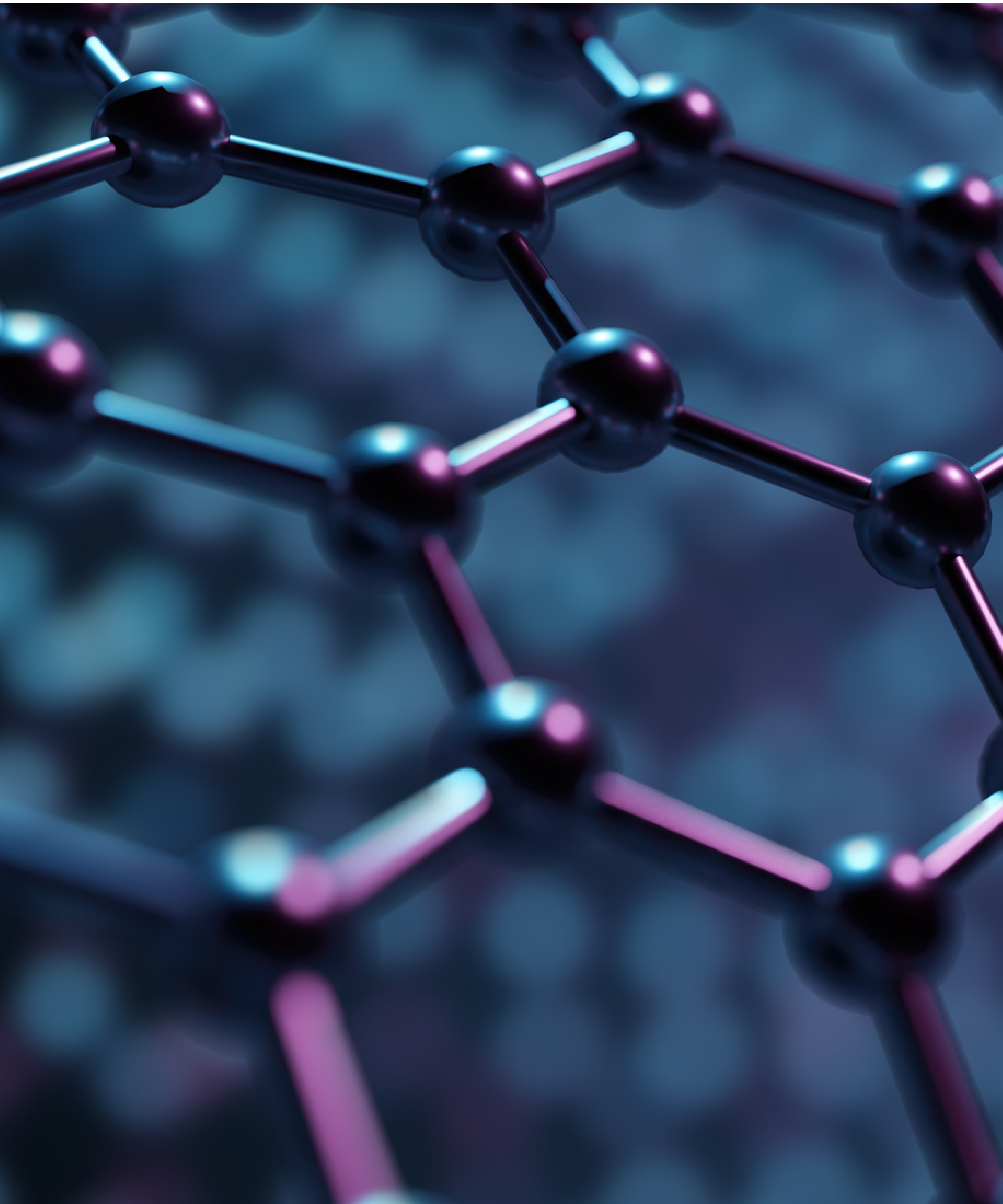
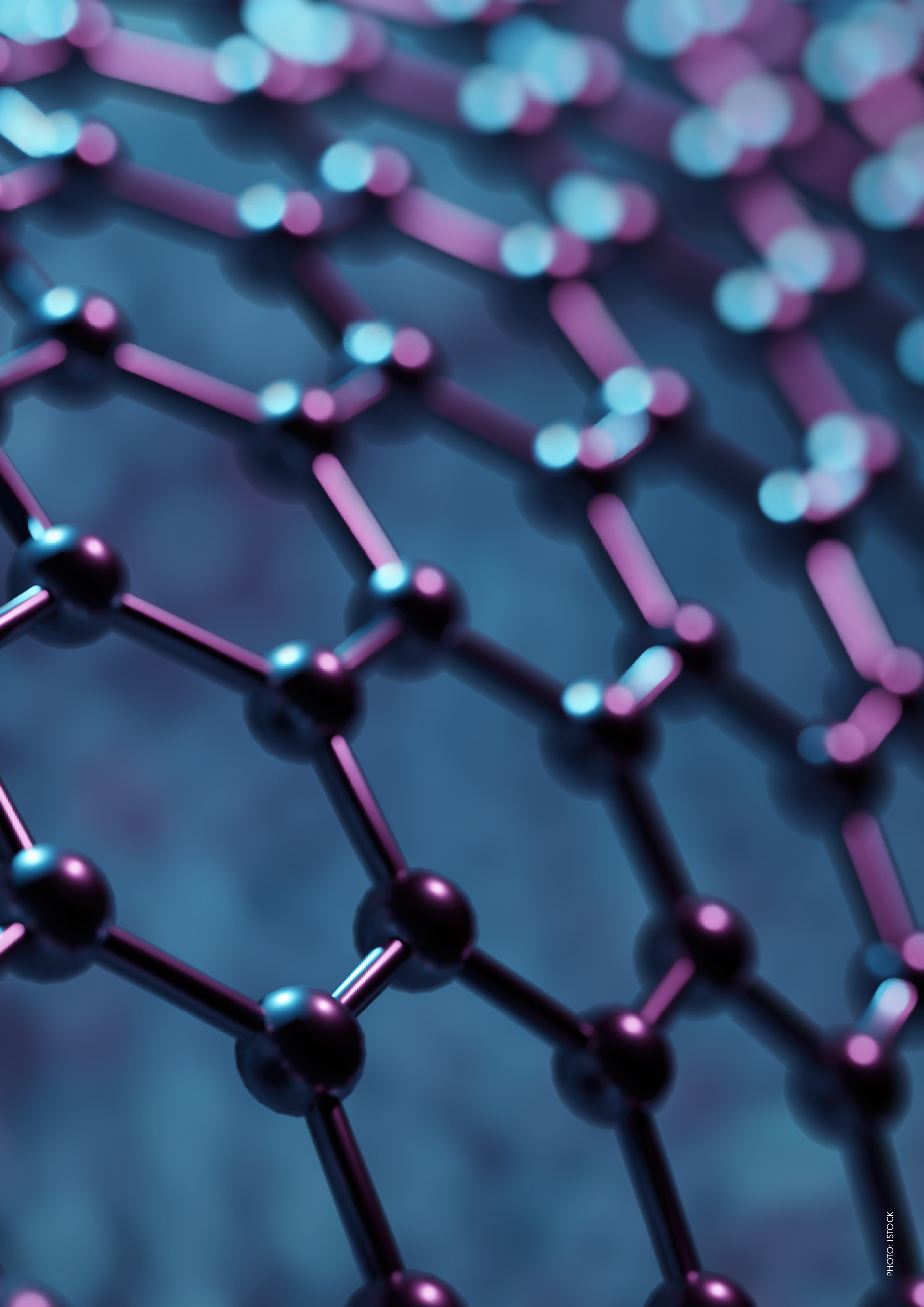
Mistra Environmental NanoSafety Phase II

ANNUAL REPORT 2020

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Content

1. Message from the Chair

Nanotechnology is often presented as the technology of the future. It has the potential to revolutionise the electronics industry, energy supply, healthcare, fashion, our leisure activities and the environmental work. In many ways it is true. Nanotechnology offers many new opportunities in all these areas. Increased efficiency of next-generation solar panels, strong lightweight materials for building taller wind turbines, climate-smart houses, nanoparticles in soil remediation, light and energy-efficient computers, smart clothing, and targeted medicine delivery. Nanotechnology provides us with many benefits and can play a major role in solving several of our major challenges. Therefore, it is important that we ensure continued and innovative and safe development and use of nanoparticles. This is the mission of the Mistra Environmental Nanosafety programme.

