Report 2021:2

SweNanoSafe

Swedish National Platform for Nanosafety

3rd Annual Workshop of the SweNanoSafe Research Network: Nanomaterials in the Environment

A report from SweNanoSafe's workshop, 17th November 2020

Nanomaterials in the Environment

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Preface

On 17th November 2020, SweNanoSafe (the Swedish National Platform for Nanosafety) together with the Swedish Environmental Protection Agency (EPA), gathered about forty participants for a virtual workshop on nanomaterials in the environment. The presentations and discussions focused on nanomaterials in the environment and also, the national plastic coordination assignment (microplastics) was introduced.

The event brought together participants from different stakeholder groups, mainly from academia and research institutes but also from national authorities, thereby providing opportunities for networking and further cooperation among stakeholders. Participants represented primarily Swedish universities, research institutes and industry, as well as one participant from a British university, one participant from a Danish university and one participant from the Norwegian Veterinary Institute.

On behalf of SweNanoSafe, we express our gratitude to all participants for their valuable contribution to the discussions and results of the workshop.

SweNanoSafe

National Platform for Nanosafety

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About SweNanoSafe

SweNanoSafe, the Swedish National Platform for Nanosafety, has a mandate from the Swedish Government to promote safe handling of nanomaterials. This assignment involves communicating and disseminating knowledge about the risks involved with nanomaterials to academia, authorities, businesses and organisations, and to identify any obstacles to safe handling. The platform aims to constitute a national forum for dialogue and cooperation where the abovementioned stakeholders can collaborate by sharing knowledge and experiences as well as discussing, developing, and influencing the implementation of nanosafety in society.

The platform was originally established in 2016 at Swetox, a former academic research centre. Since 1st January 2019, the platform is hosted by the Institute of Environmental Medicine (IMM) at Karolinska Institutet (KI).

The platform is currently managed by a Steering Group with members from KI/IMM and the Swedish Chemicals Agency (KemI) and a Coordination Group for the operational management of the platform's activities (employees from KI/IMM). The platform organisation also comprises an Expert Panel, a Council of Authorities, a Research Network, an Education Network, as well as a website (www.swenanosafe.se).

Background and aim of the workshop

Generally, a disconnection persists between the research examining risks to human health and the one examining the environmental impact due to nanomaterials (NMs). In the case of engineered nanomaterials (ENMs), this has been perpetuated by the relatively limited overlap in human and environmental exposure pathways. Hence, an overall approach is needed to manage the applications and implications of nanotechnologies. The European Green Deal is the roadmap for making the EU's economy sustainable by turning climate and environmental challenges into opportunities across all policy areas and making the transition just and inclusive for all. The One Health concept recognises that human health is tightly connected with the health of animals and the environment since they interact with each other.

Topics/themes for the workshop were:

- The identification of emissions and sources of ENMs, including micro- and nanoplastics and their environmental fate
- Mechanisms of NMs toxicity
- The transformation of NMs and microplastics in aquatic environment and their impact on aquatic life
- NMs screening methods using *in vitro* fish models
- Trophic transfer of NMs in aquatic environment

The main aim of the workshop was to gather national researchers and invited international guests in the field to overview how nanomaterials behave in the environment with regards to their fate, bioavailability and effects. As a secondary aim, the presentations and discussions at the workshop provided important input to an on-going SweNanoSafe assignment (given to Goodpoint) to overview and summarise the current knowledge on nanomaterials in the environment in a separate report.

The workshop in brief

The 3rd annual workshop of the SweNanoSafe research network was organized by members from the SweNanoSafe Expert Panel in collaboration with members from the Mistra Environmental Nanosafety programme. The agenda of the workshop included a keynote lecture on nanomaterials in the environment, the presentation of the national plastic coordination task of the Swedish EPA, several presentations from both senior and junior researchers in the field, as well as a presentation about the SweNanoSafe project on nanomaterials in the environment introducing targeted group discussions for input. For details, see the full agenda in annex 1. Concluding discussions were conducted in plenum.

Introduction to the workshop

Moderator Lennart Gisselsson, project manager at the cooperation office of the University of Gothenburg, introduced himself as a host of the day. Lennart also initiated a poll via mentimeter interactive presentation tool, to interact with the workshop participants regarding their expectations of reasons for their attendance.

Co-chair Joachim Sturve, University of Gothenburg and member of the SweNanoSafe Expert Panel, officially opened the workshop by welcoming everyone and introduced the field of nanosafety, emphasizing that despite the fact that Sweden is an active country with regard to nanosafety research, still more research is needed in this field.

Co-chair Bengt Fadeel, from Karolinska Institutet and chair of the SweNanoSafe Expert Panel, presented the background, aims and activities of SweNanoSafe, introducing also the aim of the workshop to improve our understanding of each other's needs, towards the goal of developing useful and safe nanomaterials.

Julia Taylor, from the Swedish Environmental Protection Agency (Naturvårdsverket), gave some opening remarks regarding nanomaterials and nano- and microplastics in the environment, indicating that nanomaterials are expected to be hazardous and us, as a community need to corporate on sustainable plastic use and quantify the knowledge gaps regarding their safety.

Keynote on Nanomaterials in the Environment – Professor Richard Handy, University of Plymouth, UK

The key-note speaker of the day was professor Richard Hand, from the department of Biological and Marine Sciences (Faculty of Science and Engineering) at the University of Plymouth, with a speech entitled "*Nanomaterials in the Environment*".

