatory measures. Still, technological development continues even more rapidly, possibly increasing the distance to lagging NM risk assessment [11] and regulation [12].

In addition to scientific uncertainty surrounding risk assessment, institutional and regulatory boundaries between different sectorial areas such as work environment, food safety, environmental protection, chemical regulation, and waste regulation, constitute challenges in their own right. New risks are characterized by high uncertainty and systemic complexity and can be understood to be "transboundary" in the sense that they transcend established regulatory as well as institutional boundaries and fit poorly into traditional regulatory frameworks. NMs constitute a case in point that may call for new ways of developing regulations, possibly based on methods different from traditional risk assessments[13], and new authorities, better apt to handle complex and international product chains [12][14].

NT may give risk issues where for example environmental protection may overlap with work environment, human health may overlap with environmental protection, food safety with environmental protection, and so on, in ways where benefits in one policy area can imply risk in another domain and vice versa. Such overlapping hazard-benefit-risk issues can be expected to be extremely difficult to manage by the regulatory policy apparatus within existing and well-established sectorial divisions of responsibility and authority. A product-chain perspective in legislation may be further developed from the small seeds already in place (see e.g. the REACH legislation) that challenges the structures and functions of the existing regulatory institutions.

STUDY 1: EXPERTS STAKEHOLDERS' VIEWS ON NT AND NMS

The study [15] explores the views of expert stakeholders in government, industry and non-governmental organisations on a number of topics in connection to NT innovation with a focus on perceived risk, perceived benefits, risk regulation, and risk management. In January 2017, we distributed a web-based questionnaire to 237 individual experts at government agencies, business corporations, and other relevant organisations. The experts had a self-rated interest in, or connection to, nanomaterials and nanotechnology in their work at their organization of affiliation. This study contributes to a multidisciplinary research field addressing questions about innovation and foresight, risk perception, and regulation of nanomaterials and nanotechnology in the public domain.

MAIN FINDINGS:
The topic of NMs and NT engages a broad range of Swedish stakeholders in many different ways, including, but not limited to, research and research funding, risk assessment, product development, as well as regulation and legislation. Expert stakeholders emphasize the benefits of NT and NMs, but perceived benefit and perceived risk varies with educational background and organizational affiliation.

Perceived risk and benefit vary depending on area of application (for example medicine, cosmetics, coatings, electronics, agriculture and food). Aligned with previous research, Swedish experts prefer applications in medicine to applications in food. However, the application areas considered most beneficial are those with well-functioning existing applications, that is electronics, coatings and material production. The areas of applications considered most risky are areas of applications that include body exposure and potential large cross-over into the environment (food, cosmetics and paint).

Expert stakeholders are generally supportive of regulation of NMs and NT. They are relatively negative to taxation and self-regulation as regulatory tools, and relatively positive to selective prohibition. Labelling as a regulatory measure divides the experts, where many see it as an appropriate regulatory measure while others see it as highly inappropriate for securing safe use and development of NMs and NT. There is disagreement over appropriate regulatory measures among experts from the different stakeholder organizations.

High perceived risk correlates with a more positive attitude to regulation, and high perceived benefit correlates with lower support for regulation. This result corresponds to findings in international research. A common and shared belief is that regulation should be based on science, and that public involvement is undesirable.

STUDY 2: PERCEPTIONS OF NANOTECHNOLOGY AMONG THE SWEDISH PUBLIC

The study measured the attitudes and perceptions of nanotechnology of a representative sample of the Swedish population, age 16-85 years of age. The study was part of the questionnaire distributed through the SOM institute's yearly national survey (2017). The questionnaire was distributed to 3400 inhabitants in Sweden, and the response rate was 55 percent. The questions included factors known from international research on what affects public knowledge about, and their acceptance of, NT with respect to perceived benefit and risk. It also considered that perceptions vary with area of application of nanotechnology.

It can be concluded that the Swedish public judges itself to have little knowledge of NT, although it is a term most heard of in the media. Generally speaking, the Swedish public is positively committed to NT and treasures its benefits to society at large. The public's positive attitude toward NT is in line with previous international studies [15][16].

MAIN FINDINGS:

- 61% of respondents have heard about NT in the media during the latest 12 months. 22% have not heard about NT.
- Self-reported knowledge of NT is low. Only 10% think they have good knowledge, and the rest think they have poor knowledge of NT.
- 66% are unable to assess risks with NT and 59% do not have an opinion on the benefits of NT.
- Among the 36% who do have an opinion on risks with NT, 65% think that the risks are small or very small, while 35% think that the risks are big or very big. A non-negligible part of the population thus perceives large risks of using NT.
- Among those who do have an opinion on the benefits of NT, 80% perceive the benefits to be big or very big, while only 20% perceive the benefit to be small or very small.
- Women have somewhat higher risk perception and lower benefit perception than men. People with higher education have lower risk perception and higher benefit perception.
- Attitude to NT differs greatly depending on its use. Generally, people are more positive to the use of NT in pharmaceuticals. Acceptance for uses in cosmetics and food is lower and these areas are more controversial.

STUDY 3: LITERATURE REVIEW ON COMMUNICATION OF NANOTECHNOLOGY TO THE PUBLIC

Ongoing work is based on content analysis in research articles on NMs and NT related to public perceptions, regulation, risk assessment, and media representations [17]. The aim is to identify "state of the art" knowledge within the field: What are the main challenges? What are the main obstacles? What implications from research have been identified? What recommendations are being provided?

PRELIMINARY FINDINGS:
Public understanding of NT is based on factors other than knowledge, which has implications for risk communication. If the purpose of communication is to make people more positive about new technologies or products, a key strategy might not be to provide additional information in order to raise the level of knowledge. Public confidence in regulatory authorities and science has proved to be crucial for attitude to NT. Media reporting and ways of presenting applications of NT play a major role in influencing attitudes. Since NT is complicated, far from everyday experience, the level of knowledge is low, the applications are many and heterogeneous, attitudes among the public can be expected to be unstable (even volatile) over time. If the risk perspective is highlighted and emphasized to a larger extent in the media (due to incidents, disagreement among scientists, and emerging controversy among social actors), a shift in the general attitude can be expected, especially with regard to applications that are already perceived as questionable. In addition, ethical issues and issues about fairness and social responsibility can be relevant.

STUDY 4: LITERATURE REVIEW ON SCREENING RISK ASSESSMENT METHODS

Since a number of studies have pointed out difficulties related to assessment of risks of NMs along the same lines as chemicals [18][13] there has been ongoing work to construct simplified, so-called "screening" risk assessment methods, to fill the perceived gap. A review of these screening risk assessment methods has been performed [19]. A background on frequently used screening risk assessment approaches for chemical substances was provided, and an inclusive review of screening risk assessment methods developed specifically for NMs was performed, making it possible to also discuss and suggest some developments.

MAIN FINDINGS:
At the time of the review, it was possible to identify 19 different screening risk assessment methods in the scientific literature with five main differences: (1) The object at risk, what is at risk – occupational risk, general human health risk, and/or environmental risk? (2) The risk object, what causes the risk – NMs, NPs and/or nanoproducts? (3) In which format, or on what risk scale, are the results presented? (4) What are the kinds of input parameters required to run the method? And finally, (5) how are the input parameters transformed to the scores on the constructed risk scale?

All of the methods cover human health, either in a general sense and/or seen from an occupational health perspective, while ten of the reviewed methods solely consider occupational risks (six methods cover both). Eight of the methods include environmental risk aspects.

Regarding the risk object, most methods (14 of 19) assess NMs and NPs. Four of the methods instead assess products containing NMs and NPs – called nanoproducts, while three assess the laboratories or production processes. Given the strong focus on occupational risks in the reviewed methods, the coverage of NMs and NPs is understandable, since it can be expected that NMs and NPs are handled as such in the production, or waste handling, processes, while for the general public it will be in the form of products containing NMs or NPs in more or less bound forms.

Finally, the algorithms and transformations of the reviewed methods differ widely both regarding the number of required input parameters and the complexity of the algorithms used. Therefore, data availability will influence the possibility to perform screening risk assessments. Their applicability will furthermore differ widely for different materials, and rely on the level of knowledge about specific NMs.

STUDY 5: PROXY MEASURES FOR SIMPLIFIED ENVIRONMENTAL ASSESSMENTS OF ENGINEERED NANOMATERIALS

Given the high complexity of the natural environment, particularly regarding NPs' and NMs' interactions with a number of natural factors and organisms, it was assumed that there presently is no feasible way of performing rigorous risk assessments, and therefore simplified approaches should be investigated. As major findings from the review of screening risk assessment methods indicated, there is a need for an even simpler method that combines high, and reliable, data availability, ratio scale-based indicators and relevance for risk assessment. The investigation therefore made an inventory of available "proxy measures", which are low-data option indicators applicable in simplified assessments of environmental threats and applied them in a proof of concept to a set of manufactured NMs.

MAIN FINDINGS:
A list of 18 candidate proxy measures was developed in a literature review, covering socio-technical, ecotoxicological, fate-related and particle-specific measures. These candidates were analysed for their connection to risk and data availability, resulting in the identification of two proxy measures – global production volume (tonnes/year) and aquatic ecotoxicity (as inverted EC50, LC50, or IC50 in mg/L)). Together with limits taken from regulatory documents, a proof of concept was conducted to illustrate its application. [20]

CASE STUDY 1: METALLIC NPs FROM STUDDED TIRES

How do metallic nanoparticles released from the wear of tire studs affect the environment and human health?

The use of tungsten carbide cobolt (WC-Co) nanoparticles in winter tires studs and heavy machinery used in road works results in the release of W-rich particles in road dust, which is transported through road runoff water and creeks into the sea. The environmental impact of this nanoparticle release has been investigated in Case 1 regarding environmental fate, nanoparticle transformation and toxicity to aquatic organisms as well as in cell-based assays. In addition to these experimental studies life cycle analysis (LCA) of the impact of WC-Co has been performed showing the cradle-to-gate impact of WC-Co as well as the related human health aspects. The results from these studies are described in more details here.

MAIN FINDINGS

- 67 % of WC-Co released from tire studs is released as particles.
- Human life sparing by the use of tire studs can be questioned from an LCA perspective due to the adverse human health effects in the mining and production phase.
- An analysis technique was developed to measure very low concentrations of W in samples from natural waters.
- Sample preparation affects the NP composition greatly in when analyzing reactive metal NPs such as WC-Co.
- Increased metal release observed from metal NPs exposed to natural organic molecules. WC-Co releases Co rapidly.
- An ecocorona covering the WC and WC-Co NPs reduces their ecotoxocity.
- No critical acute or long-term effects of WC-Co NPs was detected in an aquatic food chain.
- WC and WC-Co NPs show no acute toxicity to human cells (SH-SY5Y) while Co NPs show dose dependent toxicity.

HUMAN HEALTH ASPECTS OF WC-CO PRODUCTION AND USE IN TIRE STUDS

The question of how data lean and early LCA can contribute to the environmental assessment of NMs has been addressed in the program. The life cycle assessments performed on tungsten carbide cobalt alloy [21][22] comprise a conventional cradle-to-gate LCA (a study excluding use and waste phases) as well as a study focusing on human health impacts using the indicator Disability-Adjusted Life Year (DALY) that can be applied in LCAs.

The cradle-to-gate study [22] was designed to provide life cycle inventory data for further use in cradle-to-grave-studies, to include a comparison of Chinese and non-Chinese production and a life cycle impact assessment covering 8 impact categories (global warming potential, acidification potential, eutrophication potential, smog potential, ozone depletion potential, net water consumption, primary energy requirement and waste generation).

The study shows that a small number of flows contribute the dominant part of the total impact categories (e.g. the flows of kerosene, sulfidic tailings, water (use during mining) and electricity). Recycling was shown to greatly reduce the impacts, which comes as no surprise. Furthermore the non-Chinese production is using less energy than the Chinese production.

The use of tire studs is controversial in Scandinavia and debated because of human health impacts from increased road particle emissions. Does the use of tire studs in a Scandinavian studded passenger car actually avoid or cause health impacts from a broader life cycle perspective? The study focussing on human health impacts using the indicator Disability-Adjusted Life Year (DALY) [21] has shown that the health benefits in the use phase in general are outweighed by the negative impacts during the life cycle. The largest contribution to these negative human health impacts are from the use-phase particle emissions (67–77%) and occupational accidents during artisanal cobalt mining (8–18%). About 23–33% of the negative impacts occur outside Scandinavia, where the benefits occur.