Small size gives nanoparticles new characteristics

It is the small size that gives nano-sized material new characteristics and thereby the nanotechnology new opportunities. However, the small size and the new characteristics also change how the material interacts with the surroundings. It will spread, dust, bind, penetrate, dissolve, oxidise, and aggregate differently compared with larger pieces of the same material. This means that when a nanomaterial is produced, used by consumers, and in the end discarded as waste it will act with a degree of unpredictability. It is even more complicated as also the ageing of the material and its interactions with natural and unnatural matter will affect the impact on producers, consumers, and the environment. The second phase of the programme focuses on these dynamic features that arise once the nanomaterial reaches the consumers and nature.

Man-made nano-sized particles are not only created and exposed to nature by intention. Other products and human activities as the fragmentation of rubbers and plastics, combustion of oil and waste are sources for nanoparticles. Although these are unintentionally made, the evaluation of their potentially negative impact on the environment has largely the same complexity. The industrial producers of these materials are not necessarily involved themselves in nanotechnology, but their own and their consumers concerns of how the products impact on people's health and on the environment is closely related to the nanotechnology industry. This aspect of the nanosafety area increases the number of companies and products interested in the field and often involves products produced and consumed in very large quantities. One recent environmental issue is for example the possible presence of break-down nanoplastics.

Several aspects of nanotechnology and nanomaterials are important

To ensure an innovative and safe development of the biotechnology several aspects has to be taken into account. The most obvious, which the programme is not involved in, is the technological know-how to create new products. Also obvious perhaps, is the need of knowledge about how the nanomaterials behave and affect humans and the environment. This may, at first, seem straightforward but as explained above the complexity is high and there is a great need for deeper understanding. This lack of knowledge leads to other potential challenges. The regulatory demands concerning nanomaterials have increased in recent years. Today, companies producing and using nanomaterials, are obliged to in detail report the environmental impact of the material. A task that to some extent is very challenging if not impossible. Furthermore, a safe working environment has to be ensured which demands reliable measurements and measures, which is difficult if fundamental certified methods are missing.

Lack of knowledge of how safe nanotechnology is

The lack of knowledge of how safe nanotechnology is may also lead to exaggerated concerns and an unwillingness to engage in the field. This does not only apply to researchers and nanotechnology companies but also to the public. There are indications that the public opinion about nanotechnology is balancing between good and bad. The benefits with the new technology are great and obvious while the disadvantages are more obscure and maybe even scary. This division is perhaps best reflected in the results of a small study in Lund a few years ago that revealed that nanotechnology was perceived as good whereas nanoparticles were perceived as bad. The proposed explanation is that when the benefits are described in media there is at the same time news about negative environmental effects when nanoparticles are used. Therefore, a communication strategy is another area identified as important to ensure an innovative and safe development. Not the least, as there is a growing concern that the lack of knowledge will push the public opinion in a negative direction before the science is in place.

The Mistra Environmental Nanosafety Programme tackles crucial aspects

Phase two of the Mistra Environmental Nanosafety programme tackles the described environmental challenges by first in great detail characterising the chemical ageing and biological weathering of nanoparticles in simulated natural aquatic environment. The environmental fate of the transformed nanoparticles is determined in a unique wet land model system in which nanoparticles can be tested in parallel. The toxicity is tested at low concentrations on aquatic organisms and on fish and human cells with the aim to link weathering of the nanoparticles with changes in toxicity. The possibility to detect nanoparticles in the recycling process of products with embedded nanoparticles are explored. The participants in the programme aim to work with the same material to ensure that the results from each level can be coupled to each other to build a deeper understanding on how the weathering of nanoparticles affects their environmental fate and impact.

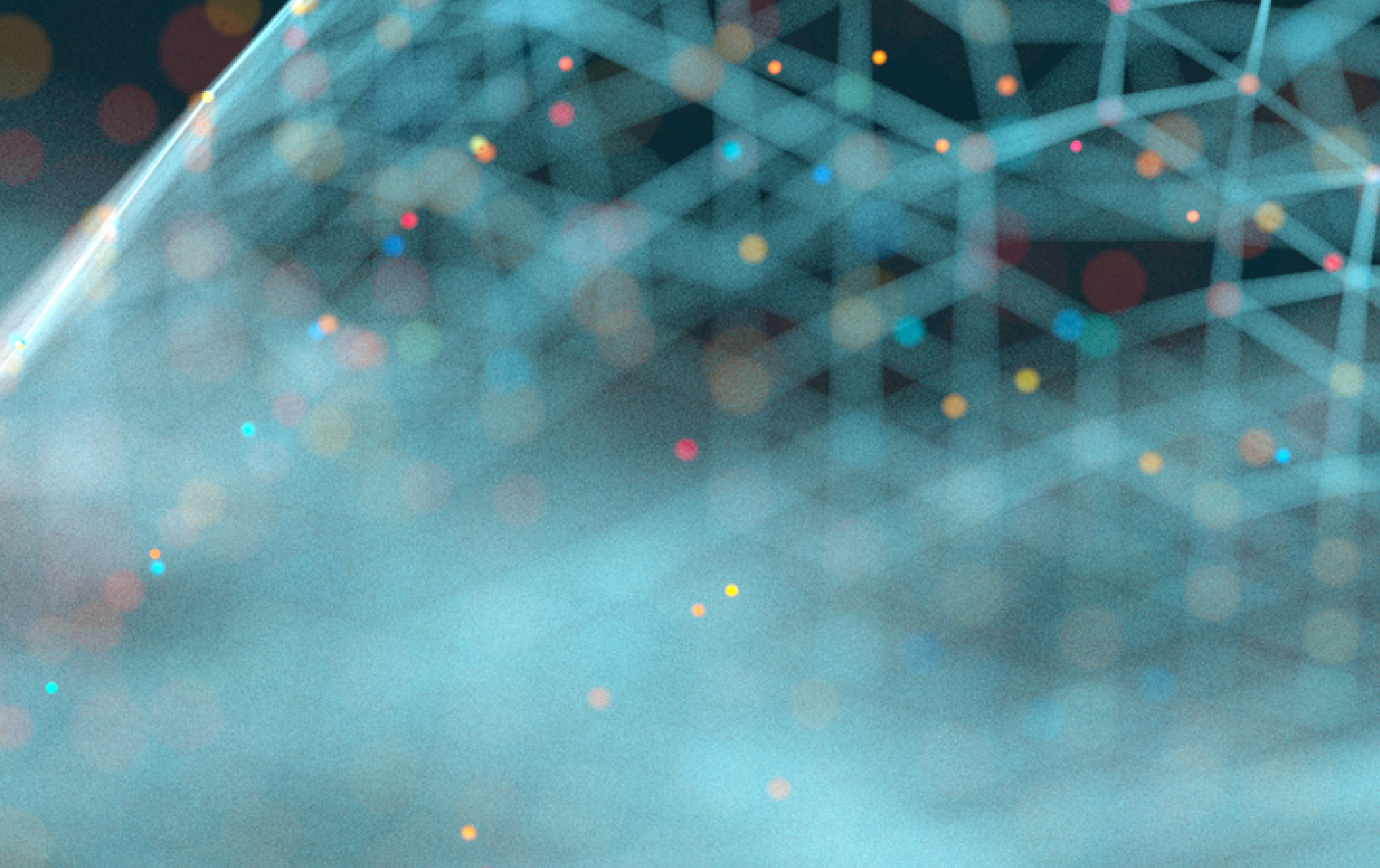
The results from the chemical and biological tests are used to suggest methods and ways to fulfill the regulatory demands. The programme is focusing on the life cycle aspects which are probably the most challenging parts of the regulation. The self-imposed mission is not limited to simply try to fulfill the regulatory demands but aim to suggest improvements and, when needed, simplifications of the regulations. We believe that this work will help already established companies to register their products and make it easier for new companies to enter the nanotechnology field. To further ensure innovative and safe development of nanotechnology the programme works with mapping all involved stake-holders, documenting their interests and needs, and create functional networks. In these two aspects not only scientists are involved, but also partner companies and a growing number of associated companies and industrial organisations.