Some highlight from the talk of Richard Handy is summarised below:

- The key fact that triggers the conduction of environmental risk assessment is chemicals being persistent, bio accumulative and toxic (PBT). The persistence of chemicals, including nanomaterials, is strongly correlated with their transformation rate and this defines their fate.
- It is very important to focus on the surface water concentration, distinguishing between the predicted and the actually measured concentration. Researchers usually heavily rely on the predicted concentration because measurements are complex. The few measurements that exist are in line with the predicted ones.
- Regarding the transformation rate – it´s rare for a nanoparticle to stay pristine, thus,

environmental levels change because ENMs are transformed (maybe multiple times) due to several environmental exposures including UV degradation, oxygenation etc. The transformation products can aggregate with each other or with other particles in the environment (e.g. sediment), resulting in a very complex system which is hard to model.

- Alterations to the surface corona can interfere with absorption mechanisms and have a toxic potential both in the environment and in living organisms.
- The key factors that influence the bioavailability of ENMs are the exposure concentration, the exposure route (water, diet), the particle setting in the exposure, factors that affect aggregation such as size, surface coatings, pH and transformation. All the processes that influence the pristine material and result in transformed derivatives, can affect the bioavailability and uptake mechanisms of the ENMs.
- Gut anatomy and different types of gut microbes affect the uptake and transformation of ENMs in different wildlife species. This is an important aspect in terms of retaining biodiversity in animal kingdom, which constitutes one of the main goals of sustainable development.
- The impact of ENMs on internal organs in an organism depends on the species, internal dose, whether exposure is chronic or acute and any potential particle transformations. In general, similar pathologies are observed for both ENM and chemicals in fishes e.g trout, but at different time frames.
    - An experiment was also presented in this context, where waterborne exposure of trout to single-wall carbon nanotubes (SWCNTs) caused acute gill injury, without time for bioaccumulation. Kidney and spleen showed minimal effects, while liver tended to be the target organ of toxicity. The most commonly used route of exposure during experiments in fishes is the chronic dietary route, because it is considered to be the most relevant (via the food chain).
- The method of single particle IC-PMS is under development for different biological matrices (animal tissues), while the traditional bioaccumulation test method (OECD TG305) is expensive, unrealistic to use for all ENMs and also unethical due to animal use. Thus, NanoHarmony is addressing these issues by developing new testing methods. Moreover, a tiered system approach for toxicity testing has already been established by the Nanomaterial Fate and Speciation in the Environment project (NanoFASE).
- Experiments also showed that CuO remained bioavailable in fresh soil, thus exposing earthworms to soil enriched with CuO NPs caused accumulation in the earthworms. However, aged soil enriched with CuO NPs caused different effects, with the CuO NPs being extracted more easily. Therefore, toxic effects depend on the type of ENPs coating (transformation) and time. Particle size, shape and coating are dependent on the type of organism, the life cycle stage and the environmental matrix that an organism lives in and constitute very important aspects that need to be considered during risk assessment.
- Exposure is never constant and varies with seasons and environmental conditions. Thus, pulse exposure and the rate of change of exposure drive the toxicity of chemicals. It is possible that the same principle also applies to ENMs, for example when pH conditions change, NM properties also change, potentially resulting in higher risk for NM toxicity.
- Dissolved metals also form particles in tissues and the severity of effects depends on the

organism, life stage, material composition etc.

Questions & answers

The keynote presentation was followed by a questions and answer session, moderated by Professor Bengt Fadeel. A summary of questions from the audience and the answers from Professor Richard Handy is provided below:

Q: How long does the coating usually last? And how is surface coating correlated to toxicity?

A: Coating is not constant – changes may happen depending on the organism, environment etc. The particle core chemistry gives the best correlations with toxicity and the coating may not be the most important determinant. So, if there is a short gap where the core chemistry is available even at short time frames. Therefore, toxicity can be caused either by coating itself (coating hypothesis) or because of the presence of small gaps in the coating which allow nanoparticles to show up and exert their toxicity

Q: So, there must be an interplay..?

A: There is always an interplay between coating composition and nanomaterial itself, coating effects may change depending on the species, environment, ageing. In some cases, coating may also mask any potential nanomaterial toxicity.

Q: How important is the morphology of ENMs, pyramids, tubes, etc – how does that affect?

A: Morphology on NM is important. The shape plays an important role in terms of toxicity exertion (rods vs spheres), even though more research is needed. No studies have been conducted in environmental media, but only in cell media and indeed, nanoparticles are taken up by cells through different ways depending on their shape. Usually cells distinguish between the different chemistry around them e.g different crystallinity, even it is unknown how, but for shape only few studies are available today. In general, different cells have been tested to understand their ability to recognize different shapes, e.g. macrophages do recognize shape…

Q: Anti-microbial NMs – how important is that effect on environmental microbiota? And how does that reflect the different functions of the microbiota? Is it a key concept to correlate metabolic diseases in humans, where microbiota plays an important role, to the NM exposure?