The LCA-studies on the net human health impacts of WC-Co in tire studs, on synthetic diamond production and on the comparison of tools based on WC-Co or synthetic diamonds will together provide a strong answer to the question "How can data lean and early LCA contribute to the environmental assessment of NMs?" We clearly need to take into consideration assessments, comparisons and recommendations coming out of these and other LCA analyses on the use of nanomaterials in specific large scale applications. For example, the methods developed and applied in the prospective LCAs on graphene nanomaterials ([23][24][25] (see Case Study 3) also contribute to this question.

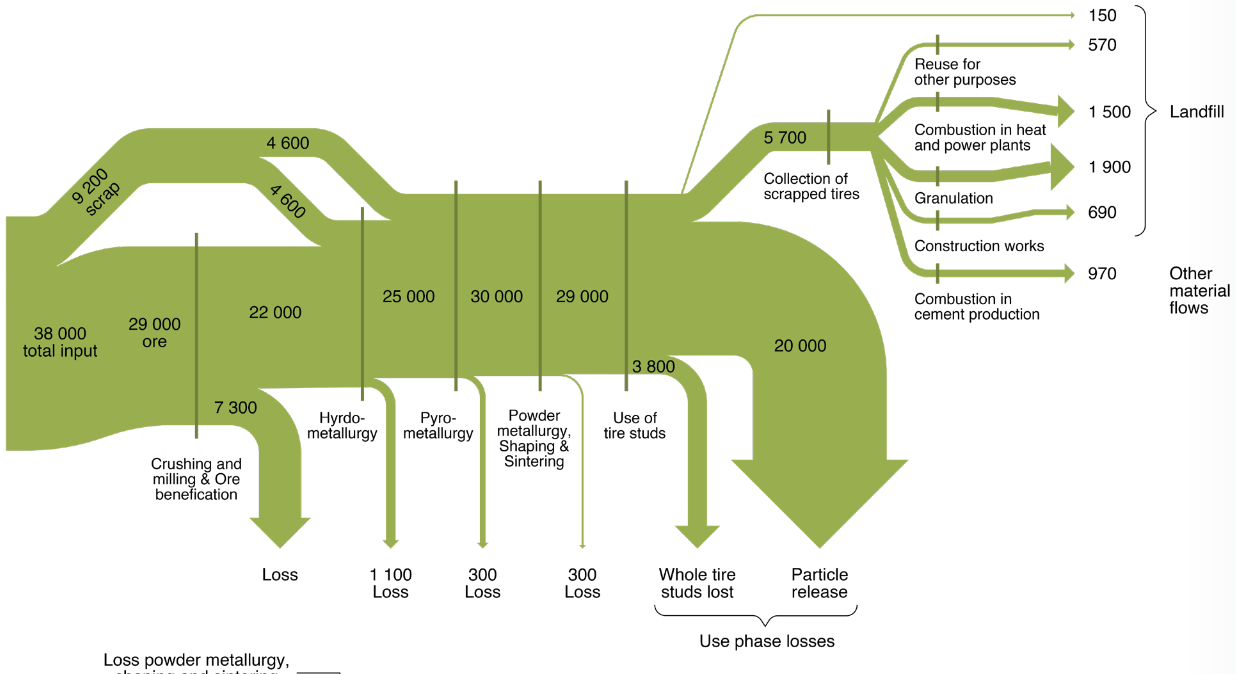
MATERIAL, SUBSTANCE AND FLOW ASSESSMENTS OF ENGINEERED NANOMATERIALS

Studies in the program have been performed applying material, substance and particle flow assessment concepts in order to find answers to the following question posed in the initial proposal:

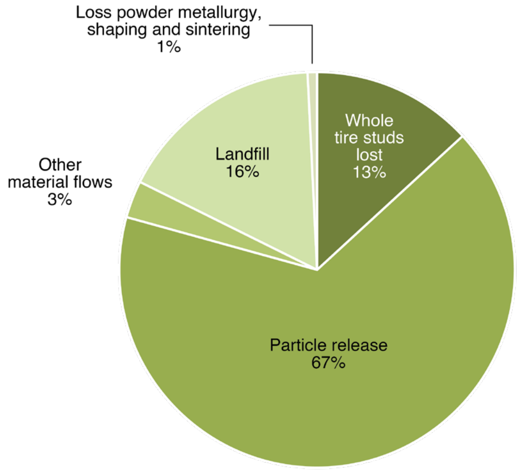
How can knowledge of engineered NM type, production rates, composition of materials and products together with particle transformations be accounted for to obtain more relevant estimates of engineered NM release, and which metrics (mass/particle number/surface area) should be used to best underpin fate, exposure, effects and risk assessment of engineered NMs?

The material flow analysis (MFA) performed approached the overarching questions with a double strategy – to perform a review of material flow studies [26] and to use the Case Study 1 on tungsten carbide as a vehicle to address the questions. The studies on tungsten

A.



B.



a. Flows of tungsten related to the use of tired studs in Sweden for a base case year 2015 [kg/year].
b. The distribution of the tungsten flow into the identified flow categories. [28]

carbide [27][28][29] give complementary information, and provide an in-depth study of the tungsten carbide flows related to use of studded tires in Sweden.

Many flow studies aim at estimating flows to waste treatment (e.g. incineration or landfills) and emissions to particular environmental compartments (e.g. via wastewater treatment plants) but given the general lack of data regarding flows from production, to products and further regarding the handling of waste streams and environmental emissions the studies deliver uncertain and partly varying results. We have to conclude that the present state-of-the-art of these methods does not support more advanced conclusions, and that the question posed is still open. The availability of data is the bottleneck, and this is due to producers' unwillingness to share production data.

Our MFA studies of tungsten carbide flows [26][[28][21] aimed at quantifying its dissipation rate and functional recycling, since tungsten is a scarce element and resource considerations therefore critical, and to assess the magnitude and relative significance of WC-Co nanoparticle release, which is related to possible environmental risks.

The results [28] are based on a steady-state mass-balance flow model. Together with the utilization of mass balances, an estimate of the annual inflow of tungsten in tire studs to the use phase and an estimate of the flow of tungsten going out from the use phase, enabled a quantification of dissipation from the use phase, and also upstream and downstream flows related to the use phase.

The results are in accordance to the general picture regarding the paucity of some data, giving high uncertainties in some respects. However, investigating a particular case, the use of tungsten carbide cobalt (WC-Co) in Sweden, makes it possible to find data that indirectly makes it possible to produce results of relevance for the posed questions. The main results (shown in figure above) indicate that the overall dissipation is 100% and that 80% of this occur during the use of studded tires (67% is released as particles and 13% is lost as whole studs).

We conclude that recovery of tungsten during waste management, research on alternative solutions to WC-Co in tire studs, and environmental risk assessments of the release of WC-Co nanoparticles are warranted.

EXPOSURE AND ENVIRONMENTAL FATE OF ENGINEERED NANOMATERIALS

Tungsten is an element with high atomic number and may thus be distinguished from naturally abundant elements that typically have much lower atomic numbers. Combined with an anthropogenic-dominated release mechanism, W-containing nanoparticles offer a unique setting in which nanomaterial analytical techniques and modeling approaches can be developed and validated.

Technological solutions for the determination of realistic exposure levels and fate of engineered nanomaterials in surface waters have been developed and implemented in the program including the following steps:

1) Review and critical evaluation of analytical techniques that may be used for nanomaterial detection and characterization in natural samples; 2) development of a sampling and analytical strategy using state-of-the-art equipment; 3) method development for analyzing samples collected from road dust, road runoff water, creek water, and sea water; 4) review current literature on the fate of engineered nanomaterials in seawater; and 5) conduct experiments to close scientific gaps.

The task faced a great challenge: the rarity of our target material. Most scientific work on the release and fate of engineered nanomaterials is based on spiking a water medium with a known concentration of model nanomaterials. The concentrations of nanomaterials used in these studies unavoidably need to be higher than the limits of detection of the analytical equipment used. As a result, the vast majority of these studies use relatively high concentrations, in the range of 0.1 – 10 mg/L. We have dealt with a real-world scenario, where the concentration of our target nanomaterial is 3 to 6 orders of magnitude lower than this range. Furthermore, natural samples contain a multitude of natural inorganic (e.g. clays, iron oxides, etc.) and organic particles (e.g. humic matter, fibrils, microorganisms, etc.) of various sizes ranging from a few nanometers to several micrometers. Natural particles are far more abundant in surface waters than engineered nanomaterials and their ratio may be 1,000:1 and often much higher. In addition, we did not artificially spike our samples; rather the release took place through normal use of winter tires and heavy machinery, so the shape and composition of our target material is unknown, but realistic. In order to deal with this challange we employed sampling techniques based on the extensive experience gained in the past in the environmental nanochemistry group (stormwater, surface water and sediments) and pushed the limits of state-of-the-art analytical equipment.

As a result, we developed a method for detecting and characterizing W-rich nanomaterials in natural waters. This method is reliable and can be used to analyze multiple samples with a high throughput, which makes it applicable for routine environmental monitoring purposes. The approach is based on sampling using membrane filters, and custom-made protocols and analysis using automated scanning electron microscopy with energy dispersive X-ray spectroscopy on the filters, and single particle inductively coupled plasma mass spectrometry on the filtrates. We have optimized conditions and materials for analyzing water samples from various sources, including low salinity and high particle content creek water, and high salinity, low particle content sea water. [30]

Further research will contribute with knowledge on the fate of metal nanoparticles in the environment and whether they are in a toxic form or not. This enables the prediction of hazards towards aquatic organisms.

UNDERSTANDING TRANSFORMATIONS OF METALLIC NANOPARTICLES IN REAL AND MODEL TEST SYSTEMS

In a collaborative effort, researchers in the program have addressed the question of how a generic, in-depth, mechanistic understanding of changes in speciation, stability, mobility, adsorption and complexation of metal-containing NPs, and their released ionic species in different aquatic settings, can be achieved.

EFFECTS OF METALLIC NANOPARTICLE DISPERSION TECHNIQUE

A main effort in the characterization of nanoparticles in the program has been how to prepare particle dispersions of reactive metals that rapidly transform/dissolve in contact with an aqueous medium.[31] Standardized protocols exist that stipulate procedures with sonication to prepare dispersions of nanoparticles (NPs) for further testing, e.g. ecotoxicology. However, these protocols are elaborated for non-reactive or poorly soluble particles and are not directly applicable for reactive and more soluble particles. They do not take into account the fact that the sonication step largely influences the particle reactivity and dissolution tendency. These aspects have been elucidated for particles of noble and base metals that have very different transformation/dissolution properties due to inherent physico-chemical differences and barrier properties of their surface oxides (shell-core-properties). These aspects, combined with strong van der Waals forces, result in rapid changes in particle size, agglomeration behavior, dissolution and rate of sedimentation. Depending on the metal and its surface oxide characteristics and the influence of the sonication step, these reactions take place already when preparing stock solutions of metal particle dispersions.