Second phase has produced good results so far

The first two years of the second phase of the Mistra Environmental Nanosafety programme have been buzzing with activities. Research has been performed and published. Regular meetings, work-shop and programme conferences have strengthened the internal and external cooperation and communication. The corona pandemic situation has influenced all activities as all meetings have been digital or lab spaces closed or have had limited access during longer and shorter periods, but so far the main activities has been carried out. The activities and accomplishments and how they are line with the programme's goals are described in the annual report.

PHOTO: KENNET RUONA

Rolf Annerberg
Chair of the programme board



2. Message from Management

Phase two of the Mistra Environmental NanoSafety programme has, besides the focus on performing excellent research within the different work packages and on publishing high quality papers, also expanded on increasing intra-collaboration within the whole programme, as well as establishing collaborations outside the programme – in spite of the very challenging situation the corona pandemic has created. We have initiated a number of activities to make our members feel that they belong to a large programme, not just their own work package.

One example is the **Mistra NanoClub,** which is a forum were members can interact and connect in new and not strictly scientific/research related manners. Within Mistra NanoClub, we organised an activity, **"Swedish Fika"** online, where all members were invited to a zoom meeting and then randomly transferred out in pairs for one-to-one sessions with other research members or partner members in 5 minute slots. This event was very successful and increased team feeling and initiated new collaborations among members.

We also laid the groundwork for a **mentorship programme** for younger researchers which is due to start in 2021. The aim is to assist our researchers to evolve at multiple levels to be able to progress both in their professional career and leadership skills.

Further, we **extended our network** with the addition of several new associated partners, including Solve Research and consultancy, CR Competence AB and Chalmers Industriteknik.

Finally, looking outwards, it is clear that a programme such as ours is **more relevant** than ever. This year, legal requirements were added under the EU chemical regulation REACH for companies that manufacture or import nanomaterials. The REACH regulation means that all chemicals in excess of 1 tonne in weight, which are either produced in the EU or imported, must be tested for health and environmental aspects. Simultaneously, innovation in the field of nanotechnology is growing rapidly. The number of products containing nanoparticles are increasing, as is the amount of microplastics in our environment; microplastics that in time could be broken down into nanoplastics with unknown impacts on our environment.

These policy and social developments bring to the fore the need for basic and policy-focused research in close collaboration with industry. The Mistra Environmental Nanosafety programme is therefore well placed to play a crucial role in identifying environmental risks of nanoparticles, and characterising their fate, as well as to support the development of effective and succinct nanomaterial regulation in the future – based on the needs from companies and existing research gaps.

PHOTO: ISTOCK

3. About the Mistra Programme

The interdisciplinary research programme, Mistra Environmental Nanosafety, aims to develop research, knowledge and best practice on risks associated with nanomaterials and their impact on our environment. This includes focused research on transformed nanoparticles, which have been altered by natural ecosystems, and on developing policies and risk assessments to ensure a safe and innovative development of nanotechnology in Sweden and internationally. The programme also works with the question of how a pioneering research and development environment can be maintained.

In phase two of Mistra Environmental Nanosafety, the programme is developing the research identified as most interesting in phase one which ended in 2018.

ILLUSTRATION: FRIDA NILSSON, MEDIA-TRYCK

Work Packages

SCALING UP: ASSESSING STRUCTURAL CHANGE AND FATE OF NANOPARTICLES WHEN ENTERING NATURAL ECOSYSTEMS

▶ **WP1** examines how different nanoparticles' fate and characteristics are changed by the natural environment. It will scale up the studies of nanoparticles performed in work packages 2 and 3 (toxicity and weathering) in order to make recommendations to regulators, industry actors and wider society on which nanoparticles should be either avoided or used with caution.

CHEMICAL WEATHERING AND BIOCORONA FORMATION ON NANOPARTICLES

▶ **WP2** focuses on generating a mechanistic understanding of what happens to nanoparticles if dispersed and weathered at natural environmental conditions, for instance how such transformation influences their particle composition/characteristics, dissolution pattern, colloidal stability, fate and toxic potency? It focuses mainly on interactions with different kind of natural organic matter and its specific biomolecular constituents (for instance secreted biomolecules). Special attention is given to materials that undergo specific transformations that change their toxic effects (investigated in work package 3). These aspects are, despite many efforts, relatively poorly understood in the context of nanoparticles transformations and toxicity.

TRANSFORMED NANOPARTICLES ON AQUATIC FOOD CHAINS AND CELL MODELS

▶ **WP3** examines the effects of transformed nanoparticles on aquatic organisms, food chains and models. Nanoparticles may enter the environment through intentional releases (e.g., environmental remediation and pesticide use) as well as through unintentional releases from nanoparticles utilisation, atmospheric emissions and solid or liquid waste streams from production facilities. Once introduced to the aquatic environment, nanoparticles will undergo a multitude of transformation via a number of processes including dissolution, aggregation/agglomeration and subsequent sedimentation, as well as interactions with abiotic and biotic components present in the aquatic system. Agglomeration followed by sedimentation of nanoparticles may lead to deposition on the sediment surface where benthic organisms may be particularly at risk of exposure.

PROACTIVE RISK ASSESSMENT, REGULATION AND CREATION OF STAKEHOLDER LEARNING ALLIANCES

▶ **WP4** works with proactive risk assessment, regulation and the creation of stakeholder learning alliances. Of importance is to modify nanosafety regulation to reflect real risks by for example include the consideration of corona formation in risk assessments. Furthermore, this work package aims to support the effective flow and use of information between experimentalists, regulators, and industry, and to help mobilise stakeholders to engage in responsible innovation and risk governance.

SAFE HANDLING OF NANOMATERIALS AFTER PRODUCT END-OF LIFE

▶ **WP5** focuses on researching and developing appropriate strategies for the end-of-life management for products containing nanomaterials. This research is critical to minimise human and/or environmental exposure. Risks include both immediate exposure of humans and a long-term exposure of the ecosystem, including humans, due to leaching and accumulation. More knowledge is needed in the area of waste management and recycling, and of risks related to handling nanomaterials, in order to develop appropriate strategies, which both foster development and minimise risks.

MANAGEMENT, COMMUNICATION, ECONOMY AND STAKEHOLDER RELATIONS

▶ **WP 6** focuses on ensuring the smooth facilitation of the programme in phase two. It focuses on supporting a steady progression of the other work packages: internal and external communication, including societal impact. Another aim is to build networks, support platform building and manage industrial, scientific, authority and other stakeholder interaction.

4. Selected Highlights 2020

IMPACT WORKSHOP

For a programme such as Mistra Environmental Nanosafety, policy, environmental and industrial impact as a result of research is key. To improve our impact work, and increase our researchers and partners' understanding of impact, the programme organised an impact workshop. The aim was to highlight key definitions of impact and to initiate discussion on what impact mean in the context of nanosafety. The workshop was organised together with Chalmers Industriteknik, which host the Sio Grafen network.

EARLY CAREER RESEARCHER MEETING

To catalyse collaboration and discussion within the programme, Mistra Environmental Nanosafety organised a meeting focusing solely on early career researchers. All the doctoral students and post-docs presented their research, as well as the methods they use. The goal was to create a better understanding of the work that is going on in the programme so that researchers from different work packages can initiate collaborations.

NANOMATERIALS IN WASTE WORKSHOP

So far very little effort has been made to investigate nanomaterials in waste. To initiate discussion and research in this area, Mistra Environmental Nanosafety co-organised a workshop highlighting risks related to end of life handling of nanomaterials. The workshop is part of the programme's aim to contribute to a better understanding of nanomaterials and where there is risk for exposure at different part of the production, use, and end of life stage.

The workshop was arranged by Avfall Sverige and SweNanoSafe in collaboration with the Mistra Environmental Nanosafety programme, and the research project: Management and recycling of composites containing carbon nanotubes – without risks for health and environment, funded by Vinnova.