A: Microbiota and NMs are not totally understood and investigated but some studies conducted on fishes, show that microbiota changes depending on the type of NM (silver, gold). For example, Ag does change the microbiome and this is different for AgNPs than for $AgNO_3$. But we don´t see changes in nutrition despite the change in microbiome – the effect on biofunction seems to be small. But there are huge gaps in the understanding of the normal microbiome. A meta-analysis is needed!

The questions and answer session was closed with a concluding remark from Bengt Fadeel saying that studies conducted on eco health are of great relevance for studies on human health.

Nanomaterials in the Environment – an interim report – Arne Wallin, Freja Milton (Goodpoint)

Freja Milton, consultant at Goodpoint AB, presented an on-going project on nanomaterials in the environment commissioned by SweNanoSafe, and introduced the interim report which was in the process of making. The presentation gave an brief overview of he sources of NMs and their emission routes, the analytical methods used for their detection, their fate and the mechanisms of their toxicity as well as considerations with regards to risk assessment and sustainable development. These topics were introduced as the starting point for further group discussions.

Selected highlights from the presentation is summarised below:

- Emissions of NMs can occur in the whole course of NM life cycle and the emissions can be both intentional and unintentional.
- Emission process can be quite complex and NMs can emit to the atmosphere, surface water, or soil. From the air NMs can be deposited further to sediment, soil, ground water and to waste incineration as well.
- There is a lack of standardized methods to measure at emission sources and in most cases, emissions are based on prediction rather than measured in real life, because it is hard to define the detection limits.
- Measurements can show variation in concentrations depending on type of water that NMs are dissolved or polluted into.
- The fate of NMs depends on:
    - Chemical transformations (Redox reactions, photochemical reactions, interaction with other pollutants, dissolution)
    - Environmental conditions (pH, temp, natural organic matter, light, NM properties, ions)
    - Physical transformations (Agglomerations, aggregations, adsorption, deposition)
    - Biological transformations (Biodegradation, Biomodification)
- NMs exert their toxicity through:
    - Formation of ROS, generating oxygenative stress
    - Release of ions (mostly happens in aquatic life cycle stage)
    - Internalization
    - Biological surface coatings (that can attach to the surface of an organism)
- Risk assessment and risk management are challenging because there is a diverse group of chemical substances and each substance tends to be assessed on a case by case basis, making standardized processes difficult.
- Limitations of the interim project include the following: application primarily to Sweden and Europe, not including human health effects, not including secondary NMs, and not covering the use of NM for remediation of contaminated soil.

Group discussions

Freja Milton, Goodpoint, then introduced the tasks for preparation in group discussions, taken place in six separate break-out rooms. The group discussions were aimed as to provide input from the workshop participants to the project on nanomaterials in the environment, initiated by SweNanoSafe.

The workshop participants were on beforehand assigned to different discussion groups. Each group appointed a rapporteur that summarized the group work in plenum. Unfortunately, not everyone participated in the discussions, hence the groups were reconstructed and one of the topics for the group discussions was left out.

Each group were provided with a few questions as basis for their discussion. Those questions together with a brief summary of the reported discussions are provided in the following paragraphs.