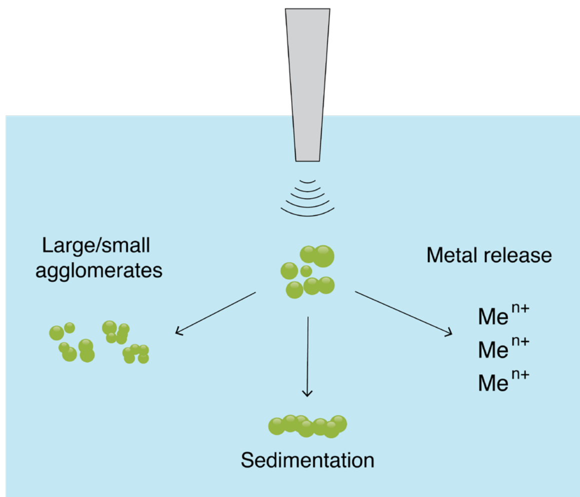
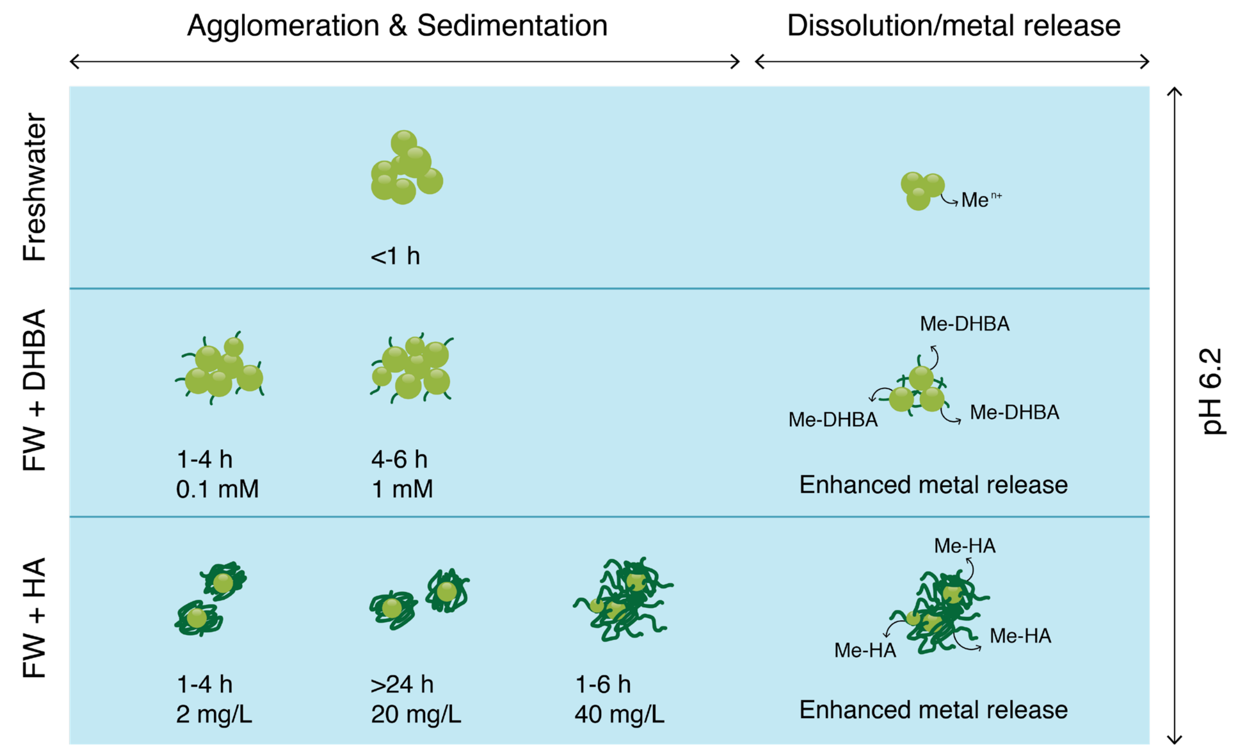


Illustration showing how sonication affects the solubility and dispersion of metal nanoparticles and the release of metal ions (Me^{n+})

As a consequence, we have shown that the administered dose of particles for further investigations is substantially lower (30-70% lower) compared with the nominal particle dose, and that NP dissolution occurs already during sonication. Standardized protocols propose bovine serum albumin to be used for particle stabilization (reduces the extent of agglomeration). However, our studies show that their presence largely influences the dissolution characteristics of the metal NPs and is highly metal specific. Except for detailed physico-chemical particle- and surface characterization, the sonication time needs to be minimized, the administered dose needs to be quantified and the particles well characterized.[31]



Increased metal release from metal NPs when exposed to DHBA or HA.

METALLIC NANOPARTICLE TRANSFORMATIONS

Changes in surface charge of particles (the zeta potential), surface oxide characteristics and transformation/ dissolution properties of metal NPs are some key factors that need to be characterized in order to understand their environmental fate.[32] Our study on the zeta potential of metal NPs describes common pitfalls for such measurements, connected to presence of biomolecules, dissolving NPs (increasing ionic strength), agglomerating NPs, and solutions of high ionic strength. Our results show that neglecting these aforementioned aspects renders the zeta potential determinations erroneous and hence also any conclusions on e.g. environmental fate and interactions with organisms.

Research has been performed on a broad set of metal NPs of different surface reactivity (Cu, Mn, Al) with the aim to investigate changes in reactivity upon environmental contact and how these differences in environmental transformation/ dissolution characteristics relates to toxicity. Our results show that oxide characteristics in terms of electrochemical reactivity (nobility), crystal structure and composition, are closely related to the induced toxicity towards human lung cells. [33]Investigations have also been conducted to elucidate how the presence of natural organic matter (NOM) in simulated surface water interacts with metal NPs (Cu, Mn, Al) of different reactivity and how these interactions influence their transformation/dissolution characteristics, agglomeration/sedimentation behavior as well as chemical speciation of released metals, as this largely governs their bioavailability.[34] From an environmental fate and risk assessment perspective, the results imply that Cu, Mn, and Al NPs dissolve relatively rapidly in the presence of NOMs such as humic acid (HA) and DHBA (dihydroxybenzoic acid) and rapidly agglomerate and sediment.

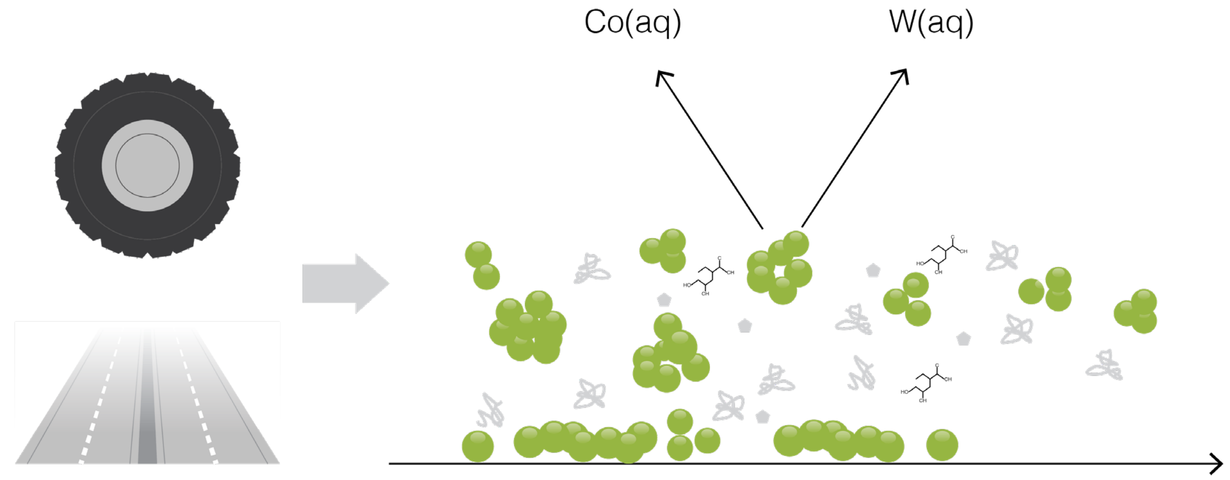
Any NP-specific risks (e.g. increased toxicity) are therefore expected to be limited to the vicinity of the dispersion source of the NPs. With no or very little NOM present, the release is much slower, but at the same time there is less stabilization of the NPs, hence limiting their mobility. Dissolved Al and Cu further rapidly form strong complexes with NOM, reducing their bioavailability, however this is not the case for Mn. Independent of interaction with NOM, the particles readily agglomerate and sediment in surface water.

FATE OF TUNGSTEN CARBIDE COBALT NANOPARTICLES

In Case Study 1 the wear of tire studs has been studied, with the aim to assess environmental interactions and fate of tungsten carbide (WC) and tungsten carbide cobalt (WC-Co) NPs dispersed into surface water settings. This has been a collaborative effort together with studies on the ecotoxicity of the same NPs. The investigated particles, both nano- and micron-sized, are relevant at realistic conditions as they have been observed in the environment adjacent to traffic areas and are known to originate from, for example, wear of studded tires.

Scarce data is reported in the scientific literature on the aquatic toxicity and/or the environmental dispersion of such particles. The WC NPs and WC-Co NPs have therefore been well-characterized from a surface and particle characteristic perspective, as well as on the extent of particle agglomeration/sedimentation, transformation/ dissolution and interactions with synthetic surface water with and without of natural organic matter.[35][36] NPs of Cu and Co NPs were included for comparison. [36]

WC NPs in surface water exhibited rapid sedimentation, slow dissolution, and very little interaction with NOM. This indicates a limited transport and mobility of these WC NPs upon dispersion in surface water. The lack of interaction and dispersion stability upon interaction with NOM is connected to the properties of the surface oxide formed on the WC NPs. The smallest fraction of the WC NPs was however more stable in solution and sedimented at a slower rate compared with the larger fraction. These smallest NPs are hence more prone to be mobile and transported into different aquatic settings of the environment and should be considered in future studies. The WC-Co released almost the entire Co content within 24 h, which is something to consider since Co soluble species may, under some circumstances, be toxic.



Wear of tire studs releases Co(aq) and W(aq) to surrounding water.

ENVIRONMENTAL CORONA: ECOCORONA

An initial characterization of NOM binding showed that humic acid and dihydroxy benzoic acid did not bind at biologically relevant pH to tungsten carbide (WC) nanoparticles. [35, 36] Simultaneously, experiments were carried out on protein binding in the presence of humic acid and dihydroxy benzoic acid at neutral pH. In accordance with the initial studies, we found no differences in either protein affinity or structure, likely because there were no interactions between humic acid and dihydroxy benzoic acid with the nanoparticle surface.

Based on these results we decided to produce two new, more complex, but biologically more relevant ecocoronas. One consisted of the breakdown products from algae and the other of excreted biomolecules from zooplankton.

ALGAE ECOCORONA

The algae ecocorona was produced by digestion of 20 or 40 liters of algae by zooplankton (*Daphnia magna*) during about 10 days. Zooplankton and larger debris were removed and the breakdown products were concentrated by strong anion exchange chromatography using 1M NaCl to elute the biomolecules. The concentrate was extensively dialyzed against milliQ water and frozen in aliquots. The UV absorbance spectrum was similar to those previously reported from lake water, suggesting that the carbonic material was similar and supporting the idea that the algae ecocorona mirrored a relevant natural environment. Moreover, NMR analysis showed that the prevalent molecules in the corona are short fatty acids and a so far unidentified molecule with at least four carbons with a methyl (CH3) group connected to a methine (CH) with a hydroxyl (OH) or nitrogen and two additional consecutive methine groups with unknown substituents. The full chemical structure of the algae ecocorona is still to be determined.

The effects of this algae corona on the toxicity of WC-Co and cobalt particles was tested on zooplankton. Interestingly, the long-term toxicity test, where the particles covered by an ecocorona were presented to zooplankton, showed that the ecocorona extended the lifetime of the animals, i.e. reduced the toxicity. Furthermore, the effect on reproduction was examined by counting the number of offsprings. Following these results, we have examined in what way the ecocorona reduces the toxicity. We found that neither the dissolved cobalt nor the bioavailable cobalt differs between particles covered and not covered with ecocorona, which excludes those two possible explanations. It was further determined that carboxyl groups bind to the surface of the cobalt, which correlates well with the presence of fatty acids in the ecocorona. This suggests that the protective effect is due to differences at the surface of the particles.[38]

Preliminary results show that the algae ecocorona also delays the acute toxic effect of 50 nm aminated polystyrene on zooplankton. The mechanism behind the delay is not yet known, but the ecocorona stabilizes the particle dispersion in the test media, suggesting that reduced aggregation makes the particles less toxic.

INFLUENCE OF BIO/ECO-CORONA ON AQUATIC ORGANISMS AND TRANSFER THROUGH A FOOD WEB

The toxicity of nanoparticles on different aquatic organisms has been tested in the program, where all organisms were part of an aquatic food chain. A combination of study organisms has been used originating from different trophic positions in the aquatic food web. For Case Study 1, studied organisms include algae (*Scenedesmus sp.*), zooplankton (*Daphnia magna*), a benthic isopod (*Asellus aquaticus*) and a fish, crucian carp, (*Carassius carassius*). Different methods have been implemented to identify the potential effects caused by the nanoparticles on aquatic wildlife. Acute toxicity studies lasting for 24-48 hours have been combined with long-term toxicity tests where organisms were monitored during the course of their whole life span. Exposure scenarios have included direct exposures (i.e. particles added directly to the organisms) and exposure where particles are transferred through a simplified aquatic food chain. For food chain transfer, we have added particles to algae, which have then been eaten by zooplankton, which in turn has been fed to fish.

Testing has focused on the metallic nanoparticles related to the materials used in car tire studs, namely tungsten carbide (WC), tungsten carbide cobalt (WC-Co) and cobalt (Co). Testing on the metallic nanoparticles in the food web clearly shows the strength of combining expertise from different academic partners and enabling focus on more aspects within a single study.