RESEARCH PAPER ON RISKS OF NANOPARTICLES TO AQUATIC ENVIRONMENTS

During 2020, researchers within the programme published a seminal publication of the importance to all the individual work packages: "Influence of natural organic matter on the aquatic ecotoxicity of engineered nanoparticles: Recommendations for environmental risk assessment" (Arvidson, Hansen, Baun). The publication succinctly outlines where the research field is at present for assessing and dealing with risks of nanoparticles in aquatic environments.

The research publication is a good example of the programme's contribution to nanosafety research.

3RD ANNUAL WORKSHOP OF THE NATIONAL NANOSAFETY RESEARCH NETWORK

The Mistra Environmental Nanosafety programme works closely with the national nanosafety platform SweNanoSafe, established by the Ministry of the Environment. Researchers from the programme contributed to the platform's third annual workshop which focused on the Swedish national plastics coordination assignment on microplastics and nanoparticles in the environment. Contributions by Mistra Nanosafety researchers included talks on the environmental impact of microplastics from consumer products, and the transformation and fate of nano and microparticles in the environment.

SweNanoSafe is a platform for collaboration between academia, authorities, business and other organisations that have an interest in sharing knowledge and experiences as well as discussing, developing and influencing the implementation of nanosafety in society. The platforms was established by the Ministry of the Environment and is coordinated by Karolinska Institutet. The goal is the safe handling of nanomaterials at all levels that protect human health and the environment!

NANOTHEATRE

Mistra Environmental Nanosafety researchers participated in an outreach effort to educate highs school youth about nanoplastics and the importance of questioning the world around them. They held interactive seminars in schools across Sweden, including in Skåne and Norrland, to accompany the performance of the play, Den Rätta vägen.

– Even though the play is based on nanoplastics, we want to highlight bigger issues than just plastics. We want young people to learn to question information, but also understand that the responsibility does not lie with them in the first place but with politicians and the adult world, says Tommy Cedervall, Director of the Mistra Environmental Nanosafety programme.

PHOTO: ISTOCK

5. A Chat to some of Our Programme Researchers

"It is curiosity that drives me. The fact that there is so much we do not know"

Mikael Ekvall
researcher at the Unit for Aquatic Ecology, Lund University

Mikael Ekvall, researcher at the Unit for Aquatic Ecology at Lund University, investigates the effects of nanoparticles on aquatic environments such as lakes and ponds. He became interested in nanoparticles during his doctoral studies on zooplankton – when he used fluorescent nanoparticles to follow the otherwise transparent zooplankton in various experiments.

Within the Mistra Environmental Nanosafety programme, he, together with his research colleagues, is developing a number of experimental wetlands in a lab in Lund. The goal? To find out how different nanoparticles are transported through the wetlands and how they affect the organisms that live there. A feasibility study has already been done. It showed that some nanoparticles are stored in wetland sediment.

– Now we are doing new experiments on a larger scale to get a better picture of how the nanoparticles are transported through the wetlands and affect the organisms that live there.

Concentrations of nanoparticles are important

Mikael Ekvall emphasises that the concentration of nanoparticles is something that interests him. In the lab, high concentrations are often added to explore effects on eg zooplankton. But these levels do not correspond to what you would typically see in nature.

– With nano, we know that the material gets completely new properties. But we do not know much about the risks yet. Therefore, we need to have more knowledge about nanoparticles in natural systems to be able to identify which properties are actually dangerous when the particles end up in nature.

The challenges of researching nanoparticles in natural environments are today many. In nature, there are already a lot of naturally occurring nanoparticles. They are composed of different carbon compounds – as well as the plastic that is thrown in nature. Water also contains more organic carbon than, for example, air, which makes it extra difficult to measure how nanoparticles affect aquatic organisms and environments.

Size matters for toxicity

In spite of the challenges, Mikael Ekvall and his colleagues have established some key research results.

– We can safely say that there is a size dependence between toxicity and size. In our experiments we can see that smaller particles are more dangerous than larger ones. We think it can look the same in nature.

Another important discovery, which surprised Mikael Ekvall and his group, is that nanoparticles become less toxic when they form a so-called ecocorona. Ecocorona is formed from organic material and attaches around the nanoparticle itself. The zooplankton that swam with nanoparticles with ecocorona lived longer than the zooplankton that swam with nanoparticles without.

– We do not know what happens inside the zooplankton; why do they live longer? It was something that made me surprised and curious to find out more.

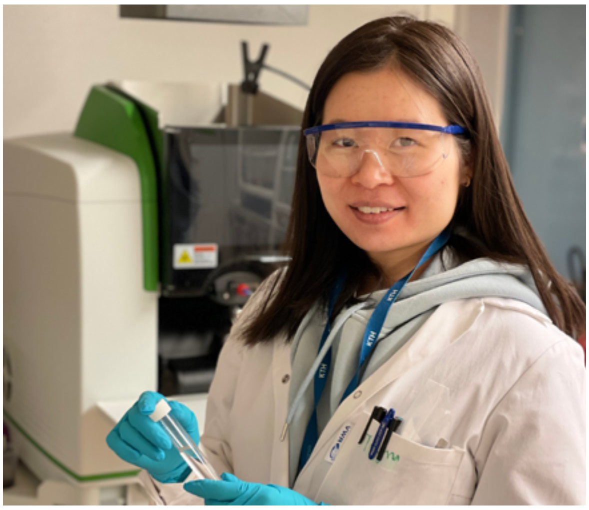
The goal is to find answers to unknown questions

The goal of Mikael Ekvall's research, not only within the Mistra programme, is to find answers to these types of questions. Why is the toxicity of nanoparticles changing? What are the far-reaching effects of nanoparticles that end up in nature? How do specific shapes, curvatures, and time intervals affect the toxicity of nanoparticles?

– The fact that there is so much you do not know is exciting. It drives me. A lot of research will be needed to measure natural concentrations in nature. I want to be involved in conducting that research, he concludes.

Mikael Ekvall is active in work packages one and three within Mistra Environmental Nanosafety.

"I am interested in how nanomaterials behave differently in many aspects"



Tingru Chang researcher at the division of surface chemistry and corrosion science , KTH

Tingru Chang is a researcher at the division of surface chemistry and corrosion science at KTH. For her scientific curiosity and freedom is key to new research results and breakthroughs.

– The fact that many things are unknown makes you feel excited. As a researcher you have the possibility to try out many different things, as well as your your fresh ideas, and go where your curiosity takes you. I think this is extremely important for any research progress, especially in a new area such as nanosafety, where we still know so little.

Characterisation of nanomaterials, metal release and their behaviors in various conditions

Her research focuses on the characterisation of nanomaterials, metal release and their behaviors in various conditions: what are the characteristics of different nanomaterials; what happens to them as they aggregate or disperse in different mediums; how do their properties affect the possible adsorption of natural organic matter or biomolecules in aquatic environments, and vice versa?

– To me it is really interesting that the property of nanomaterials, especially surface chemistry, is so size dependent and is important for how they affect the environmental toxicity and nanosafety.

Tingru Chang explains that her current research is quite closely related to her PhD-study on corrosion and characterisation of metal and alloys. For instance, copper alloys can be mixed with different metals that have copper as their principal component. But there are some important differences.

– It is much more challenging to study nanoparticles than massive materials because of their size. They are also more active which presents another difficulty. The kinetics of how they behave in liquids is also widely divergent.

Aim to group nanoparticles together

Together with her research group, Tingru Chang are in the process of scanning and examining different metal and metal oxides such as cobalt, copper and yttria to characterize how they behave in different environments, and how they interact with environment eco-ecorona or natural organic matter. The aim is to be able to group nanoparticles together – based on their properties, toxicity and the risks posed to the environment.