Group 1 - Sources and emission routes

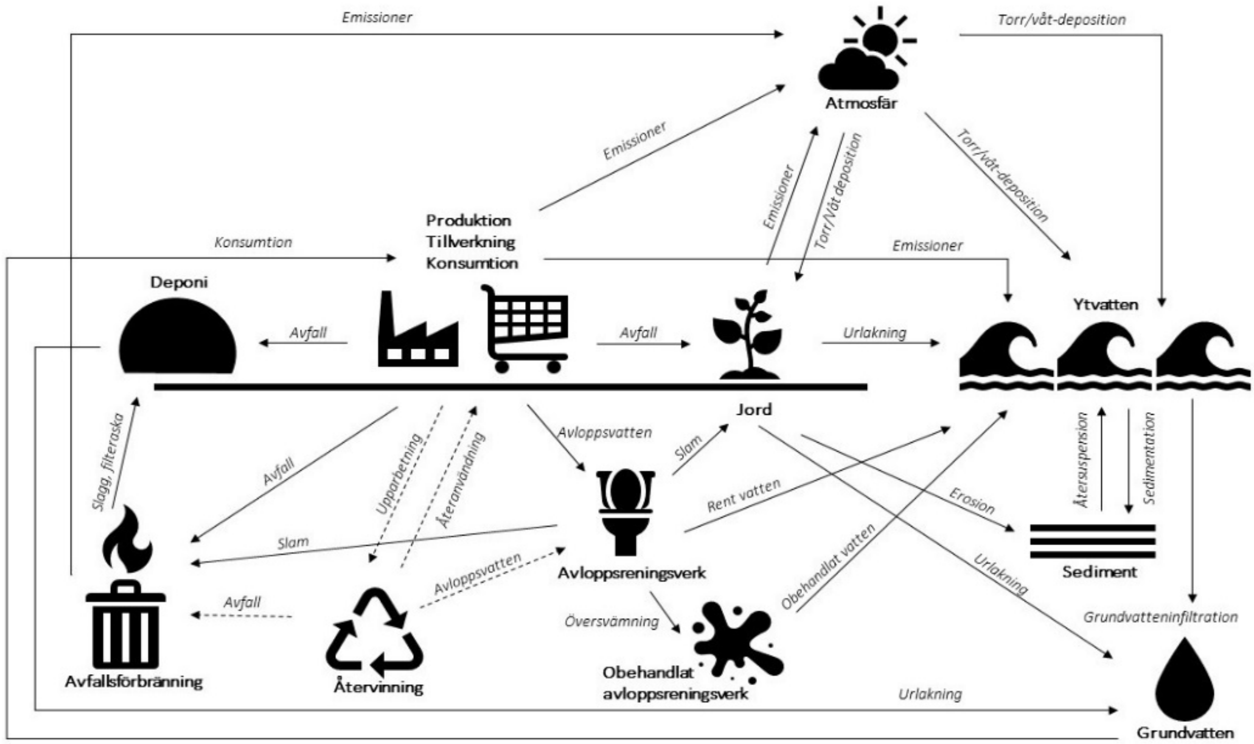

Figure 1. Illustration to overview the sources and emission routes of nanomaterials in the environment, provided as a discussion material for Group 1.

- **Based on your experiences, where can emissions of ENM occur?**
  During the whole life cycle chain, but maybe emission is higher during production and waste management.
- **What emission routes would you consider as the most important? (Do you miss any other transmission paths / sources in the figure?)**
  There is a need to test and follow NMs throughout their life cycle to see where emission occurs.
- **What challenges and knowledge gaps exist in this area? Speculate and discuss.**
  Not that much, unfortunately industries themselves may not have that much knowledge about emissions.

A question whether there are specific NMs that are more important and more often emitted was raised and it was concluded that there are very few measurements hence there is a lack of data.

Group 2 - Analytical methods

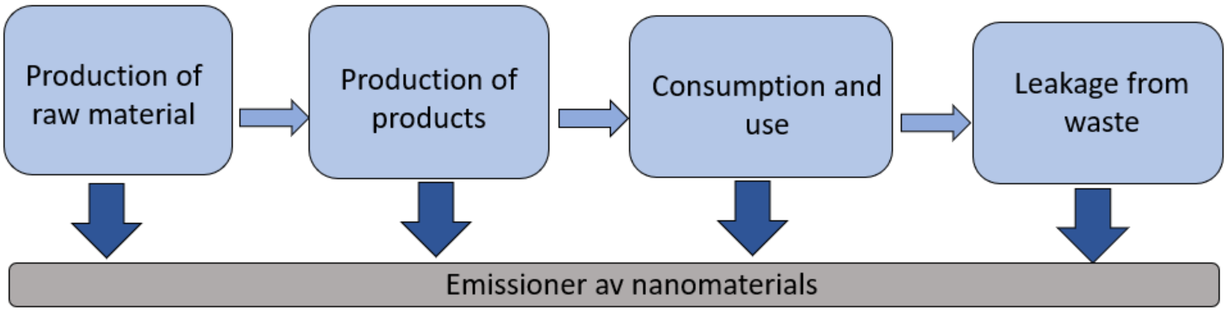

Figure 2. Illustration of the different stages in the life cycle of ENM were there is need for adequate methods of detection and measurement, provided as a discussion material for Group 2.

- **What data is needed to model flow, distribution, estimate quantities and preform LCA and risk assessments of ENM?**
  Production rates as a starting point would be really important to know, followed by baseline characterization to trace in environmental matrices. Moreover, it is important to gain knowledge on application of ENMs and their possible emission routes throughout the life cycle, monitore specific ENMs at different stages in the emission route and develop methods on how to distinguish between ENM and naturally formed NM. Put priorities in risk assessment according to the available data and their analysis.
- **How are the possibilities to analyze ENM in different environmental matrices different?**
  Generally, it is more difficult to analyze ENM in soil than air. Usually this depends on materials, metals more easily distinguished from an organic matrix, while polymers would be difficult Isotopes or labelled ENM can be used in controlled experiments to trace them at different stages of life cycle. However, fate is dynamic, effects depend on the properties of the matrices.
  Easier analysis: air>water>soil
- **What challenges and knowledge gaps exist in this area? Speculate and discuss.**
  Soil is indeed the hardest to measure – a suggestion is to manufacture materials that are easy to trace.

Group 3 - Fate (nobody was involved)

Group 4 - Toxic mechanisms

- **Are there other toxic mechanisms related to ENM in addition to those described in the figure?**
  The picture does not cover all the known mechanisms. All conventional mechanisms are relevant, including genotoxicity. Biotransformation is missing which is a mechanism of toxicity, while internalisation is not. The figure appears to described 4 mechanisms: ROS formation (however, note that the formation of ROS per se is not a toxicity mechanism; the authors need to replace this with: oxidative stress); ion release (which refers to the release of toxic metal ions from metal or metal oxide NPs); internalization (note: not clear why this is a mechanism of toxicity; there may also be uptake without toxicity; the authors need to consider the biotransformation of NPs that may occur once the

NPs are internalized); and surface coating (according to the figure, the surface of the organism is coated with NPs meaning that this is a physical mechanism leading to a disruption of the vitality of the organism, but surface coating may also refer to the fact that natural organic substances or NOM may adsorb to the surface of the NPs leading to the formation of a so-called eco-corona which in turn may affect the responses to the NPs). Overall, the group felt that in terms of biochemical mechanisms, the list is too limited as there is more to nanotoxicity than oxidative stress; in principal all of the classical mechanisms or endpoints of toxicity are relevant including cyto- and genotoxicity etc.