ACUTE AND LONG-TERM TESTING

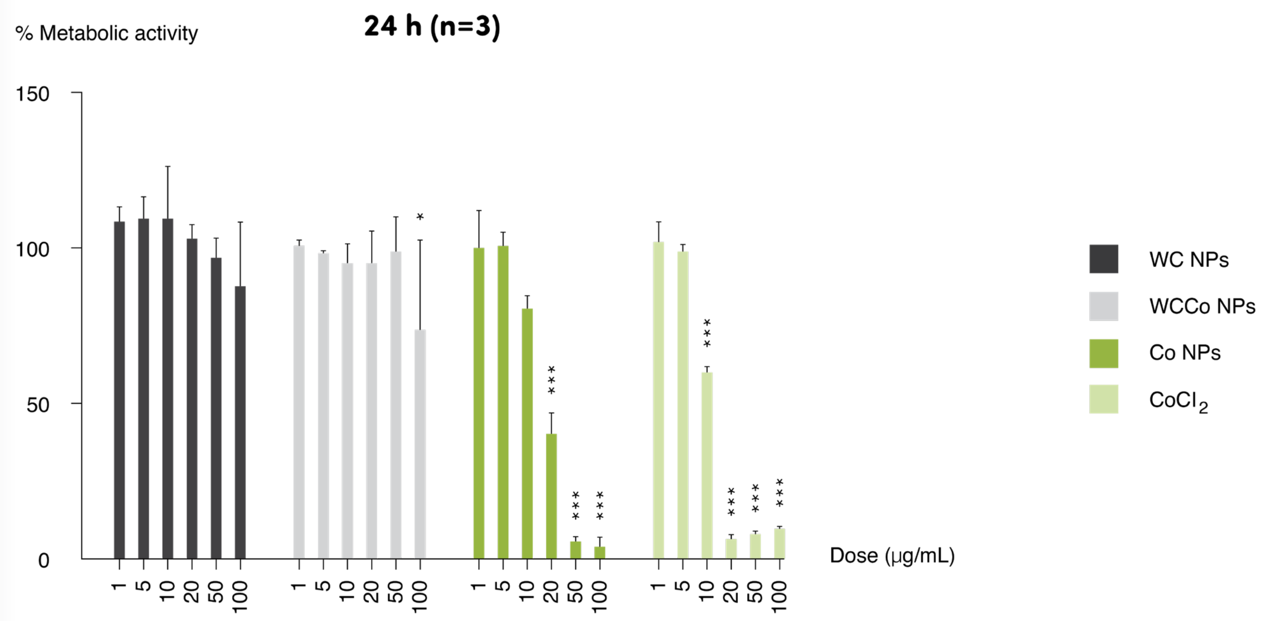
Acute (24 h) exposure of *D.* magna to the metallic nanoparticles revealed no effects on survival. To confirm exposure during the 24 h experiment, the amount of WC taken up by Daphnia was analyzed and it was concluded that the organisms indeed accumulated WC during the exposure. Increasing the exposure time, following the individuals during their whole life, revealed a reduction in survival in all three metallic nanoparticle treatments compared to an unexposed control treatment. For WC nanoparticles, long-term exposure to the particles resulted in an increased time to the first reproduction. During the characterization of the WC particles, it became evident that the particles sediment very fast. Based on the fast sedimentation, we decided to include an additional exposure scenario focusing on benthic (bottom living) organisms and exposed the isopod *A. aquaticus* to WC for two months. We found that *A. aquaticus* accumulated WC during the exposure, however, we could not find any effects on either survival or reproduction, nor on leaf decomposition, an important ecosystem service provided by *A. aquaticus*.

As no acute lethal effects could be observed we also performed a behavioral assay on *D. magna* following acute (24 h) exposure to WC nanoparticles to assess any sublethal effects on the natural migration behavior. No effects on the migration behavior could be detected after 24 h of exposure.[29][35]

The observed differences when comparing acute and long-term testing as well as the difference in response when comparing aquatic species lead us to conclude that there is a need for more long-term testing. This test should also include more species and quantify their potential exposure situation in order to get a more detailed picture of how particles could affect aquatic organisms once released into nature.

FOOD CHAIN TRANSFER OF PARTICLES

Two experiments focusing on the exposure of nanoparticles through trophic transfer of WC nanoparticles have been conducted. Organisms were exposed by introducing particles together with algae, that were then eaten by zooplankton, which were then given as food to crucian carp (*Carassius carassius*). No effects on the survival of crucian carp could be seen after exposure to WC introduced via the food chain.



Non-differentiated SH-SY5Y cells were exposed for 24 h to the indicated nanoparticles or to CoCl2 and cell viability was assessed by using the Alamar blue assay. Nanoparticles were provided by KTH.

INTEGRATING THE DEMANDS FOR HUMAN HEALTH AND ENVIRONMENTAL ASSESSMENTS: IN VITRO HUMAN CELL TOXICITY TESTING

The metallic nanoparticles in Case Study 1 have also been studied to assess human cell toxicity. We have applied conventional *in vitro* cell model systems as well as high-throughput-based approaches. The test materials were carefully characterized in the respective cell culture media and we have also evaluated the impact of the bio-corona on toxicity. We found that the cytotoxicity of cobalt nanoparticles is likely to result from the release of cobalt ions. Tungsten carbide and tungsten carbide-cobalt nanoparticles, on the other hand, were non-cytotoxic in the model that we have used.

METAL NANOPARTICLE EFFECT ON DIFFERENTIATING HUMAN CELLS

Cytotoxicity testing has focused on the tungsten carbide (WC), tungsten carbide cobalt (WC-Co) and cobalt (Co) metal nanoparticles. Tungsten carbide and tungsten carbide cobalt nanoparticles have been studied previously in different cell models [39][40][41]. However, it is not known whether these nanoparticles could impact on cell *differentiation*, nor their toxic effects during different stages of differentiation. We selected the human neuroblastoma-derived cell line SH-SY5Y as a model of a differentiating cell type. These cells can be differentiated with retinoic acid resulting in cells with cholinergic and dopaminergic neuronal properties. We evaluated the potential cytotoxicity of the nanoparticles as well as CoCl2 as an ionic control under three different scenarios: non-differentiated SH-SY5Y cells, differentiated SH-SY5Y cells, and SH-SY5Y cells undergoing differentiation by retinoic acid exposure during exposure to particles.

We found that the WC nanoparticles and WC-Co nanoparticles were not cytotoxic, whereas Co nanoparticles triggered a pronounced dose-dependent cytotoxicity with similar results seen for the CoCl2 salt. The differentiating cells were more sensitive to cobalt than the non-differentiated or already differentiated cells. A manuscript containing these results is currently in preparation: "Neurotoxicological evaluation of cobalt-impregnated tungsten carbide nanoparticles versus cobalt nanoparticles", G. Gupta, A. Gliga, J. Hedberg, A. Serra, D. Greco, I. Odnevall Wallinder, B. Fadeel. The same particles and cobalt salt have also been evaluated in a zebrafish embryo model in order to determine whether the results that we have obtained in cell culture would translate into *in vivo* effects. These results are still in the process of being analysed.

CASE STUDY 2: COMMERCIAL SILICA NPs

While silica NPs are benign in ecotoxicological models, surface chemical modification can mitigate observed effects in cell culture

Manufactured silica nanomaterials are amongst the nanomaterials with the highest global production volume (several 10 000 tons/year) and are widely used in numerous applications in society such as paints, coatings, cosmetics, textiles and food. Consequently, these nanoparticles were selected as one of the priority substances by the OECD Working Party on Manufactured Nanomaterials (WPMN) in 2010 as a part of the global effort to increase our understanding of the safety aspects of nanomaterials. The WPMN concluded that silica nanomaterials are not yet studied sufficiently, and only few relevant publications were identified. Indeed, in a meta-analysis of 38 published toxicity studies of silica nanomaterials, Schrurs and Lison [42] concluded that consistency across different studies was poor thus precluding any firm conclusions with regards to the potential toxicity of this class of nanomaterials. On the other hand, due to the high abundance of silica in the earth crust and its wide use in commerce since many decades, silica nanomaterials are often perceived as being non-toxic.

In order to increase our understanding of the potential environmental hazards and risks of these nanoparticles, researchers in the program have conducted risk assessment on a series of systematically varying silica (SiO_2) nanoparticles. Our commercial partner Nouryon has produced a library of silica nanoparticles of varying size and surface modification for study in a variety of model test systems of environmental and cell toxicity. The aim was to develop a quantitative structure-activity relationship (QSAR) model for integrated model test systems, including *in vitro* and *in vivo* tests. However, since these silica particles were found to be non-toxic in half of the model systems tested, it was not possible to perform the integrated QSAR modeling. Instead, experiments on metallic nanoparticles and subsequent QSAR modeling are underway. Here we summarize some of the main findings to date on the characterization and risk assessment of SiO_2 nanoparticles.

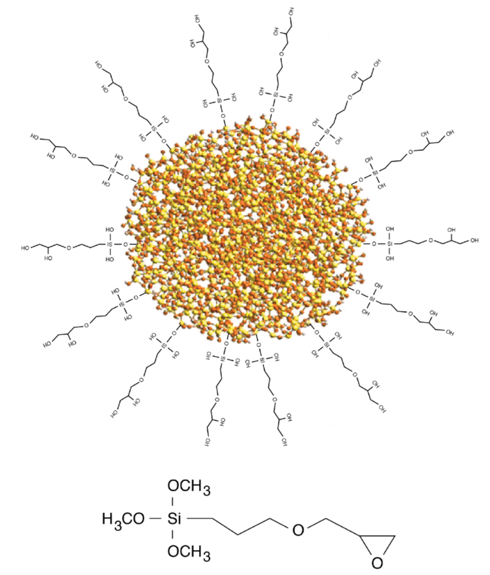
MAIN FINDINGS

- Colloidal silica NP library produced with different sizes, surface chemical modifications (bare, Al, silane), and with/without fluorescent core
- NP characterization by SEM, TEM, DLS, CLS, ES-DMA and zeta potential gave particles ranging from 7nm to 100nm, and -11mV to -39mV
- No dissolution, agglomeration or sedimentation of silica NPs observed in aqueous solutions
- Surface negative charge in salt solution increases as silica NP size decreases
- No surface interaction with natural organic matter detected experimentally with silica NPs
- Protein exchange (Vroman effect) observed on silica NPs. Protein binding differs for curved versus flat NP surfaces. Protein adsorption on single NPs monitored in real time using nanoplasmonic sensing.
- No acute toxicity detected in the aquatic organisms algae, bacteria, zooplankton
- Cell culture of fish gill, human lung and human immune cell lines show total surface area and surface chemistry determining cytotoxicity for bare NPs. Smaller NPs are most cytotoxic and silane coating mitigates toxicity. Cytotoxicity only observed at very high NP concentrations.
- Blood protein or lung surfactant biocoronas can either promote or reduce silica NP cytotoxicity observed in human lung, gut and immune cell lines

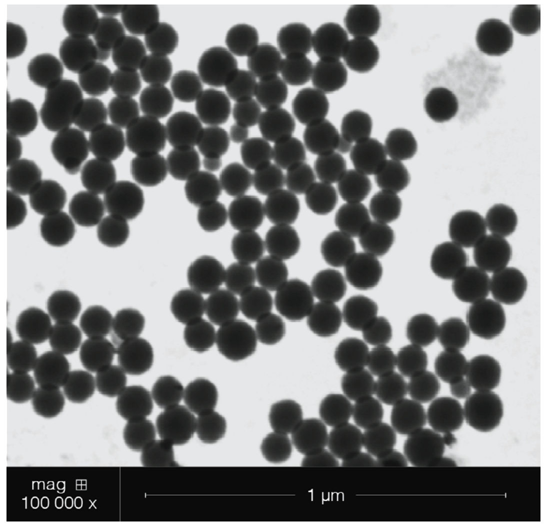
PRODUCTION AND CHARACERIZATION OF COLLOIDAL SiO_2 NPs

A LIBRARY OF SYSTEMATICALLY VARYING SILICA NANOPARTICLES

Commercial silica nanoparticles were custom-made with systematically varying properties and separated into two series. The Series A of silica nanoparticles were based on the same silica nanoparticle, having a specific surface area of 360 m^2/g corresponding to a mean particle size of 7 nm (equivalent spherical diameter - ESD, which is the mean particle size calculated from specific surface area assuming monodisperse spherical particles). Three different surface chemistries were prepared on these nanoparticles in order to modify their surface charge density: Unmodified (bare) silica; silica with a surface modification of aluminum substituted into the silica structure; and silane modified silica. The silane modified silica particle is sterically stabilized with glycerol-propyl tails. With the steric stability, the particles are colloidal stable at increased electrolyte content. Zeta potentials of the silica nanoparticles ranged from -11mV to -39mV. Series B of silica nanoparticles were unmodified colloidal silica of three different sizes: 7, 25 and 100 nm ESD. An example of the 100 nm colloidal silica particles produced and used in the program is shown in the adjacent SEM image. See the adjacent tables for a summary of the properties of the particles.