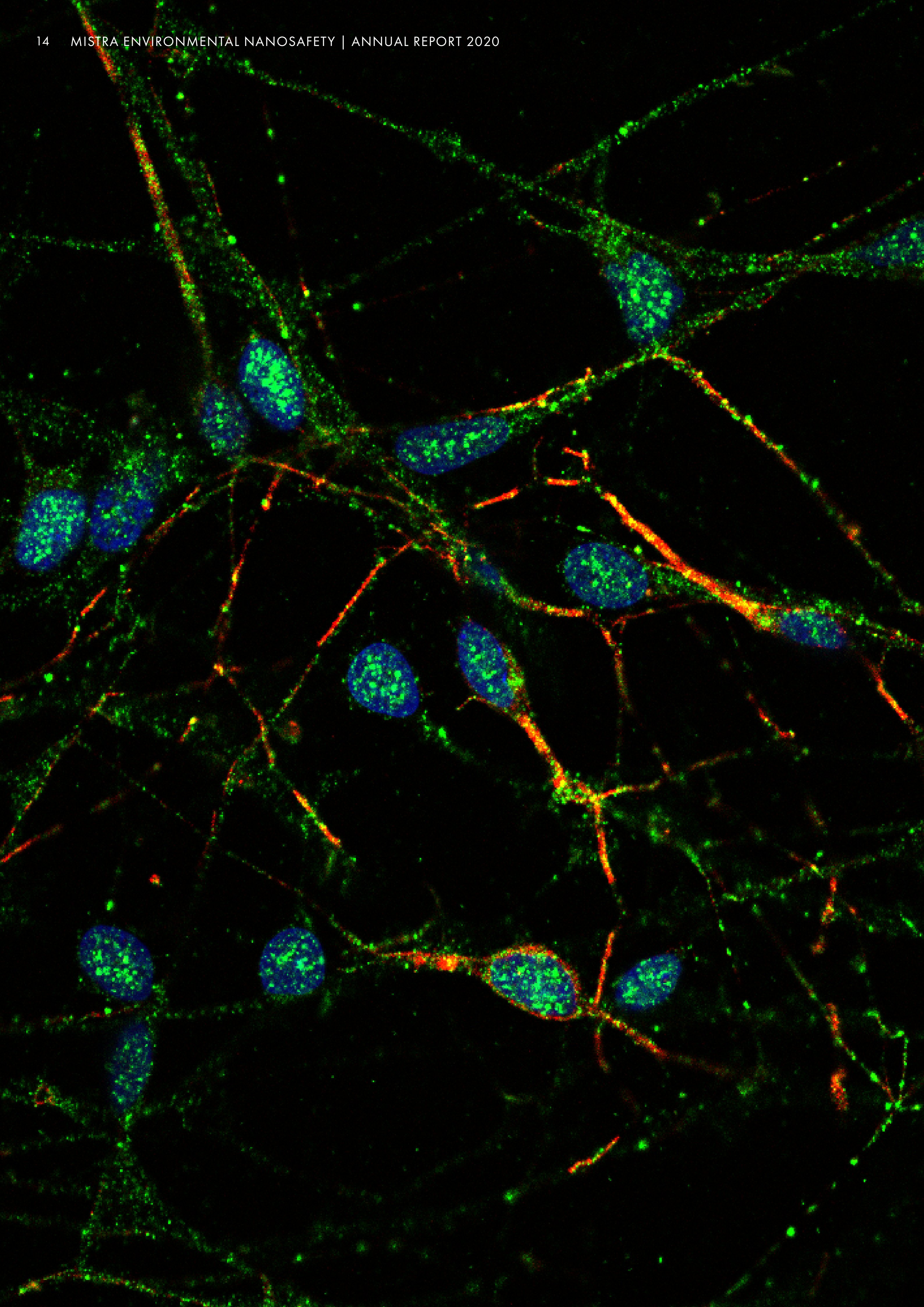
Another goal is to establish under what conditions the nanoparticles become toxic.

– For me it is not enough to just say that nanoparticles are harmful or not. I want to find out under what conditions they become more or less toxic. What actually happens when nanoparticles present in natural environments, and at what stage in the process when they become toxic?

Tingru Chang says that she hopes her research can support regulation and standardization of nanoparticle use.

– Working with nanosafety is about identifying exposure risks – both to the environment and to health. Our work can help in identifying the fate and characteristics of nanoparticles as they enter natural environments. If we can categorise nanoparticles together in different groups, we have come a long way in helping industries have better knowledge on how they might use similar new type of nanoparticles.

Tingru Chang is active in work package two of the Mistra Environmental Nanosafety programme.



"We still know very little about nanoparticles and their effect on the human body"

Govind Gupta postdoctoral researcher
Institute of Environmental Medicine, Karolinska Institutet

When Govind Gupta, postdoctoral researcher at the Institute of Environmental Medicine, Karolinska Institutet, arrived in Sweden to study how nanoparticles affect human health, he didn't know much about human cells as his thesis project had focused on aquatic organisms.

Today, he is developing advanced cell culture models to be able to ascertain the effects of nanoparticles on the brain, the lungs and the liver.

– I always felt that if you are a toxicologist you should know about the human consequences of exposure to different materials. It was the logical next step from my PhD studies on eco-toxicity.

Earlier research focused on effects of cobalt and tungsten

Govind Gupta's previous research, under phase one in the Mistra Environmental Nanosafety programme, focused on investigating the effects of cobalt and tungsten particles on brain cells, specifically on neurons that are involved in the development of Parkinson's disease. The results showed that exposure to cobalt particles or cobalt particles mixed with tungsten leads to a loss of cell viability of these neurons.

– The results highlight the potential harm to the brain from nanoparticles made of cobalt and cobalt mixed with tungsten. However, the concentrations we used in our experiments are much higher than what you might get in the human body. We performed our work using cell cultures.

As part of his work in phase two of the Mistra programme, Govind Gupta is now working to develop more advanced *in vitro* models of other organ systems. He will test a wider range of nanoparticles, including plastics, and they will also focus on cells from the lungs and the liver.

– The most important thing for me to find out is the mechanism that leads to cell damage or cell death. How are cells exposed to nanoparticles, and how do cells die; what actually happens in the cell?

The goal: to create a better understanding of processes

Govind Gupta says that these questions really drive him forward as a researcher: to create a better understanding of the processes under which nanoparticles affect the cells in the body. A big part of this work is to develop approaches to study nanoparticles under more realistic conditions.

– *In vitro* models, where you grow cells in the lab can never compare to the complexity of the human body. To be able to extrapolate results to human health you therefore need to use in vivo models, for example mice. But developing more advanced *in vitro* models can reduce animal testing.

The 3D cell culture models that Govind Gupta is now developing with his supervisor, Prof. Bengt Fadeel, are one step closer to using in vivo models. With the 3D models, he hopes to be able to get more in-depth results of how nanoparticles can potentially impact the human body.

– It is all about developing more realistic models and to use concentrations of nanoparticles that are closer to the exposures in real life. It is not enough to only establish that nanoparticles are toxic – you need also to be able to explain why and how.

He continues:

– Overall, we still have limited information about the safety of nanoparticles, and I hope I can contribute to more knowledge, and be part of developing more accurate and more refined models.

Govind Gupta is active in work package three in the Mistra programme.

"I want to push the discussion on nanomaterial regulation forward"

Maria Bille Nielsen PhD Department of Environmental Engineering, Technical University of Denmark

Before Maria Bille Nielsen started her PhD at the Department of Environmental Engineering at the Technical University of Denmark, she worked with water quality management in the Danish Environmental Protection Agency. But the interest in research, and the passion to gather knowledge and data brought her back to the academic field.

– What I like is that you can be curious in your work. You can adapt your scientific direction to societal changes and needs and hopefully provide useful contributions and solutions. You also have opportunity to really delve into specific aspects, and explore things in depth.

In her research, she focuses on environmental regulation of nanoparticles – an area that is becoming increasingly relevant as new nanomaterials are being developed, and products containing nanoparticles on the market are counting in the thousands.

– My overall research aim is to contribute towards developing the next generation of nanomaterial regulation. By identifying gaps in current regulation, I want to help ensure that nanomaterials are produced and used in a way that is safe and where environmental risks are properly assessed and controlled. Simultaneously, I want to highlight how certain regulations might be a hinder rather than an aid to a thriving nanomaterial industry.

Analysing the regulation gap

Maria Bille Nielsen and her colleagues are currently working on analysing how the EU chemical legislation REACH is regulating nanomaterials. In 2018, the EU adopted a revised annex under REACH, which came into effect in 2020. The annex contains a long list of information requirements that manufacturers and importers of nanomaterials or products containing nanomaterials need to comply with.