- **What are the most important toxic effects on the environment?**
  Bio-accumulation, persistence in fish and marine organisms, eutrophication, and perhaps biomagnification through the food chains!
- **What challenges and knowledge gaps exist in this area? Speculate and discuss.**
  There is a lack of biochemical mechanistic work (however, not everyone in the group agreed) and in most cases researchers tend to use the same guidelines and testing approaches. Despite the available omics data, there are very few new biomarkers gained from them so far. The nutritional status of the organism and its immune defence are usually impaired by ENMs. Moreover, ecosystems or communities of organisms need to be looked at, in order to examine the effect on biodiversity!

  It is important to look beyond oxidative stress. This often pops up, but is not the only mechanism. Furthermore, apart from acute toxicity, the group felt that long-term effects also warrant attention – including for instance NP impact on the nutritional status or pathogen defense. Additionally, it is important to consider ecological (community) effects and not only effects on individual organisms. The discussion on NPs in the environment should also cover the possibility of bioaccumulation of NPs and biomagnification along the food chain (to humans).

Group 5 - Risk assessment and risk management

- **What information is needed to perform reliable environmental risk assessments of ENM?**
  Depends on the level of information required. Simplified/ screening risk assessment methods are available (Nanoriskcat, proxy measures).
- **How can risk management of ENM be implemented?**
  Stepwise procedure with an initial screening risk assessment of the ENM itself, followed by a life cycle assessment of the specific ENM product. The stepwise procedure is aimed at a company which is wondering about the use of a material and the evaluation of it before engaging in its use.
  Also, reiterative sampling of the environment is important to see how it is cleaned up.
- **What challenges and knowledge gaps exist in this area? Speculate and discuss.**
  There are many challenges, especially in decision making.
  ENMs is a very diverse group of chemical substances, and until now evaluation is performed on a case-by-case basis. Larger companies have resources but most likely not SMEs, what about down-stream users?? Also, ECHA released an update on ENMs in risk assessment so there is also some new information on that.

Group 6 - Sustainable development goals and ENM

Figure 3. The Sustainable development goals, provided as basis for the discussion in group 6.

- **Can ENM contribute to achieve the sustainable development goals? Give examples.**
  There are lots of knowledge gaps. Maybe there is no strong link between nanosafety and the SDGs. There is higher correlation between nanotechnology and the goals.
  SDG 16 AND 17 through strong institutions and partnership
  SDG 9 AND 12 through implementation of safe by design concept (more correlated to nanosafety)
  SDG 6 AND 14 (clean water and no marine pollution) also via lignin NPs??
- **How can we utilize the potential of nanotechnology but at the same time minimize the risks (think from a safe-by-design perspective)?**
  By SIA approach- Safe by design perspective and regulatory preparedness, Safe from the beginning concept. Innovation processes need to consider safety!
- **What challenges and knowledge gaps exist in this area? Speculate and discuss.**
  There are many gaps. No proper regulatory framework, definition of nanomaterials varies among different sectors and legislative frameworks, lack of a coherent risk assessment system to evaluate their safety on human health and the environment.

It was commented that addressing the SDGs is often linked to simpler solutions than bringing in nanotechnology. Many SDGs are interlinked and solving one (eg. SDG 16 – strong institutions) indirectly supports/solves the others. However, in Iraq, for example, there are attempts to support water management systems for supporting crops etc using nanotechnology for cleaning the water and for supporting the growth.!

Thereafter it was concluded that it would be important to know about the emissions in such nanotechnology applications to support the SDGs!

National Plastic Coordination – an overview – Åsa Stenmarck, Julia Taylor (Swedish Environmental Protection Agency)

The next speakers, Åsa Stenmarck and Julia Taylor, both working at the Swedish Environmental Protection Agency (EPA) held a talk about plastic and the National Plastic Coordination. Åsa gave firstly an overview, emphasizing that one of the main goals is to gather and spread the knowledge on plastic, to create meeting possibilities and to act as an independent actor, basing the decisions on facts. National Plastic Coordination focuses its action on plastics, microplastics, road and tyre wear but not nano-plastics or textiles (unless textiles are turned into plastics). However, it was mentioned that even though they are not currently working with nanoplastics, they would like to collaborate with SweNanoSafe since they want to set new goals and roadmaps by developing and strengthening networks.

Then, Julia talked about Swedish EPA's work on microplastics. A lot of the previous work focused on two government assignments (finished 2017, 2019), mapping the most important sources/pathways of microplastics in Sweden. She said that innovation is a very important problem-solving tool and thus, grants are needed for actions leading to solutions.

Previous work had focused on emissions from artificial grass pitches, playing fields and other outdoor sports and play facilities. The investigations were performed by the IVL Swedish environmental research institute, in cooperation with KTH, including measurements on artificial grass with and without granules. Unfortunately, few alternatives to these materials are available on the market today, and thus, innovation competition has been set for developing sustainable alternatives to artificial grass and rubber surfaces in school yards and pre-schools, aiming at promoting the use of sustainable new materials and products and reducing microplastic emissions.

The aim of the report would be useful not only to EPA itself but also to other institutions for obtaining a more clear view of the needs in this area and creating a research agenda for microplastics, valuable for all relevant stakeholders and research funders. Moreover, inspired by SweNanoSafe`s action, the aim is also the creation of a research network and the collaboration with experts within macro/micro plastics field, facilitating dialogue and knowledge exchange, as well as promoting science-based decision making.