Schematic image of silane modified silica, and chemical structure of glycidoxypropyltriethoxysilane



TEM image of colloidal silica nanoparticles studied in this program (100 nm, 30 m^2/g). Scale bar 1 µm.

PRODUCTION OF LABELLED SILICA NANOPARTICLES

Various methods of labeling silica nanoparticles have been discussed in the Mistra Environmental Nanosafety program in order to be able to detect and monitor the fate of the particles in environmental and biological test models. The main experimental focus regarding labeling of silica nanoparticles has been on fluorescent silica nanoparticles.

The silica nanoparticles were produced having organic fluorophores FITC or RITC incorporated into the silica nanoparticle core. Trapping of fluorophores inside the particle has the benefit of reduced dye leakage. This preconjugate is then incorporated in a silica matrix formed during the hydrolysis and condensation of TEOS in a mixture of water, ethanol and ammonia, thus forming fluorescent colloidal silica. Particles were then coated with an additional TEOS layer. This second coating of TEOS, or water glass, also made the particle suspensions more homogenous, i.e. less aggregated, which seemed to imply that an additional silica layer rendered the particles more stable. The synthesis resulted in particles with the main fraction having size around 40 nm. By using water glass for the outer surface layer, the surface chemistry was very similar to the commercial silica and to the silica nanoparticles already used in the program. The fluorescently labeled silica nanoparticles have been used in nanoparticle uptake studies of cell culture models (see below) and zooplankton, and some of this work is still in progress.

FATE OF SiO_2 NANOPARTICLES IN AQUEOUS SYSTEMS

The silica nanoparticles studied in this program showed no agglomeration or sedimentation in any experimental model system, regardless of surface modification. No dissolution of particles was observed, however only particle size was measured before and after experiments. We cannot exclude the possibility of some particle dissolution, especially for the smallest silica particles. This could be investigated further in the future.

Experimental studies have been performed to describe the interaction of both small and large organic molecules naturally found in runoff waters, streams and lakes and how they interact with silica nanoparticles depending on their size and surface charge, as well as pH and salt concentration. It was found that the presence of the small molecule dihydroxybenzoic acid (DHBA) in the aqueous solution significantly affects the surface charge of silica nanoparticles at environmentally relevant pH values. [34] However, characterization of the interactions of the natural organic matter DHBA and humic acid with nanoparticles showed no binding to silica at biologically relevant pH. [34] They had a negligible effect on the silica nanoparticle size distribution. Neither molecule induced agglomeration or sedimentation of the SiO_2 NPs during the 21-day test period. [34] The SiO_2 NPs are more stable than metallic NPs and therefore more mobile in aqueous solutions, and could, if dispersed in a given environmental exposure scenario, potentially be transported to other environmental aquatic settings.

NANOPARTICLE FATE MODEL BASED ON NEW THEORETICAL APPROACHES

To compliment the experimental studies [34, 37], theoretical models that describe the surface charging of silica nanoparticles of various sizes at different pH as well as in different salt solutions, as well as the interaction of organic molecules with the silica surface, have been developed. The fate models employ a multilevel and novel modeling approach and include using Monte Carlo (MC) and molecular dynamics (MD) simulations and analytical theory. The modelling was divided into four stages: (i) behavior of model organic molecules in aqueous solution, (ii) adsorption of organic molecules on planar surfaces, (iii) adsorption of organic molecules on single particles and (iv) aggregation of multiple particles. The first three parts are simulated using molecular dynamics (MD), whereas the last one involving Monte Carlo (MC) simulations.

We have applied the Corrected Debye Hückel (CDH) theory for predicting the surface charging behavior of silica nanoparticles of various sizes in different salt solutions as well as at different pH values. Based on the information gained by theory along with experimental data, we developed MD model of charged silica surfaces. The predictions from this theory are that the surface charge of silica nanoparticles in a salt solution increases as the particle size decreases [43]. Furthermore, the density functional theory (DFT) was used to calculate the pKa values of natural organic matter: 2,3-dihydroxybenzoic acid (DHBA), phthalic acid, trimelltic acid and Suwannee river fulvic acid (SRFA).

Series A Silica nanoparticles: Same particle size/different surface chemistries

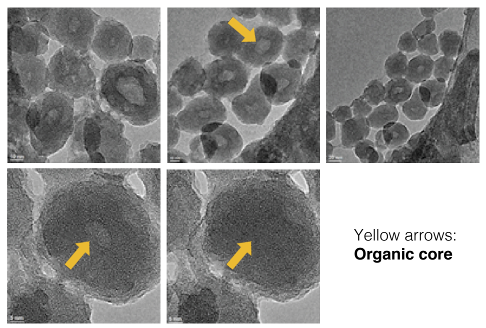
Silica	Particle size ES-DMA number mean (nm)	Specific surface area (m2/g)	Equivalent spherical diameter* (nm)	Counter ion: Na (wt-%)	Relative charge density	Colloidal stability at neutral conditions
Bare silica	16	360	7	0.60	Medium	Limited
Al-modified	16	360	7	0.60	High	Improved
Silane modified	16	360	7	-	Low	Sterically

**equivalent spherical diameter – ESD, i.e. mean particle size calculated from specific surface area assuming monodisperse spherical particles*

Series B Silica nanoparticles: Different particle sizes/same surface chemistry

Particle size: ES-DMA number mean (nm)	Specific surface area (m2/g)	equivalent spherical diameter* (nm)	Counter ion: Na (wt-%)
16	360	7	0.60
34	130	100	0.18
100	30	7	0.10

**equivalent spherical diameter – ESD, i.e. mean particle size calculated from specific surface area assuming monodisperse spherical particles*



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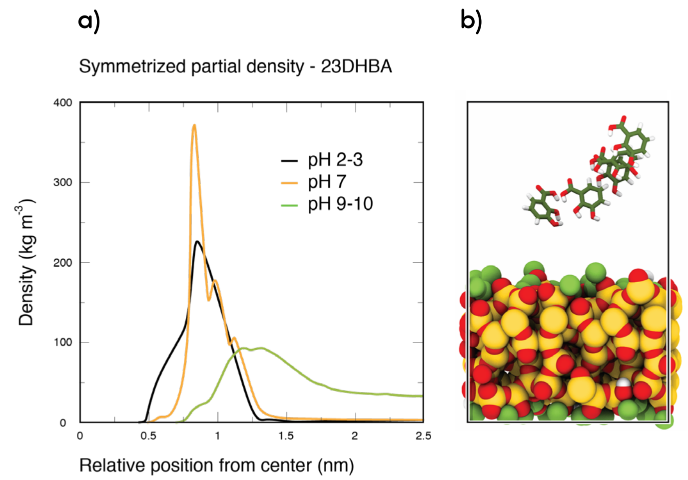
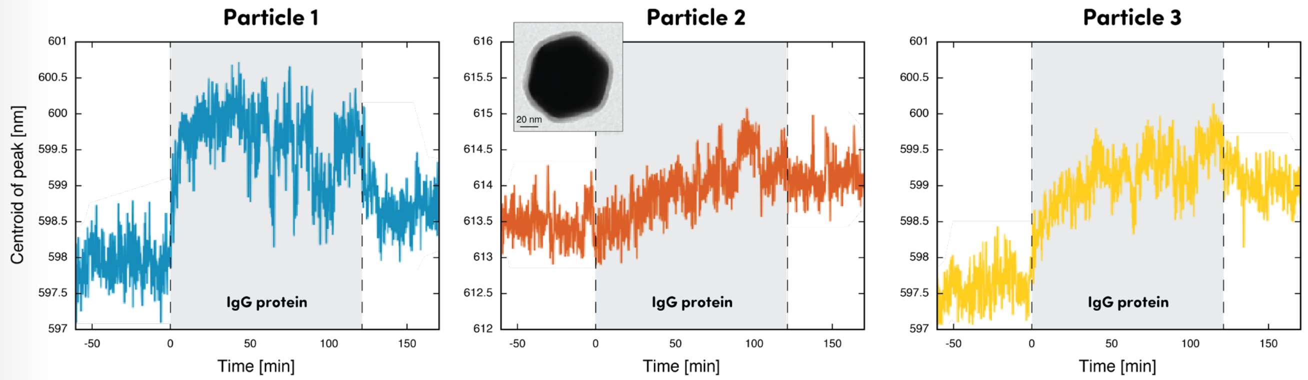
TEM images of fluorescent silica nanoparticles, ca 40 nm in diameter. Arrows point to the organic core of the particles. A water glass coating is visible surrounding the organic core.



(a) Partial axial density of organic molecules as a function of distance from the center of silica as a function of pH.
(b) Snapshots from MD simulations showing simulation box with silica surface, organic molecules and ions. [43]



Preliminary unpublished data showing the temporal evolution of the IgG protein corona formation kinetics on three nominally identical Au core–SiO_2 shell nanoparticles measured simultaneously by single particle plasmonic nanospectroscopy.

Recently, we have been focusing on MD simulations of the adsorption of small organic molecules on the charged silica as well as titanium oxide surface and comparing simulations data with the experimental results. [44] [45] [43] In MD it is possible to see contributions from the interactions between organic molecules themselves as well as with the surface (see figure p 33). Such an approach takes into account that, at certain conditions (pH, salt concentration), there may be strong competition between clustering of molecules and their adsorption on the surface. MD simulation conducted in such way allow to qualitatively estimate the behavior of the system by following the axial densities of organic molecules (normal to the surface) and the distances between molecules. We have performed such MD simulations for four different small organic molecules in water and in 1M NaCl for three different silica surfaces that correspond to pH 2, 7 and 9. One finding, as shown in the figure, is that under basic conditions, DHBA is positioned farther from the surface and in lower density compared to neutral and acidic conditions. [43] In the future, we plan to include simulation of larger systems, such as aggregation of many bare and coated particles, as well as how the shape of particles affects the adsorption of organic molecules and aggregation behavior.

BIOCORONA FORMATION ON SiO_2 NANOPARTICLES

Nanoparticles can obtain a molecular coating during transport in the aquatic system, or in cell culture, prior to uptake by aquatic organisms and cells. This is called particle transformation. It is important to understand this transformation and the effect of the adsorbed molecular coating on the uptake and toxicity of the particles. Experiments were carried out to study protein binding to silica NPs alone and in the presence of humic acid and dihydroxy benzoic acid at neutral pH. As for the metallic NPs in Case Study 1, no differences were found in either protein affinity or structure with or without NOM, most likely because there were no interactions between humic acid and dihydroxy benzoic acid with the silica nanoparticle surface in the first place.

NANOPLASMONIC SENSING TO DETECT BIOCORONA FORMATION IN REAL TIME

A nanoplasmonic sensing methodology was used to describe, in real time, protein binding to faceted Au core–SiO_2 shell nanoparticles of different size, which results in different surface facet-to-edge ratios. The edge is a curved surface with the same curvature in all different particle sizes, whereas the facets are flat surfaces that decrease in size as the particle size decrease. The formation of a complex protein corona and the real time exchange of proteins was also monitored on colloidal silica nanoparticles in suspension, and the results compared to the surface-bound nanoplasmonic particles.[46] Pre-adsorbed serum protein albumin and/or immunoglobulin G (IgG) were replaced over time by other proteins when blood serum was introduced. These results were confirmed in equilibrium experiments using silica nanoparticles with similar size in dispersion. Furthermore, IgG was found to bind differently to the curved and flat surfaces of the nanoparticles. The nanoplasmonic sensing methodology enables the investigation of the initial formation and further development of the corona in real time and, since no particle aggregation can occur and obscure the results, it guarantees experiments on reasonably monodispersed samples. For similar studies on nanoparticles dispersed in solution at relative high concentrations, the proteins will always affect the results and the corona development over time is difficult to follow in the same conditions.