– Basically, there are many challenges in regulating nanomaterials. One has to do with the definition of nanomaterials, where room for interpretation still exists and it may therefore not always be clear whether a certain material should be covered by the nano-specific requirements. Another, huge factor is that much of the information that is being asked for under REACH, seems to be very challenging for companies to gather at this point.

Methods lack or only exist in scientific literature

In their analysis, they reviewed all the nano-specific information requirements under REACH and found that for some areas, there were methods or guidelines that companies could use to analyse their products. For other areas, however, methods lack, are under development or only exist in scientific literature, and are therefore not available as internationally accepted test guidelines or standards yet.

– It is clear that there currently is a gap between regulation and the methods and guidelines actually available. Today, it seems that companies are facing a huge challenge trying to comply with what the EU is asking for.

Why is the EU asking for information that companies are currently not able to provide? Maria Bille Nielsen reflects that you can see the gap between regulation and the market in two ways.

– By having a lot of requirements, EU is setting a gold standard and shows ambitiousness. EU sets out an urgent need and pushes forward the development of new methods for assessing safety and other aspects. Yet, on the other hand, placing a too heavy burden on the industry might serve as a barrier to innovation of new and novel nanomaterials.

Aims to identify areas of special importance in terms of risk

She hopes that her and her colleagues' research can help identify the areas which are most important to focus on – and as such support both regulators and industry.

– What is needed is a targeted effort to develop new methods and tests, and make them available to companies; especially in relation to determination of nanoparticles and material adsorption, degradation and exposure scenarios. A big part of that is also to show where methods available in scientific literature could be made use of as well as ensuring regulatory reliability and relevance of the information submitted by registrants.

Finally, Maria Bille Nielsen wants to push the discussion on regulation forward:

– It is important to keep adapting regulations to the specific properties of nanomaterials and figuring out how to do this in smart ways, which is definitely not an easy task. To really support both nanosafety and nanomaterial development, it is key that both scientists, regulators and companies are on the same page, she concludes.

Maria Bille is active in work package four of the Mistra Environmental Nanosafety programme.

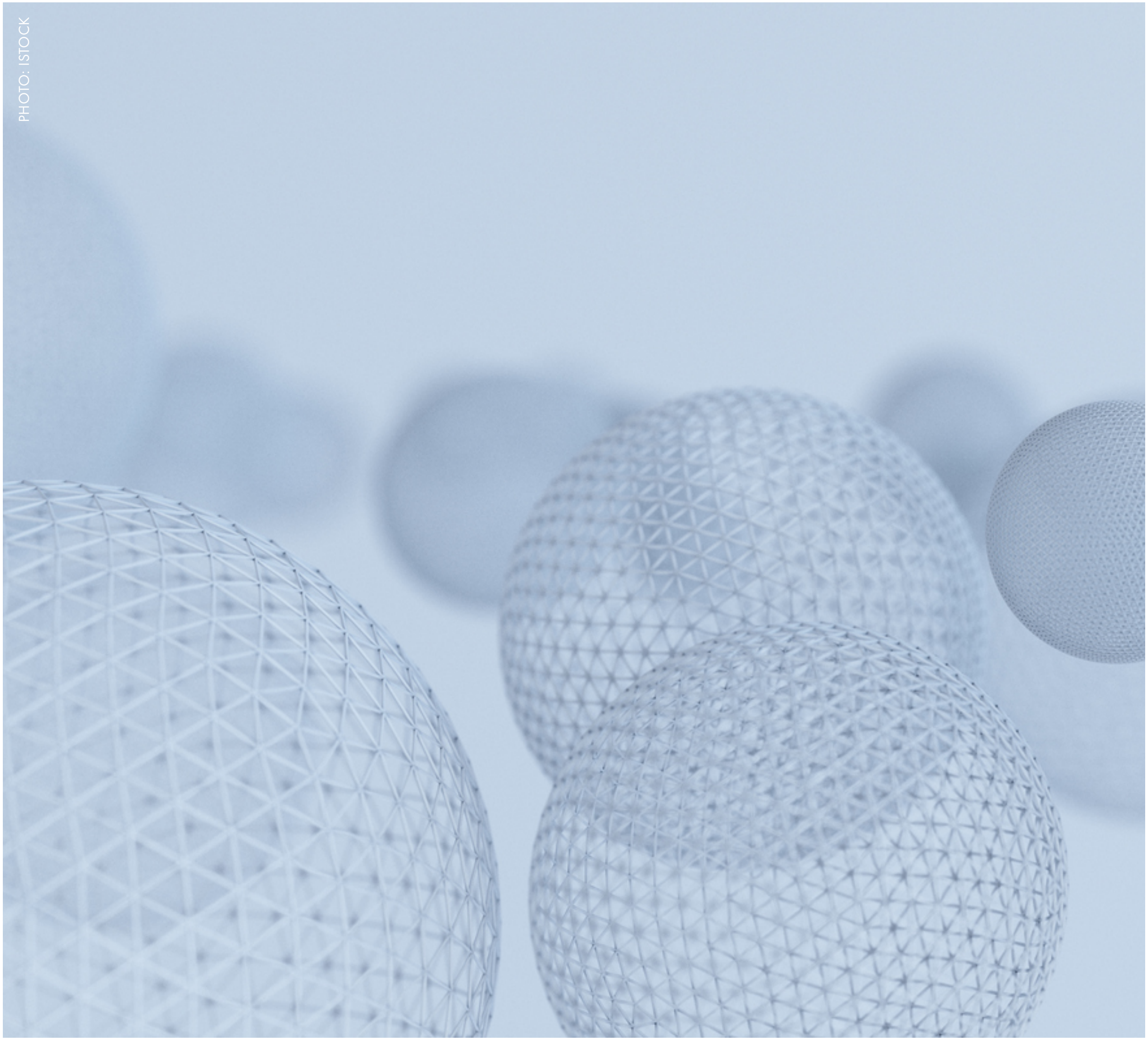


PHOTO: ISTOCK

5. A Chat to some of Our Programme Partners

"We are part of the programme to get more knowledge!"

Alejandra Castro Nilsson
CEO, SOLVE Research and Consultancy

SOLVE Research and Consultancy, founded in 2012, joined the Mistra Environmental Nanosafety programme in 2020. SOLVE supplies a number of services for companies working with nanomaterials, from formulation and aggregation analysis to stability testing, screenings and size and molecular weight determinations. SOLVE is the only company in Sweden to use the asymmetric flow field-flow fractionation (AF4) methodology. It allows for separation and analysis of macromolecules and particles in solution according to differences in size.

– While we do a full measurement of nanoparticles as part of our services, we want to find out more about the fate of nanoparticles. What happens to them when they enter the environment? Would they become a risk for living organisms?, says Alejandra Castro Nilsson, CEO at SOLVE Research and Consultancy.

Pharmaceutical and food companies are major customers

Many of the companies approaching SOLVE are pharmaceutical companies. They need support to characterise engineered nanoparticles – which are used for example as drug carriers and for cancer treatment.

Recently, SOLVE has also started to work with companies within the food industry. They are eager to find out whether nanoparticles can unintentionally end up in food products – for example from the soil the vegetables are grown in, or from processing and packaging.

– With the technology that SOLVE has we can analyse food, water and soil samples to see if they contain non organic or organic nanoparticles and determine their concentration. But to determine where these particles came from, and if they are toxic or not, is a more challenging task. This is a big issue, and something which is becoming increasingly important to companies all over the world.

Alejandra Castro Nilsson explains that water and soil samples presents particular challenges since these can contain a lot of different nanoparticles, some of them in low concentration but in sufficient amount to be a concern. Added to this there is also the need to distinguish between naturally occurring nanoparticles, engineered nanoparticles and degraded nanoparticles from for example plastics or pesticides.

– For us to develop our services, we are really eager to get access to knowledge and tools that can help us analyse toxicity and composition of nanoparticles. What techniques can we use, and how could we incorporate them into our services? We also want find out more about how nanoparticles affect the environment.

The Mistra programme is a natural fit for SOLVE

Alejandra Castro Nilsson and her collegues started SOLVE while they were still at university. They saw a niche to develop refined characterisation services for companies, using the asymmetric flow field-flow fractionation technique.