Regarding the current microplastic projects within the Swedish EPA, it was mentioned that five research projects on the environmental impact of nanoplastics are under development, paving the way for defining microplastics criteria and supporting filter solutions for household plastics. Also, five smaller projects (funding of 2 pre-procurement purchasing groups, funding zero microplastics challenge 2020 and a translation of the government assignment "microplastics in the Environment 2019") are underway and available soon, as well as a collaboration with the OECD regarding NMs from textiles, tire and road wear.

Highlights from the joint discussion on nano- and microplastics (in plenum)

It was pointed out that further collaboration with other municipalities (e.g Stockholm city) is needed to spread the knowledge, although the questions of how to do it still remains. Hence, clearer processes and strategies should be defined on how to reach out other stakeholders. It was also mentioned that besides the funding of research projects, there are also research workshops organised to increase the dialogue and present the ongoing applied research and results to the relevant stakeholders. Furthermore, it was proposed that a workshop focusing on the applicability and advantages of microplastics would be also interesting and valuable in order to define exactly the balance between benefit and risk, resulted from microplastics use.

EPA has not fully formulated yet whether people can live with nanoplastics or they need to be very cautious about them, since more knowledge on the actual environmental effects is needed. Hence, a broader understanding of what the major challenges are, is required as well as the identification of the relevant solutions and the priority areas on which research should begin with.

Environmental impact of microplastics from consumer products – Tommy Cedervall, Lund University

The next speaker, Tommy Cedervall who is an associate professor and the head of the Department of Biochemistry and Structural Biology at Lund University, held a talk entitled *"Environmental impact of microplastics from consumer products"*. In the presentation, Tommy described how nanoplastics affect nature, using polystyrene as a model. He pointed out that everything we know so far is almost from polystyrene models, since polystyrene is the most commonly used type of plastics. Today, ongoing research is conducted about defining and mapping the toxicity effects of different types of nanoplastics through acute and chronic toxicity tests, as well as characterization methods.

Important key aspects of his presentation were:

- The smaller the plastic, the further it gets down in the food chain. Even though it is difficult to detect nanoplastics, methods are emerging.
- There are many different sources emitting nanoplastics such as styrene, coffee cups, pacifiers, bottles, fruit bags, eco bags, package bags etc.
- Many different types of food processers have been used to "degrade" nanoplastics and see whether they are released, but only certain types of processers eventually produced nanoplastics.
- It is important to know how nanoplastics are formed before we know if there are effects. So far, effects of synthesized polystyrene have been noticed, hence it is reasonable to assume that there may be effects by other degraded plastic NPs as well. Moreover, polystyrene might not cover all types of effects, so there may be also unknown effects originated from nanoplastics.
- Different types of polystyrene show different toxicity. For example, aggregation of polystyrene particles did not seem to affect the toxicity which was surprising – aggregates were not forming a new type of surface but rather formed loose aggregates that were acting almost as individual particles.
- There is a large difference between acute and chronic testing – long-term experiment with polystyrene-loaded algae feeding to zooplankton and fish showed effects regardless of the surface chemistry.

Transformation & fate of nano & microparticles in the environment – Julian Gallego, University of Gothenburg

The next speaker was Julian Gallego Urra who is a post-doctoral researcher at the University of

Gothenburg. His research at the department of marine sciences, in the Kristineberg research marine station, focuses on the marine environmental nanochemistry, including natural and anthropogenic NPs found in the aquatic environment, as well as microplastics. He is also participating in the MISTRA Environmental Nanosafety program- Phase II regarding the detection and the fate of NMs in the aquatic environment, using new analytical techniques.

He opened his speech entitled *"Environmental fate of nano- and micro-plastics in the aquatic environment"*, pointing out that even though there is an agreement on the definition of microplastics as objects with one of the three dimensions below 100 nm, there is still slight confusion when it comes to the definition of microparticles. Important questions that should be answered during the exposure assessment included the following: how much are being released (in soil, water, air), which form they have (primary particles, aggregates, dissolved), where they go (transport mechanisms: aggregation, settling, resuspension), how long they remain (persistence, dissolution, sediment burial), and how much of them are eventually present (predicted environmental concentrations). Then, he talked about the possible pathways of environmental nanoparticles (ENPs) release and their transport and transformation in aquatic environments, emphasizing that a lot of research is conducting today and thus, there is a lot of information around that topic.

Furthermore, he presented an example from his ENP research referring to nanosilver (nanoAg) as one of the most studied NPs. Important aspects of his speech were:

- Silver nanoparticles are highly dynamic in the environment, and many possible reactions can occur on the surface of nanoparticles (e.g oxidation followed by release of Ag ions, Ag ions form insoluble AgS (anoxic), Ag can also form complexes with Cl making it stable… but not bioavailable etc.
- Released Ag ions is believed to be the cause of toxicity, but aggregation of particles is believed to determine their ultimate fate. OECD has put some ideas forward e.g. OECD aggregation test No. 318 which measures the dispersion stability of NMs in simulated environmental media and evaluates how different NPs behave in different environmental matrices. However, this test is mainly designed for homo-aggregation and only can be used for very simplified water chemistry.
- In case of multiple components (suspended particulate matter), there are multiple pathways and numerous different types of aggregations that may take place during their mixture.
- Also, the fate of gold (Au) NPs in seawater during algal blooms has been examined. The result of the study showed that the seawater composition does affect the aggregation rate, namely, the seawater composition in the spring and summer resulted in aggregation rates reduced by more than 60% compared to the winter seawater composition. Thus, algal blooms can have an impact on the aggregation of NPs.
- Future research should be focused on the development of more standardized tests, the study of potential cotransport of contaminants, mechanisms and rates of degradation and transformation and also, particle-mediated transformation of other compounds.
- Detection in the environment remains still challenging since little is known about the natural components as well (organic matter and suspended inorganic colloids).