To further evolve this experimental methodology, we have recently succeeded with similar proof-of-principle experiments at the level of individual nanoparticles. These preliminary data were obtained by simultaneous measurements of the optical scattering response from three individual faceted Au core–SiO_2 shell nanoparticles upon exposure to IgG. As seen in the adjacent figure, the temporal evolution of the binding is very different for the three nanoparticles measured simultaneously at exactly identical conditions. This result highlights that studies of single nanoparticles in the nanosafety context will be able to completely eliminate ensemble-averaging artifacts, which to-date deny access to the understanding of important details related to nanoparticle size, shape, microstructure, aggregation and composition. Hence, we predict that by applying this new experimental methodology, we will be able to address questions like: (i) Are all nanoparticles in an ensemble toxic or only a small fraction of them? (ii) What is the structural reason that causes the toxicity in that fraction? (iii) Does it matter whether a nanoparticle is single- or polycrystalline? (iv) Does difference in microstructure affect the stability of a nanoparticle in a certain chemical environment?

INTEGRATED HAZARD ASSESSMENT

The Mistra Environmental Nanosafety program is addressing the relation between physico-chemical properties and the environmental hazards of selected nanoparticles, and aims to broaden the scope of quantitative structure-activity relationship (QSAR)-analyses with respect to biological complexity. Overall, the results show that the environmental chemistry and the organisms in the environment affect nanoparticles in multiple and sometimes unpredictable ways. The effect is different from nanoparticle to nanoparticle. How the organisms are affected by the nanoparticles depends not only on the specificity of the nanoparticle, but also on how the nanoparticles transform if released into nature. It is neither a simple task to mimic realistic environmental conditions at laboratory conditions nor to predict relevant parameters in toxicity tests. The conclusion is that more focus should be on the prevailing mechanisms that drive toxicity in order to understand how to design and evaluate possible toxic effects induced by nanoparticles. Regarding colloidal silica nanoparticle toxicity, our findings in the different model test systems show a generally low ecotoxicity of silica nanoparticles and indicate that silica particles of different sizes but identical surface chemistry could potentially be grouped into an assessment group under regulation such as REACH.

ECOTOXICOLOGICAL TESTING OF SILICA NANOPARTICLES IN AQUATIC ORGANISMS

The ecotoxicological hazard of the colloidal SiO_2 nanoparticle library to algae *Raphidocelis subcapitata* (as representatives of aquatic primary producers), bacteria *Pseudomonas putida* (as representatives of aquatic destruents) and freshwater zooplankton *Daphnia magna* was characterized and provides information for three of the main trophic levels in an aquatic ecosystem. Substantial effort was invested in order to complement the tests with investigations using the fish embryo model, an OECD-standardized assay. Unfortunately, those experiments were unsuccessful, due to technical problems.

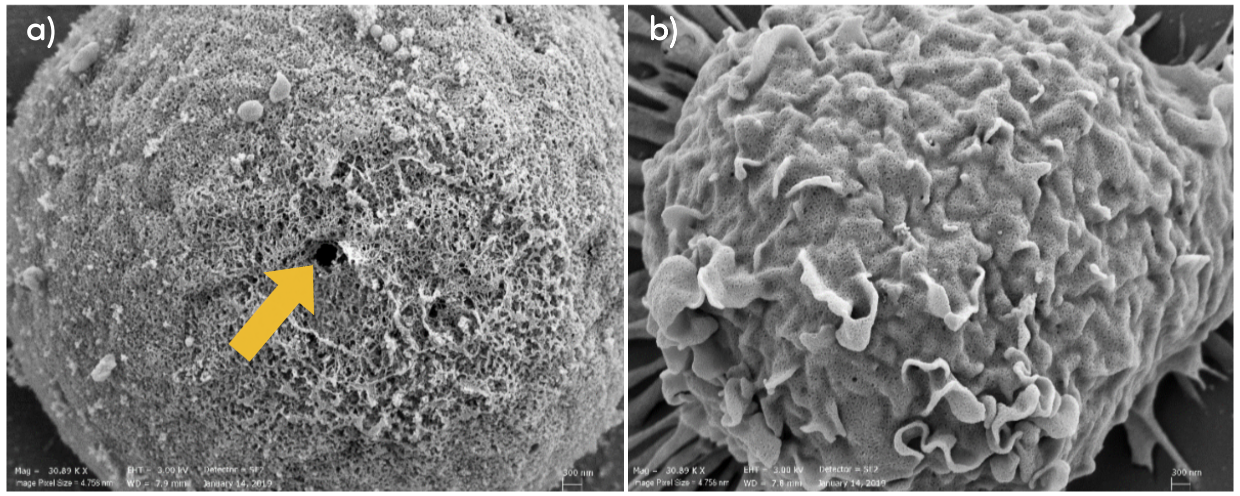
Additionally, the combined effect of SiO_2 particles and a common herbicide was investigated in some instances in order to shed light on more complex, environmentally realistic exposure situations

All silica nanoparticles used were analysed with a Malvern Zetasizer in order to characterize their size in relevant culture solutions. Determination of the size of all particles in the three different test media was performed at the beginning of the test and at the end. This was done in order to support the later elaboration on whether the size of the particles actually matters for their ecotoxicity, and in order to check for potential agglomeration during the test. No change in particle size was detected.[47] All seven nanoparticles were re-tested in combination with paraquat (a herbicide) in the algal bioassay, in order to investigate the ecotoxicological consequences of sorptive processes, i.e. the interactions between particles and a “classic” chemical pollutant. We could demonstrate that SiO_2 nanoparticles show strong sorptive behaviors, which might potentially open up routes for using SiO_2 particles to clean-up wastewater streams and processed waters from micropollants.

No acute toxicity was observed by silica nanoparticles in algae, bacteria or zooplankton.[47] Specifically, no effects on the survival of the zooplankton could be observed during a 24-hour period even at very high concentrations of 1 and 10 mg/L, respectively. A second experiment involved the use of the high concentration of 10 mg/L and increasing the exposure time to 48 hours. The second experiment also revealed an overall low toxicity of the particles, except for the Al modified 7nm (smallest, most negatively charged) particle size tested, which displayed an increased toxicity compared to the other particles. Due to the very high concentrations used and low effects on the zooplankton, it was decided to not continue with further toxicity tests using the silica nanoparticles at this stage.

INTEGRATING THE DEMANDS FOR HUMAN HEALTH AND ENVIRONMENTAL ASSESSMENTS: IN VITRO CELL TOXICITY TESTING

The library of silica nanoparticles has been studied to assess cell toxicity in fish gill, human lung and human immune cell lines. We have applied conventional in vitro model systems as well as high-throughput-based approaches. The nanoparticles were carefully characterized in the respective cell culture media and we have also evaluated the impact of the bio-corona on toxicity. Overall, we have found that the smaller the silica particles, the more toxic they are, yet this is only observed at very high nanoparticle doses. The total surface area and surface chemistry determine toxicity for silica nanoparticles. The silica particle coatings tested were able to modify the toxicity response. For example, while the smallest particles tested (7nm) were most toxic, the silane coating was able to mitigate this toxicity. Note, however, that cell toxicity was observed only at very high nanoparticle concentrations and non-relevant exposure levels.



Scanning electron micrographs of THP-1 cells exposed to SiO_2 NPs. Cells exposed to cytotoxic (a) and non-cytotoxic (ethoxy-silane modified) SiO_2 NPs (b). Yellow arrow in (a) shows hole on the surface of cells created due to the NPs. Note also that the normal membrane ruffles are lost under these conditions.

CONVENTIONAL TOXICITY TESTING OF SILICA NANOPARTICLES

As a first step, as mentioned above, we performed characterization of the silica nanoparticles dispersed in cell culture medium by using a Malvern Zetasizer in order to determine both hydrodynamic size and surface charge. The nanoparticles were also evaluated for potential endotoxin content.

Six silica particles were found to be toxic to the fish gill cell line RTgill-W1 from Rainbow trout (*Oncorhynchus mykiss*), showing a clear concentration-response relationship with EC50 values between 13 and 92 mg/L. [47] Toxicity in the fish cells decreases with increasing hydrodynamic size and was dependent on particle surface area. Surface modifications clearly impact toxicity, with ethoxy silane-modified particles showing no cytotoxicity.

Toxicity screening of the silica nanoparticles was also conducted using two different cell lines of human origin, the monocyte-like THP-1 cell line and the bronchial epithelial cell line BEAS-2B. The studies were performed across a broad range of silica nanoparticle concentrations. The results obtained in the two cell models were largely concordant and revealed that particle size and surface modification play an important role for cytotoxicity of silica nanoparticles. Hence, small particle diameter correlated with increased cytotoxicity, and uncoated silica particles were more cytotoxic than particles modified with ethoxy silane groups. [48]

We also determined cellular uptake of the silica nanoparticles by using transmission electron microscopy and we used the FITC-modified silica nanoparticles to demonstrate the subcellular localization of the internalized nanoparticles (see adjacent figure). Confocal microscopy of THP-1 cells exposed to FITC-labelled nanoparticles with co-staining of different cellular organelles revealed that the silica nanoparticles co-localized to a considerable extent with mitochondria.[48]

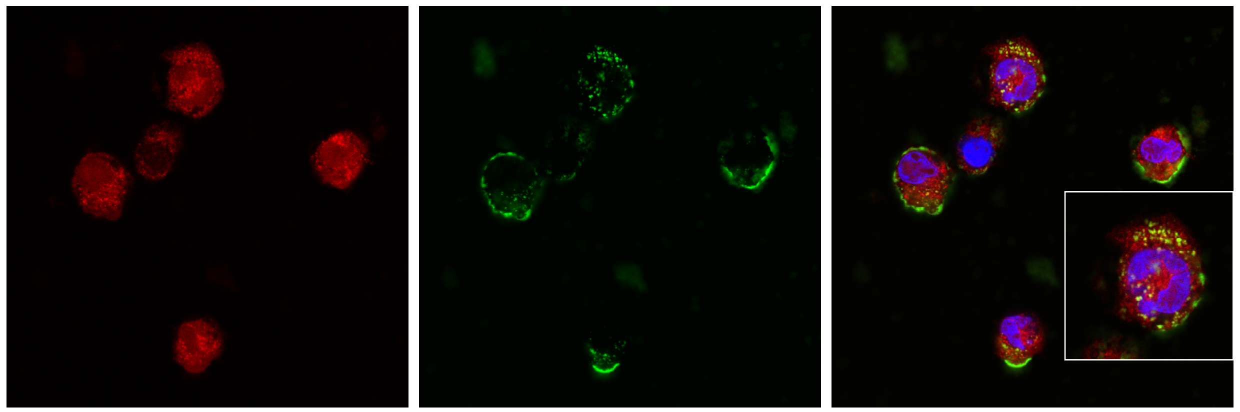
Further studies were conducted using the THP-1 cell model in order to address the possible underlying mechanism of cytotoxicity of the smallest nanoparticles. Our team has previously shown that silica nanoparticles from a commercial source exerted potent cytotoxicity *via* lipid peroxidation. [49]Using the library of silica nanoparticles produced within the program, we have now shown that these particles also exert cytotoxicity with lipid peroxidation, as revealed by using the fluorescent dye BODIPY, and we could show that the toxicity was mitigated to some extent by adding Trolox, a water-soluble vitamin E analog. [48] Furthermore, using the RAW264.7-Difluo™ LC3 cell line, a mouse macrophage cell line expressing RFP::GFP::LC3 fusion protein allowing for the fluorescent tracking of autophagic flux, we found that silica nanoparticles triggered autophagy. [48] The data also demonstrated that cells respond with autophagy induction which could protect cells from particle toxicity or contribute to cell death. [50] Taken together, we have provided evidence for size- and surface-dependent cytotoxicity of silica nanoparticles. Importantly, toxicity was abrogated by surface modification and these studies therefore may support a safe-by-design approach for silica particles.