– It is a natural fit for us to be part of the Mistra programme. As a company we want to contribute towards the safe use of nanoparticles.

– Personally, think the whole area is really interesting. How can you manage nanoparticles better? How can you get more knowledge now, and avoid safety issues and public concerns in the future? she concludes.

"It is a huge benefit to be part of a larger network of contacts"

Erik Nilebäck
project manager at Chalmers Industriteknik

Chalmers Industriteknik, which among other things leads the national innovation programme SIO Grafen, joined Mistra Environmental Nanosafety 2020. The purpose of SIO Grafen is to create a pool of engagement and community around graphene and enable practical applications by gathering important actors from academia, industry and institutes.

Graphene is a nanomaterial with only one atomic layer thick carbon layer. It is stronger than steel, transparent, flexible and at the same time one of the best thermal and electrical conductors. Graphene can be used in the manufacture of electronics, surface layers, composites, sensors and for energy solutions for electricity generation, storage and cooling.

Erik Nilebäck, project manager at Chalmers Industriteknik, says that they have already begun to see benefits from the collaboration with the Mistra programme.

– We have started a strategic project within SIO Grafen that will focus on work environment and safety. We are interested in the needs of our members and what the handling of graphene looks like in the industry. This work now includes researchers from the Mistra programme.

Access to researchers and experts key to ensure innovation and development

Erik Nilebäck believes that access to researchers and experts is important for companies that produce and use graphene. Especially since a lot is happening in the field with regard to how nanomaterials should be registered. In 2020, legal requirements were added under the EU regulation REACH for companies that manufacture or import nanomaterials. Companies are now required to report specific information on nanomaterials. The REACH regulation means that all chemicals in excess of 1 tonne in weight, which are either produced in the EU or imported, must be tested for health and environmental aspects.

– From an industrial perspective, there is a need to be able to ask questions about what applies. How do you register correctly under REACH? Can the product be included in the graphene registration that already exists or should it be registered as a new nanomaterial?

He hopes that the collaboration between Chalmers Industriteknik and Mistra Nanosafety will contribute to increased knowledge among companies regarding the entire chain, from the production of graphene, production of graphene applications and recycling of materials and products. It can be about putting industry representatives in contact with researchers within the Mistra programme, or about conducting different types of workshops to raise the state of knowledge. Different types of collaborations and joint research applications may also be relevant.

– The recycling perspective is becoming increasingly important. And the work environment perspective. Therefore, it is good that we are now part of a larger network of contacts as we have access to researchers within Mistra. It will be interesting to see how we can further develop the collaboration in the future.

The wish – a national collective resource for companies

In the future, however, Erik Nilebäck wants to see a collective resource or platform that can function as a complement to authorities such as the Swedish Chemicals Agency or the Swedish Maritime Administration. The platform should preferably be national and function as a sounding board and support for companies working with nanomaterials. The long-term perspective and the practical perspective are important.

– There is a need to group nanomaterials that are similar to each other. Companies need to be able to ask questions and get help with different parts of the management. Today, we have many good initiatives, such as the Mistra programme and SweNanoSafe, but we still lack something more lasting – which has the industry in focus.

– I believe that we need a national investment to be able to strategically support the continued development of nanotechnology and nanomaterials in Sweden, he concludes.

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7. Communication and Impact Work

Communication, collaboration and impact are key features of the Mistra Environmental Nanosafety programme. In 2020, a number of strategic communication activities were organised to increase knowledge and understanding of nanosafety, and the research within the programme. Many of the activities moved online due to the ongoing corona pandemic – a working practice that offers many benefits in terms of gathering people from different parts of Sweden. Some key communication highlights include a workshop on impact pathways to support our researchers to work with outreach and research impact, our public event on nanosafety and nanoplastics during Lund Future Week, targeted at the general public, and our strategic media work to highlight the need for more targeted research on nanoplastics and risks to the environment. We have also put effort on building networks with strategic partners and partner universities as a way to increase visibility and make sure that we are reaching our prioritised target groups. Finally, we have focused on highlighting key researchers and research undertaken within the programme in a series of articles to showcase both the people, and the research questions of key relevance to both our programme and the wider field of nanosafety.

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Scientific conferences and seminars

17th Nov 2020:
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Surber, N. 2020 "GoNano Winter School: Learning to Create Social Value with Nanotechnologies." 4–7 February 2020. RMIT Europe, Barcelona, Spain. Participant.

Surber, N. 2020 "GoNano Webinars on Co-Creation." May 28, June 4 and June 11, 2020. GoNano Project, Zoom. Participant.

Surber, N. 2020 "The GoNano Online Conference: Responsiveness to Societal Needs and Values in Nanotechnologies and Beyond." 29 October, 5 November and 12 November, 2020. GoNano Project, Zoom and YouTube. Participant and Discussant (5 November.)

Surber, N. 2020 "Science, Precaution, Innovation: towards the integrated governance of new technologies." 14–15 October, 2019. Center for Interdisciplinary Research, Bielefeld University. Participant.

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Annex 1. Progress Report for the Mistra Environmental Nanosafety Work Packages

SCALING UP: ASSESSING STRUCTURAL CHANGE AND FATE OF NANOPARTICLES WHEN ENTERING NATURAL ECOSYSTEMS

WP1 examines how different nanoparticles' fate and characteristics are changed by the natural environment. It will scale up the studies of nanoparticles performed in work packages 2 and 3 (toxicity and weathering) in order to make recommendations to regulators, industry actors and wider society on which nanoparticles should be either avoided or used with caution.

In 2020, the work in WP1 progressed on developing methods for analyses of the fate of nanoparticles when passing through a natural wetland ecosystem, as well as on modeling the interactions of organic molecules interactions with the nanoparticles. Molecular dynamics (MD) simulations methods were developed for modeling the interaction of organic molecules with SiO_2 nanoparticles of different shapes and variable charge density. Our results clearly show that particle shape has a considerable effect on the interactions with organic molecules and a manuscript addressing those advancements is under submission. Furthermore, MD simulations of polystyrene nanoparticle interactions with organic molecules show that surface functional groups, as well as ionic composition, regulate the interaction strength. The next step is to compare simulation results with experimental data. Another highlight from WP1 is that we have successfully designed, developed and tested a unique, replicated wetland systems where experimental studies on nanoparticle fate, aggregation, as well as effects on plants and animals will be studied. Preliminary results from a pilot study are very promising, showing that:

- we can detect the nanoparticles (polystyrene with gold core);
- the particles are distributed unevenly in the wetland environments and in the sediments,
- some types of organisms, such as the digging invertebrate *Asellus*, takes up more particles than others.

Hence, our preliminary results indicate that we will be able to predict the distribution of nanoparticles in natural ecosystems, and thereby also their potential toxic effects. This wetland-infrastructure will be utilised by several other WP´s within the project.

CHEMICAL WEATHERING AND BIOCORONA FORMATION ON NANOPARTICLES

WP2 focuses on generating a mechanistic understanding of what happens to nanoparticles if dispersed and weathered at natural environmental conditions, for instance how such transformation influences their particle composition/characteristics, dissolution pattern, colloidal stability, fate and toxic potency? It focuses mainly on interactions with different kind of natural organic matter and its specific biomolecular constituents (for instance secreted biomolecules). Special attention is given to materials that undergo specific transformations that change their toxic effects (investigated in work package 3). These aspects are, despite many efforts, relatively poorly understood in the context of nanoparticles transformations and toxicity.

The main focus during 2020 has been to investigate how the presence of natural organic matter (NOM) and its surface interaction/adsorption and exchange with 14 different metal and metal oxide nanoparticles (NP) influence their short-term environmental transformation and dissolution properties in synthetic freshwater. The study comprises both less investigated metallic NPs (Sb-, Sn-, Co-, Ni-, Mn-containing and Y_2O_3) and more extensively studied metal oxide NPs (ZnO and CeO_2). Parallel ecotoxicological studies are on-going in collaboration with WP3 on a few selected metal NPs (e.g. Mn, Sn, and Ni) which showed no effect, increased release or reduced dissolution patterns in the presence of NOM. A manuscript in preparation.