Questions & answers

The presentations from Tommy Cedervall and Julian Gallego was followed by a questions and answer session moderated by Professor Joachim Sturve.

Q: Is there a possibility of looking through data archives in order to model seasonal variation and different aggregation rates in seawater or river water?

A: maybe yes, e.g. algal blooms with chlorophyll measurements – so, maybe we can use this environment as model.

Q: Is there a way to use standardized methods for risk assessment or is the system too complex?/ Could we test eco-corona formation for the needs of RA?

A: There is too much information and thus, it is too early to know, however maybe there is a possibility of having a standardized method that can be used for the needs of RA if all the various environments and conditions can be represented and tested.

Q: Could nanoplastics and microplastics in water resources, act as vectors for microorganisms?

A: this depends on the real situation and condition exist inside the water. Organic pollutants-contaminants that may be present in water, can compete microplastics and nanoplastics for bioaccumulation in fish. What really occurs depends on their concentration and the total chemical equilibrium. Moreover, since NMs have a large capacity to adsorb organic pollutants, it is possible either organic pollutants to be attached to NMs consumed by fish or vice versa (through trophic transfer). Nanoplastics and viruses have the right size domains to attach. And again, whether the virus will be active or compete the nanoplastic particle over any other particle depends on the equilibrium force.

Also, discussion was taken place about the transport of other chemicals or microorganisms (e.g viruses, eco-corona, bio-film). A model representing all these components would be extremely useful but the same time really challenging to be created.

Nanoparticle screening using *in vitro* fish models – Marianne Brookman-Amissah, University of Gothenburg

Marianne Brookman-Amissah, a PhD student from the department of biological and environmental sciences, at the University of Gothenburg held a talk regarding nanoparticle screening using *in vitro* fish models. She opened her presentation, mentioning that pollution by nanomaterials is a major growing concern, particularly the one caused by nanoplastics (both engineered and from microplastic degradation). She also pointed out that growing concerns come always with the need for knowledge on potential unknowns (toxicity, adverse effects and routine ways for testing).

Marianne firstly talked about polystyrene, the synthetic polymer that is commonly used in the food and packaging industry, its sources (natural breakdown processes, engineered) and the different sizes used (25,100, 2000nm). She mentioned also that polystyrene nanoform is used in biosensors and drug delivery. Then, she talked about piscine cell lines and how feasible it is to establish cell lines from normal tissue, since fish naturally have high telomerase activity. Moreover, these cell lines constitute the first choice when it comes to NPs toxicity testing since they are cost-effective and devoid of some ethical issues, implementing the 3Rs principles.

In addition, an overview of a screening of polystyrene NPs method was provided, focused on acute cytotoxic assays and toxicity mechanisms. The screening process described, included the following biochemical assays: alamar blue (cell viability), CFDA-AM (membrane integrity) and neutral red (lysosome integrity). None of these fluorescence signals overlapped with each other so it was possible to be conducted at the same time. The fact that the results did not show that much difference between the different sizes of nanoparticles used, was possibly due to various factors including possible low concentration studied, short-time exposure or the formation of aggregate(s) which decreased the total amount of surface area available to the cells.

Finally, she talked about the Sph3roiD project which refers to the development of a liver trout cell culture (3D aggregate/spheroid culture), aiming to examine potential long-term toxicity and bioaccumulation of chemicals and NPs.

Trophic transfer of nanoparticles in aquatic environments - Amalie Thit Bruus Jensen, Roskilde University, Denmark

The final presentation was held by Amalie Thit Bruus Jensen, assistant professor at the Department of Science and Environment in Roskilde University. She held a talk entitled: `` Trophic transfer of nanoparticles in aquatic environments``. The main topic of her presentation was the trophic transfer of copper oxide (CuO) NPs from sediment to worms and fish.

Important key aspects of her talk were:

- CuO NPs are released into the aquatic environment from food packages and then it is possible that they agglomerate or aggregate and then, deposited as sediment. Since the NPs accumulate in the sediment they may pose a risk to the organisms living in the sediment. Indeed, uptake in sediment-dwelling biota (worms) has been observed and thus, a trophic transfer to fish is likely to occur.
- The exposure and the uptake of CuO NPs in water is different than in sediment. It has been observed that when worms were exposed via water, the accumulation of copper chloride (CuCl) was higher than CuO NPs, whereas, when worms were exposed via the sediment, the uptake mechanisms were observably different.
- To conclude, trophic transfer does occur for both CuO NPs and CuCl, and their accumulation is measureable in both worms and fish. Intestine was the main site of copper accumulation.
- There is high egestion of Cu in fish especially for NPs and also, altered gene-expression levels for both CuO NPs and CuCl have been observed in fish.
- Sediment is an important route of uptake and should be included in future studies

Conclusions - Final discussion in plenum

All participants concluded that there was valuable information provided by the presentations and important knowledge exchange during the workshop. The fruitful group discussions covered various topics including the sources and the environmental fate of nanomaterials, nano and micro plastics and the related environmental aspects, trophic transfer and their risk assessment approach.