HIGH THROUGHPUT SCREENING (HTS) FOR THE ASSESSMENT OF ADVERSE EFFECTS OF NPs ON CELL LINES

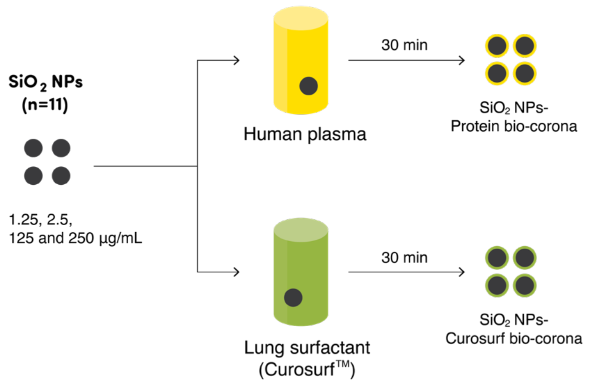
With the growing numbers of nanomaterials, there is a need for rapid and reliable ways for screening nanomaterials using *in vitro* approaches [51]. The adoption of high-content analysis (HCA) and high-throughput screening (HTS) for nanomaterial toxicity testing allows the testing of numerous materials at different concentrations and on different types of cells, reduces the effect of inter-experimental variation, and makes substantial savings in time and cost. HTS/HCA approaches should be coupled with characterization of nanomaterials in relevant exposure media taking into account the possible role of the bio-corona of protein and/or lipids (of particular importance in the lung). Our library of silica nanoparticles was analyzed along with another library of silica particles obtained from the Joint Research Centre (JRC) of the European Commission [52]– altogether 11 different nanoparticles – using an established HTS/HCA platform. The particles were assessed across a range of concentrations in 3 different cell lines representative of the lung, the gut, and the blood/immune system, and cells were exposed to nanoparticles in the presence or absence of human plasma or porcine lung surfactant (Curosurf®) to assess the role of the corona. (See adjacent figure)

Our HTS approach provides a valuable set of data on the toxicity of silica nanoparticles and the importance of the biocorona for the biological effects. The work is currently being finalized and we are analyzing the data. There are already interesting results. While the benchmark nanomaterials from JRC were largely non-cytotoxic for cell lines, the toxicity towards the broncheo-alveolar cell line H441 was enhanced in the presence of lung surfactant while, in contrast, the toxicity of the JRC particles towards THP-1 monocyte-like cells was mitigated in the presence of human plasma. [53] Thus, the biocorona can either promote or reduce NP toxicity. This observation can help to improve the toxicity testing of nanoparticles using *in vitro* models. After evaluation of the HTS testing for evaluating effects of nanoparticles in human cell lines, consideration will be made on the applicability and relevance for other vertebrate cell lines, such as fish. However, these tasks will be performed after the end of Phase 1 of the program.

MitoTracker Red | FITC-labeled SiO_2 | Merge + DAPI [blue]



Confocal microscopy of THP-1 cells exposed for 3 hours to FITC-labeled silica nanoparticles (50 µg/mL). The particles co-localized with mitochondria as shown by yellow (red+green) punctate staining.

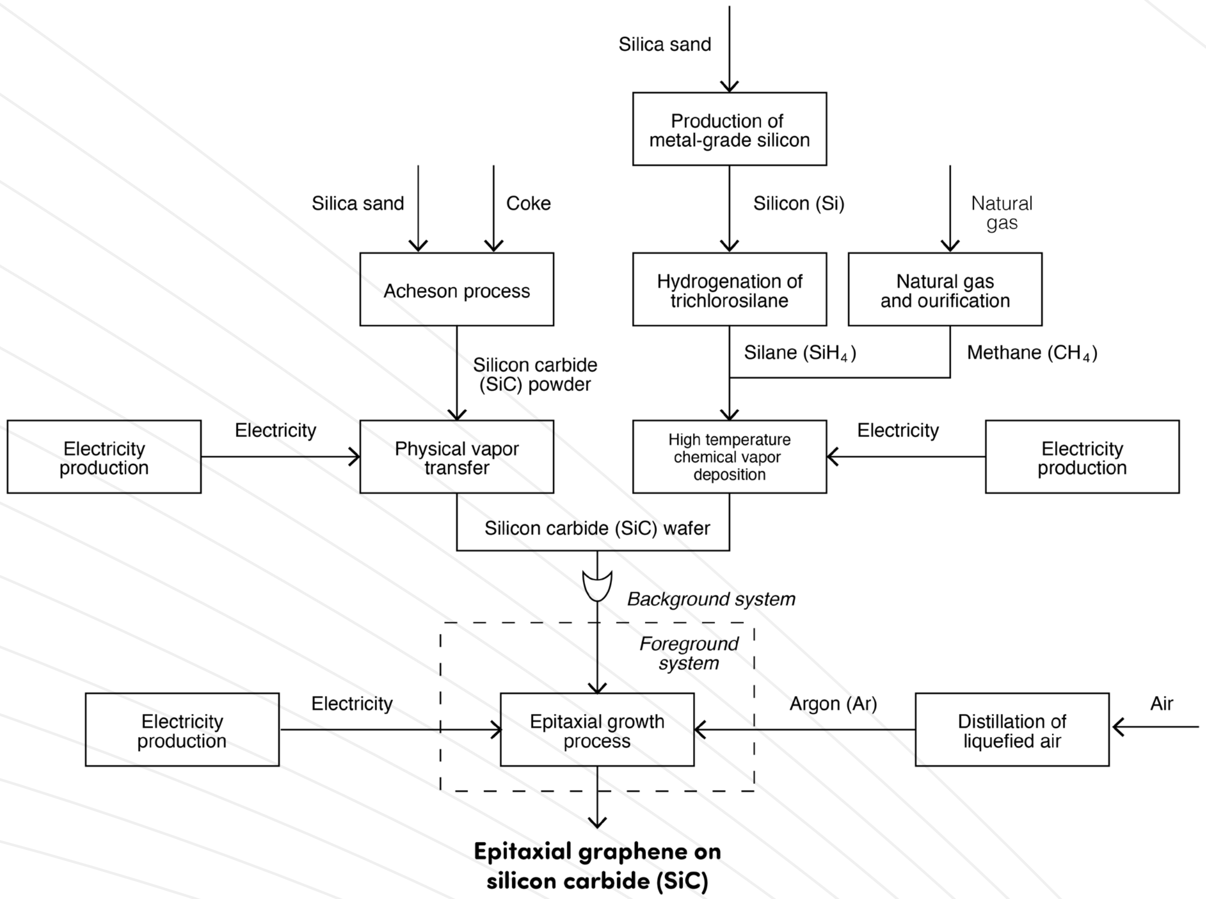


Silica NPs were pre-incubated in human plasma or porcine lung surfactant prior to cytotoxicity screening in order to evaluate the possible contribution of the biocorona on cytotoxicity.

As the toxicity of silica nanoparticles is very low at environmentally-relevant concentrations, as experienced in our *in vitro* and *in vivo* test models, the data does not warrant the application or development of QSAR modeling in this system. We are currently in the process of producing a critical review on the environmental hazards and risks of SiO_2 particles, by amending our own data with the ecotoxicological and exposure data described in the open scientific literature. [54] This will provide an in-depth ecotoxicological hazard profile of SiO_2 nanoparticles.

CASE STUDY 3: FUTURE NANOMATERIALS

Environmental impact assessment of epitaxial graphene production methods



Flow chart describing the main processes of the cradle-to-gate life cycle of epitaxial graphene. The dashed line shows the boundary between the foreground and background system.

The life cycle analysis (LCA) of graphene builds further on studies performed both during and prior to the start of this Mistra program [23][24][27] and extends the coverage to *graphene production methods.* The results have been published as R. Arvidsson and S. Molander, "Prospective Life Cycle Assessment of Epitaxial Graphene Production at Different Manufacturing Scales and Maturity," J. Ind. Ecol., vol. 21, no. 5, pp. 1153–1164, Oct. 2017.

The study was a prospective LCA, and in this kind of LCA the aim is to *assess emerging technologies*, which differs from conventional LCA studies. This led to a reliance on data from laboratory and pilot-scale investigations and assumptions regarding future technological development. In this study of epitaxial graphene production (a kind of graphene of high quality for use in electronics), three scenarios were applied representing laboratory, pilot-scale and industrial production. The adjacent flow chart illustrates the main processes of the cradle-to-gate life cycle of epitaxial graphene.

Epitaxial growth is a potential production process for graphene, where it is grown on silicon carbide (SiC) wafers at high temperatures. Results from the epitaxial graphene-LCA are counter-intuitive since the three scenarios have similar impacts, despite increased scale of production. This is due to the fact that use of electricity related to production of SiC wafers dominates all impacts, and that this production is considered to already be a mature production process. Therefore, the only case with diminishing impacts is the use of thinner SiC wafers in the industrial scenario, where a substantial reduction can be obtained. From a methodological perspective, the study points to the importance of a temporal robustness of LCA studies, including knowledge and assumptions regarding different background systems, scale of production and technological maturity. The approach with a prospective LCA will, as all studies of future states, bring uncertainties, but also possibilities to guide the current technological development to avoid future environmental impacts.

OUTREACH

WEBSITE AND SOCIAL MEDIA

Outreach has been a prioritized activity throughout Phase 1 and for that reason we have had a dedicated communications officer from Gothenburg Center for Sustainable Development (GMV) linked to the program (Ida Brattström (2015-2017), Anna Wallin Adersjö (2017-2018)). Our main channel for communication has been our website: http://www.mistraenvironmentalnanosafety.org/en, which has been kept up to date with the main news and activities in the program, related activities in the field on nanosafety and publications generated in the program. The website has also acted as a verified source of information about the program containing our annual reports as well as summarizing the different case studies and work packages. A lot of effort has gone into building up the website as it is today and therefore it can be seen as a deliverable in itself that most likely will be the main source of information for interested parties looking for nanosafety research from Sweden outside of academia.

The program is also present and active on Facebook (https://www.facebook.com/mistrananosafety/) and Twitter (https://twitter.com/mistrananosafe) to share our latest news and to be part of the ongoing discussions related to nanosafety.

CONFERENCES AND WORKSHOP ORGANIZATION

WORK-SHOP: FRONTIERS IN SILICA (2015)
Frontiers in Silica Worskhop 2015, March 19- 20, 2015 Chalmers University of Technology, organized by Michael Persson, AkzoNobel and Chalmers University of Technology. Several MISTRA program scientists presented results at this workshop.

WORK-SHOP: SYMPOSIUM "EXPERIMENTAL AND THEORETICAL STUDIES OF NANOPARTICLES AND THEIR INTERACTIONS WITH AQUEOUS SOLUTIONS AND ORGANIC MOLECULES" (2015)
Mini-symposium targeting processes at the water-mineral interface at the University of Gothenburg, Organized by the Caroline Jonsson Research Group, Environmental Nanochemistry, Dept. of Chemistry & Molecular Biology, University of Gothenburg, 22 September 2015.

CONFERENCE: 2ND NANOSAFETY FORUM FOR YOUNG SCIENTISTS (2016)
Prof. Fadeel organized a meeting for young scientists in the field of nanosafety in Visby, Gotland in 2016 – a meeting where all the oral and poster presentations are held by students and post docs. It is noteworthy that a PhD student from the MISTRA program at KTH won the award for best poster presentation. The conference was organized by the EU Nanosafety Cluster and the FP7-eNanoMapper project.

WORK-SHOP: NANOMATERIALS CHARACTERIZATION WORKSHOP (2017)
This workshop took place at the Sven Lovén Marine Research Center, in Kristineberg, Fiskebäckskil, November 15 – 16, 2017. The workshop consisted of lectures on the theory behind analytical techniques, sample preparation methods for the characterization of nanomaterials and hands-on demonstration of state-of-the-art equipment available to characterize nanoparticles in the environmental nanochemistry lab of Prof. Hassellöv. The lectures were conducted by several partners in Mistra Environmental Nanosafety (GU, AkzoNobel, KTH). Participants were students and researchers in the Mistra program together with some external participants. The participants and the teachers were very satisfied with the joint learning experience.

WORK-SHOP: DELIVERING SAFE NANOTECHNOLOGY TO THE MARKET (2018)
Prof. Fadeel has been actively involved in the EU nanosafety cluster (www.nanosafetycluster.eu) for many years and he organized and co-chaired a one-day workshop in June 2017 at the EURONANOFORUM 2018 meeting in Malta. The workshop entitled "delivering safe nanotechnology to the market" was dedicated to discussion and interaction with nano-sector players from the European Commission, regulatory authorities, large and small companies, and academic researchers, including representatives of several EU-funded projects. The workshop was co-organized with the Nanotechnology Industry Association and Dr. Georgios Katalagarianakis from DG Research & Innovation of the European Commission presented the opening remarks.