Other activities during 2020 include in-depth fundamental transformation/dissolution studies of Co and Ni NPs and their binary combinations (Co_3Ni, CoNi, and $CoNi_3$) in synthetic freshwater with and without NOM or ecocorona biomolecules (excreted from Daphnia).

These studies address aspects of stability and mobility as well as importance of NP synthesis method, microstructure, phase composition, crystalline structure and corrosion properties and kinetic adsorption effects on the NP biotransformation/dissolution characteristics. A manuscript has been submitted for publication.

The results of the screening study formed the basis for which NPs (Y_2O_3, CeO_2, Sb_3O_4, Co) to focus on in forthcoming in-depth studies and testing in WP2, WP3 and later on also in WP1. These studies are on-going. Initial pilot studies of single particles of Co, Ag and Cu have been initiated to further assess the interaction with NOM with respect to molecular species adsorption and metal dissolution.

TRANSFORMED NANOPARTICLES ON AQUATIC FOOD CHAINS AND CELL MODELS

WP3 examines the effects of transformed nanoparticles on aquatic organisms, food chains and models. Nanoparticles may enter the environment through intentional releases (e.g., environmental remediation and pesticide use) as well as through unintentional releases from nanoparticles utilisation, atmospheric emissions and solid or liquid waste streams from production facilities. Once introduced to the aquatic environment, nanoparticles will undergo a multitude of transformation via a number of processes including dissolution, aggregation/agglomeration and subsequent sedimentation, as well as interactions with abiotic and biotic components present in the aquatic system. Agglomeration followed by sedimentation of nanoparticles may lead to deposition on the sediment surface where benthic organisms may be particularly at risk of exposure.

The overall work carried out in the last year in WP3 includes screening of selected nanoparticles, nanoplastics, after collaboration meetings within the work package and studies were carried out on polystyrene nanoparticles (PSNPs) to begin with. Screening for cytotoxicity and other sub lethal endpoints [ROS, EROD activity] induced by polystyrene nanoparticles of 3 sizes (25, 100 and 2000nm) after short term exposure (24 hour) with rainbow trout gill and liver monolayer cell lines. The same particles were tested on a variety of human cell lines derived from the gastrointestinal tract and the liver and we did not observe any cytotoxicity. A study of CuO nanoparticles in which the WP could show that these particles dissolved in the acidic environment of the lysosomes leading to oxidative stress and cell death was completed (poster abstract). Additionally, WP3 finalised and submitted a study of a panel of amorphous silica particles prepared by our partners at Nouryon. This work has shown that small, amorphous silica particles elicit pro-inflammatory effects while silane modification of the particle surface serves to mitigate these effects.

Ongoing work involve following long term metabolic changes in exposed D. *magna* by NMR based metabolomics and to link the binding of proteins in D. magna intestine to nanoparticle toxicity. In the collaboration with the Sph3roid project (Lead by Tobias Lammel) development of a standardised method of culturing 3D rainbow trout liver cells is ongoing for further in vitro screening and long term studies. Plans are made to screen a number of rare earth metals nanoparticles using similar methods with and with the addition of ecocorona supplied by Lund University but is yet to be carried out. The WP is planning to extend the polystyrene material and have had discussions with the company Cellevate on who will prepare PS fibers using the electrospinning method.

In addition to ongoing post doc work in the programme, Dr. Gupta received a research grant from the Institute of Environmental Medicine for investigations of metal nanoparticles against SARS-CoV-2. Prof. Fadeel is also Chair of the scientific expert panel of the national nanosafety platform, SweNanoSafe, hosted at the Institute of Environmental Medicine at Karolinska Institutet, and co-chaired the workshop on nanomaterials in the environment in November 2020 with Prof. Joachim Sturve.

PROACTIVE RISK ASSESSMENT, REGULATION AND CREATION OF STAKEHOLDER LEARNING ALLIANCES

WP4 works with proactive risk assessment, regulation and the creation of stakeholder learning alliances. Of importance is to modify nanosafety regulation to reflect real risks by for example include the consideration of corona formation in risk assessments. Furthermore, this work package aims to support the effective flow and use of information between experimentalists, regulators, and industry, and to help mobilise stakeholders to engage in responsible innovation and risk governance.

Key highlights in 2020 include: the publication of a study with regard to the correlations between natural organic matter (NOM) or "ecocorona" and nanoparticle aquatic ecotoxicity in which the WP found that ecotoxicity without NOM is a conservative proxy for ecotoxicity with NOM. This work was supplemented by a study on the availability and suitability of methods needed to comply with the new regulatory provisions on NMs for physico-chemical characterisation and environmental fate and effects. Here the WP found that 80 percent of the information requirements and a bit more than 40 percent of the waiving criteria in the new REACH Annexes are supported by available methods and that most of the relevant methods are included in recent OECD guidance documents or ECHA guidance.

The WP also published a paper in *Nature Nanotechnology* on the fact that the number of consumer products in Europe has surpassed 5,000 in which we discussed the advances and challenges towards consumerization of nanomaterials. We have yet to find any nanorobots commercialized for consumers, but in a review of current regulations of nanorobots, the WP finds that it is unclear which specific regulations might be applicable and recommend nanorobots should be subject to broad, risk-related studies as well as dialogues with stakeholders and the public about the definition, purpose and controllability of nanorobot applications.

With regard to stakeholder analysis, the WP reviewed the state-of-the-art of SA within environmental management and regulation and found that there is a lack of clear definitions of key-terms such as "Stakeholder" and "Influence" and that transparency with regard to methodology, results and decisions made is of paramount importance as it otherwise undermines the credibility of Stakeholder analysis.

WP4 also presented a paper titled "Sculpting Responsibility? Historicising Nanoscience and Technology Development in Attendant Research and Innovation Ethics Practices" at the 4S Annual Meeting.

Finally, the WP organised the annual Mistra Environmental Nanosafety meeting, during which the question of research impact was discussed with various stakeholders within the programme.

SAFE HANDLING OF NANOMATERIALS AFTER PRODUCT END-OF LIFE

WP5 focuses on researching and developing appropriate strategies for the end-of-life management for products containing nanomaterials. This research is critical to minimise human and/or environmental exposure. Risks include both immediate exposure of humans and a long-term exposure of the ecosystem, including humans, due to leaching and accumulation. More knowledge is needed in the area of waste management and recycling, and of risks related to handling nanomaterials, in order to develop appropriate strategies, which both foster development and minimise risks.

Key achievements in 2020 include: finalising experimental studies on emissions from pilot scale combustion experiments under conditions resembling waste incineration (e.g. following the European directives for waste incineration). The materials combusted were two types of CNT reinforced polymers. A paper was submitted in October 2020 and is still under review.

The WP performed experimental studies of pyrolysis as a tool for separating CNTs from the polymer matrix. The goal for 2020 was to optimise the experimental settings to generate a CNT product that later can be re-introduced in a new polymer, evaluated by comparing the properties of a polymer with new CNTs with that of a polymer reinforced with recycled CNTs, illustrating the proof-of-principle. The proof-of-principle experiments will take place 2021.

The WP also analysed the handling chains at a waste recycling facility from a risk perspective of emissions of nanomaterials, and performed exposure/emission measurements at the same recycling facility recycling electronic waste and car scrap – two waste flows identified as likely to contain nanomaterials in the future. The measurements will primarily be analysed from the perspective of nanoparticle emissions today.

WP5 published its first tests using XAS (synchrotron based spectroscopy) as a tool for determining speciation of trace metals in ash from waste incineration in the journal Energy and Fuels. Finally, the work package organised a workshop on NanoWaste on 4th of November, attracting a broad audience including representatives both from industry, academia and the waste industry organisation Avfall Sverige.

MANAGEMENT, COMMUNICATION, ECONOMY AND STAKEHOLDER RELATIONS

WP 6 focuses on ensuring the smooth facilitation of the programme in phase two. It focuses on supporting a steady progression of the other work packages: internal and external communication, including societal impact. Another aim is to build networks, support platform building and manage industrial, scientific, authority and other stakeholder interaction.

During 2020, the work package organised a number of programme meetings online due to the ongoing corona pandemic, as well as focused on planning and launching the mentorship programme for younger researchers. Effort was also put into assisting other work packages in organising targeted events, as well as on outreach events on nanoplastics aimed at the general public.

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