One of the most important concluding remarks was also the need for further collaboration between academia, governmental authorities and industry. They should equally take the necessary actions, in order to define the sources and environmental fate of NMs including microplastics as well as, to develop more standardized and related to real-world settings methods for toxicity testing.

Annex 1. Agenda

3rd Annual Workshop of the National Nanosafety Research Network: Nanomaterials in the Environment
Organized by the National Nanosafety Platform (Swenanosafe) and the Swedish Environmental Protection Agency

Date: November 17, 2020
Location: Online *via* Zoom (registration required)
Time: 09.30 – 15.30
Participants: academia, governmental authorities, funding agencies, non-governmental organizations
Moderator: Lennart Gisselsson, Lund University

09.15 ONLINE MEETING OPEN

09.30 Welcome and introduction – prof. Joachim Sturve, GU; prof. Bengt Fadeel, KI (SweNanoSafe)

09.40 Opening remarks – Julia Taylor, Swedish Environmental Protection Agency

09.45 Keynote on Nanomaterials in the Environment – prof. Richard Handy, Univ. of Plymouth, UK

10.15 Questions & answers (moderator: Bengt Fadeel, KI)

10.30 Nanomaterials in the Environment – an interim report – Arne Wallin, Freja Milton, Goodpoint

10.45 Group discussions: input on the report on nanomaterials in the environment [break-out]

11.30 Presentation of group discussions (in plenum) [each group to elect a rapporteur]

12.00 (VIRTUAL) LUNCH BREAK

13.00 National Plastic Coordination – an overview – Åsa Stenmarck, Julia Taylor (Swedish EPA)

13.15 Discussion on nano- and microplastics (in plenum)

13.45 Environmental impact of microplastics from consumer products – Tommy Cedervall, LU

14.00 Transformation & fate of nano & microparticles in the environment – Julian Gallego, GU

14.15 Questions & answers (moderator: Joachim Sturve, GU)

14.30 Nanoparticle screening using *in vitro* fish models – Marianne Brookman-Amissah, GU

14.45 Trophic transfer of nanoparticles in aquatic environments - Amalie Thit Bruus Jensen, RUC

15.00 Discussion - Lennart Gisselsson, moderator

15.30 CLOSE OF WORKSHOP

Annex 2. List of participants

NAME		AFFILIATION
Arvidsson	Rickard	Chalmers
Surber	Nicholas	Chalmers
Nilebäck	Erik	Chalmers Industriteknik
Milton	Freja	Goodpoint ab
Wallin	Arne	Goodpoint AB
Brookman-Amissah	Marianne	Göteborgs Universitet
Carney Almroth	Bethanie	University of Gothenburg
Gallego	Julian	University of Gothenburg
Mattsson	Karin	University of Gothenburg
Sturve	Joachim	Göteborgs Universitet
Haasmark	Sara	ICA Fastigheter
Alenius	Harri	IMM, Karolinska Institutet
Keshavan	Sandeep	IMM, Karolinska Institutet
Gupta	Govind	Karolinska Institutet
Peng	Guotao	Karolinska Institutet
Nilsson	Fritjof	KTH
Odnevall Wallinder	Inger	KTH
Pylypchuk	Ievgen	KTH
Karlsson	Helen	Linköpings Universitet
Cedervall	Tommy	Lunds universitet
Gisselsson	Lennart	Lund University
Lundqvist	Martin	Lund University
Kammereck	Stefanie	Materials and Environmental Chemistry, SU
Rahmani	Roja	Materials and Environmental Chemistry, SU
Kryuchkov	Fedor	Norwegian Veterinary Institute
Bredberg	Anna	RISE
Bohlén	Martin	RISE - Research Institutes of Sweden
Rissler	Jenny	RISE/LU
Thit	Amalie	Roskilde University
Almstrand	Ann-Charlotte	Sahlgrenska Universitetssjukhuset
Gorokhova	Elena	Stockholm University
Ivanov	Mikhail	Stockholm University
Lyubartsev	Alexander	Stockholm University
Elihn	Karine	Stockholms Universitet
Andersson	Åsa	Naturvårdsverket
Latvala	Siiri	Swedish EPA
Stenmarck	Åsa	Swedish EPA
Taylor	Julia	Swedish EPA
Athanasiou	Marietta	SweNanoSafe, IMM, Karolinska Institutet
Fadeel	Bengt	SweNanoSafe, IMM, Karolinska Institutet
Hanberg	Annika	SweNanoSafe, IMM, Karolinska Institutet
Jackson	Charlotte	SweNanoSafe, IMM, Karolinska Institutet
Midander	Klara	SweNanoSafe, IMM, Karolinska Institutet
Nymark	Penny	SweNanoSafe, IMM, Karolinska Institutet

Miliutenko	Sofiia	Trafikverket
Handy	Richard	University of Plymouth