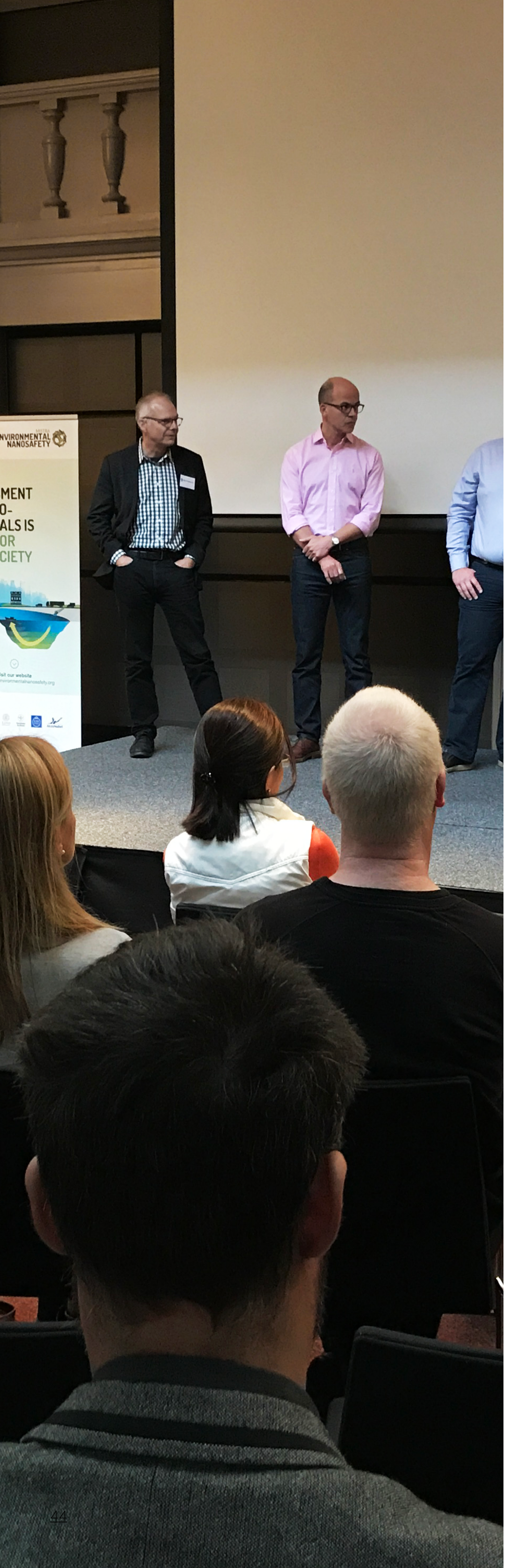
CONFERENCE: TOWARDS NANOTECH SAFETY (2018)
For the final meeting of Phase 1 Mistra Environmental Nanosafety, a scientific conference was held in Gothenburg 13-15 November. During these days the results from the program and related topics of nanotech safety and toxicological were discussed. Prominent external speakers were invited and gave an even wider perspective with Keynote speaker Prof. Mark Wiesner from Duke University, USA. Stakeholders in the field of nanosafety also attended and discussed how regulation and policies of the future should be constructed to allow for an effective and safe innovation and development of nanomaterials. More info: http://conference.mistraenvironmentalnanosafety.org/

CONSORTIUM MEETINGS
Consortium meetings inviting everyone in the program have been organized twice a year. They have been a valuable tool to increase translation and collaboration over the different scientific WPs:

- Kick-off Mistra Environmental Nanosafety meeting, April 22, 2015, Chalmers University of Technololgy, Gothenburg.
- Mistra Environmental Nanosafety Consortium meeting, in conjunction with the Visualization Workshop, Nov 12-13, 2015, Visual Arena, Lindholmen Science Park, Gothenburg
- Mistra Environmental Nanosafety Scientific meeting, May 19, 2016, Lindholmen Science Park, Gothenburg
- Mistra Environmental Nanosafety Consortium meeting and Communications Workshop, Nov 24-25, 2016, Karolinska Institute, Stockholm
- Mistra Environmental Nanosafety Consortium meeting, Apr 26-27, 2017, Lund University, Lund
- Mistra Environmental Nanosafety Consortium meeting and nanoparticle characterization workshop, Nov 14-16, 2017, University of Gothenburg, Sven Lovén Marine Research Center, in Kristineberg, Fiskebäckskil,
- Mistra Environmental Nanosafety Scientific meeting and writing workshop for Phase 2 application, Apr 11-12, 2018, KTH, Stockholm
- Mistra Environmental Nanosafety Conference "Towards Nanotech Safety", Nov 13-15, 2018, Post Hotel, Gothenburg

UNDERGRADUATE AND GRADUATE COURSES

GRADUATE COURSES ON NANO(ECO)TOXICOLOGY
Members of MISTRA Environmental Nanosafety organized and lectured in a graduate course on nanotoxicology at Karolinska Institutet, April 20-24, 2015: Nanotoxicology - potential risks of engineered nanomaterials to human health and the environment, 1.5 ECTS. Course organizers: Bengt Fadeel (Karolinska Institutet) and Antonio Pietroiusti (University of Rome Tor Vergata). Course amanuensis: MISTRA Asst Prof Kunal Bhattacharya, Karolinska Institutet. The course was organised by the Advanced International Training Programme in Health Risk Assessment at IMM in collaboration with the Doctoral programme Environmental Factors and Health (EFH) at Karolinska Institutet. Lectures by MISTRA Environmental Nanosafety members included Bengt Fadeel, Overview



of Nanotoxicology: Principles & Paradigms, and Systems Biology Approaches in Nanotoxicological Research; Kunal Bhattacharya, Nanotoxicology: Focus on Inflammation and Biodegradation; Thomas Backhaus, Eco-Toxicology of Engineered Nanomaterials (1); Joachim Sturve, Eco-Toxicology of Engineered Nanomaterials (2)

Prof. Fadeel at Karolinska Institutet (KI) is the organizer of a one-week annual graduate course on nanotoxicology at KI entitled "Nanotoxicology - potential risks of engineered nanomaterials to human health and the environment" and the most recent edition of the course took place in 2017. Several members of the MISTRA project participated in the course as teachers including Prof. Fadeel (KI), Prof. Backhaus (GU), Dr. Sturve (GU), and Dr. Arvidsson (Chalmers). In addition, two graduate students from Chalmers and GU, respectively, both members of the MISTRA program, attended the course.

Prof. Fadeel also took part in a one-day course on nanotoxicology in Goiania, Brazil in 2017 in conjunction with the 20th Brazilian Congress of Toxicology and presented a lecture on toxicology of nanomaterials while Dr. Sturve (GU) presented a talk in the same course on ecotoxicology of nanomaterials. The course was open to students and post docs.

MASTERS COURSE ON METAL DISPERSIONS (2015, 2016)

Inger Odnevall Wallinder lectured on metal dispersion in a masters course at KTH: KD2420 *Environmental Aspects of Atmospheric, Aquatic and Terrestrial Chemistry* 7.5 ECTS in 2015 and 2016

PUBLIC PRESENTATIONS, PRESS RELEASES, ARTICLES, INTERVIEWS AND EXHIBITIONS

Participation in The International Science Festival, Gothenburg: "Nanomaterials: Risks and possibilities", Rickard Frost, Vetenskapsrouletten. Oral discussion with public while riding the ferris wheel at Liseberg Amusement Park, April 16, 2015.

Presentation for, and supervision of, high school students in Stockholm: Prof Inger Odnevall Wallinder, *Metalliska (nano)partiklar i vår miljö*, Presentation for students from Åva High School, Nov 8, 2016.

Supervision of a high school senior project on silver nanoparticles at KTH, Jonas Hedberg and Inger Odnevall Wallinder, students: Edit Wallman and David Magnusson, *Värmdö Gymnasium*, 2016

WORK SHOP FOR HIGH SCHOOL STUDENTS IN LUND

During 20 weeks, from mid-October 2017 and forward, Tommy Cedervall and Mikael Ekvall, researchers at Dept of Biochemistry and Structural Biology at Lunds University, met with students from high schools in Lund. The students were introduced to the field of nanotechnology and nanosafety in experiments and lectures.

PARTICIPATION IN "SIXTH CONTINENT" EXHIBITION, EDMANSKA HUSET, LUND

The MISTRA research-group from Lunds University also participated in an exhibition at Edmanska huset, Folkparken in Lund during the fall 2017. The exhibition topic was plastics in the sea and its target group was students from primary school up to high school. The students could also watch a theatre about the same subject, "The sixth continent" performed by Teater Sagohuset, in the same building. The aim was to inspire students to take actions to save the threatened environment, but also to create an interest about chemistry and the significance of water.

PRESS RELEASES

- Press release, 1 October 2018. "Dubbdäck tar fler liv än de räddar", Available at: https://www.chalmers.se/sv/institutioner/tme/nyheter/Sidor/Dubbdack-tar-fler-liv-an-de-raddar.aspx [2018-10-25]
- Press release, 19 October 2018. "Slutsatsen står fast: Dubbdäck tar fler liv än de räddar", Available at: https://www.chalmers.se/sv/institutioner/tme/nyheter/Sidor/Slutsatsen-star-fast-Dubbdack-tar-fler-liv-an-de-raddar-.aspx [2018-10-25]

LETTERS TO THE PRESS

Furberg, A. Arvidsson, R. Molander, S. "Så räknade vi", published in Göteborgsposten 6 October 2018.

INTERVIEWS IN MEDIA

- Sverker Molander, interviewed by TT. 2018-10-01. Articles published in a large number of newspapers and webpages.
- Anna Furberg, interviewed by Ekot. 2018-10-01
- Anna Furberg, interviewed by P4 Göteborg. 2018-10-01
- Anna Furberg, interviewed by P4 Östergötaland, 2018-11-20
- Anna Furberg, interviewed by Göteborgs-posten 2018-10-01. Article named "Forskare: Dubbdäck kostar fler liv än vad de räddar" published in Göteborgsposten 2018-10-02.
- Anna Furberg, web interview by program communications officer Anna Wallin Adersjö: http://www.mistraenvironmentalnanosafety.org/en/blog/studded-tires-take-more-lives-they-save-0.

RADIO INTERVIEW ON NANOSAFETY, VETENSKAPSRADION SR

On May 28, 2018, Vetenskapsradion (Swedish Science Radio program) in Sveriges Radio published a news piece about research on safety assessment of nanomaterials, "Säkerhetsforskningen kring nanomaterial går långsamt". They highlighted the fact that despite millions spent on research there is no new legislation on nanoparticles or nanomaterials in the EU. The European Union has spent 600 million SEK during the latest years on this research. Tommy Cedervall, principle investigator in the program, was interviewed and shared his thoughts on this topic.

GOVERNMENTAL OUTREACH AND PARTICIPATION IN STAKEHOLDER NETWORKS

The Mistra Environmental Nanosafety program was presented by Program Manager Julie Gold in a booth at the "Speakers Corner" during The Swedish Water Management Conference, 16–17 May 2017, Gothenburg. The theme for 2017 was " From Source to Sea " with a focus on the Global Goals for Sustainable Development. Annual conference organized by the Swedish Agency for Marine and Water Management.

Bengt Fadeel delivered a short lecture for the Board of the Institute of Environmental Medicine (IMM) in 2018. The Board of IMM comprises of representatives of Swedish Government agencies including the work environment agency, the public health agency, the environmental protection agency, and several others.

Prof. Fadeel is a member of the steering committee of the National Nanosafety Platform, SweNanoSafe, inaugurated in 2016 and supported by the Swedish Ministry of the Environment and Energy. He also chairs the scientific expert panel of SweNanoSafe. The panel comprises several national experts in nano(eco)toxicology and risk assessment including MISTRA members, Asst Prof. Rickard Arvidsson and Assoc Prof. Joachim Sturve. He convened the inaugural meeting of the newly formed national network for nanosafety researchers in June 2018, and he is currently working with other members of the steering committee to produce a report on the state-of-the-art of nanosafety commissioned by the Ministry of the Environment and Energy. He is also actively involved in presenting current nanosafety activities via the platform's webpage (www.swenanosafe.se). Prof. Fadeel has also compiled a comprehensive report on nanosafety research with other researchers at IMM and this will be printed soon.

In addition, Prof. Fadeel has been engaged since 2015 as a member of an expert group on nanotoxicology convened by the World Health Organization (WHO). The task has been to develop a new Environmental Health Criteria (EHC) document on principles and methods to assess the risk of immunotoxicity associated with exposure to nanomaterials. The target audience for the document is risk assessors in a regulatory setting, researchers, and industry that needs to provide data for assessment, and the document has recently been open for public consultation and will be published soon.

SCIENTIFIC OUTPUT

PUBLICATIONS IN PEER-REVIEWED JOURNALS

Arvidsson R., Baun A., Furberg A., Hansen S. F., and Molander S., "Proxy Measures for Simplified Environmental Assessment of Manufactured Nanomaterials," *Environ. Sci. Technol.*, 4;52(23):13670-13680, 2018

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THESES

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